



## Abstract

Tongguanteng (*Marsdenia tenacissima*), which is mainly distributed in the Yunnan and Guizhou provinces of China, was first recorded in *Diannanbencao* by Lan Mao of the Ming dynasty of China. According to recent pharmacological studies, the chemical composition of Tongguanteng (*Marsdenia tenacissima*) is complex and contains C<sub>21</sub> steroidal saponins, polysaccharides, alkaloids, and other molecules, which show anti-cancer effects on various tumor cell lines. It inhibits tumor cell proliferation and growth mainly by increasing the expression of apoptosis- and cell cycle-related proteins to promote apoptosis and arrest tumor cells in the G2/M or S phase. Downregulation of the expression of vascular endothelial growth factor-2/A and matrix metalloproteinase-2/9 suppresses the formation of the tumor microvasculature, leading to tumor malnutrition, increased expression of interleukin-2, glutathione peroxidase, catalase, and superoxide dismutase, and decreased interleukin-10 and malondialdehyde expression, thereby enhancing immunity and antioxidation in the body. Additionally, inhibition of epidermal growth factor receptor, hepatocyte growth factor receptor, and tyrosine-protein kinase receptor activation enhances the anti-tumor efficacy of epidermal growth factor receptor-tyrosine kinase inhibitors as well as inhibits P-glycoprotein and cytochrome P450 to increase the concentration of anti-tumor drugs in tumor cells.

**Keywords:** *Marsdenia tenacissima*, Anti-tumor, Hematological tumor, Lung cancer, Gastrointestinal carcinoma

## 摘要

通关藤最早记载在明代兰茂所著《滇南本草》，主要分布于中国的云南和贵州等地，作为一种抗肿瘤药物在当地广泛使用。现代药理学研究表明，通关藤中的化学成分很复杂，包括 C<sub>21</sub> 甾体皂苷、多糖、生物碱等，对多种肿瘤细胞有良好的抑制作用。本文通过查阅中国知网、PubMed 等大型数据库，整理归纳近年来报道的关于通关藤体内外抗肿瘤方面的文献，发现通关藤的主要抗肿瘤机制包括：调节凋亡和细胞周期相关蛋白的表达，促进凋亡，将细胞周期阻滞在 G2/M 或 S 期，进而抑制肿瘤生长；下调 VEGF-2/A 和 MMP-2/9 的表达，抑制肿瘤微血管的生成，导致肿瘤营养不良；提高 IL-2、GSH-Px、CAT、SOD 的表达，下调 IL-10 和 MDA 的表达，增强机体免疫和抗氧化能力。此外，通关藤还可以通过抑制 EGFR、HGFR 和酪氨酸蛋白激酶受体的活化来增强 EGFR-TKI，以及通过阻止 P-糖蛋白和细胞色素 P450 来增加抗肿瘤药物在肿瘤细胞内的浓度，逆转肿瘤的多药耐药性。

**关键词：** 通关藤；抗肿瘤；血液肿瘤；肺癌；胃肠道肿瘤

**Abbreviations:** Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated x protein; Bcl-xl, B-cell lymphoma-extra large; CCL5, C-C chemokine ligand 5; CCR, Chemokine receptor; CDK, Cyclin-dependent kinase; CYP450, Cytochrome P450; EGFR, Epidermal growth factor receptor; ERK, Extracellular signal-regulated kinase; FAK, Focal adhesion kinase; IL, Interleukin; MDA, Malondialdehyde; MTE, Tongguanteng (*Marsdenia tenacissima*) extract; MMP, Matrix metalloproteinase; P-gp, P-glycoprotein; Rho, Ras homolog; TNF, Tumor necrosis factor; VEGF, Vascular endothelial growth factor; c-Met, Hepatocyte growth factor receptor.

**Funding:** This study was supported by the Creative Item of Innovative Team of Ministry of Education (No. IRT-13R63).

**Competing interests:** The authors report no conflicts of interest in this work.

**Copyright:** © 2018 TMR Publishing Group Limited. This is an open access article distributed under the terms of the Creative Commons Attribution Non Commercial License.

**Executive Editor:** Cui-Hong Zhu.



## Background

Tongguanteng (*Marsdenia tenacissima*) belongs to the family Asclepiadaceae mainly distributed in the Guizhou, Yunnan, Sichuan, and Guangxi provinces of China and other areas in Southeast Asia [1]. It was first recorded in the ancient book of *Diannanbencao* by Lan Mao of the Ming dynasty of China (1436 A.D.) as bitter, light astringent, sub-cold, homing to the lung meridian, clearing heat and detoxifying, relieving cough and asthma, and eliminating stagnation and pain [2], which is consistent with the description in the *Yunnan Zhongcaoyao Xuan* (1970 edition) and *Quanguo Zhongcaoyao Huibian* (1975 edition). Additionally, the *Yunnansheng Yaopin Biaozhun* (1977 edition) stated that Tongguanteng (*Marsdenia tenacissima*) decreased inflammation and had anti-cancer effects [2]. Tongguanteng (*Marsdenia tenacissima*) contains C<sub>21</sub> steroidal saponins, which are considered to exert anti-tumor effects; it also contains polysaccharides, alkaloids, and resins [3]. This plant has long been used as a traditional medicine to treat various types of cancer and shows potential curative effects, mild side effects, and good value for development by targeting multiple components of different signaling pathways [1] based on studies conducted since the early 1970s [4, 5].

Clinically, Tongguanteng (*Marsdenia tenacissima*) is the main raw material of compound preparations applied for anti-tumor treatment, and Xiaoaiping injection (Approval number: Z20025869) is a prescribed total water extract preparation of Tongguanteng (*Marsdenia tenacissima*) used to treat cancer. Based on clinical research conducted in the Henan province, Tongguanteng (*Marsdenia tenacissima*) was prepared as tablets and injections by the Henan Xinxiang Qianwei Pharmaceutical Factory [4, 5]. Since then, in combination with other anti-cancer drugs, it has been widely used to treat lung cancer, colon cancer, esophageal cancer, and other cancers and has been shown to increase progression-free survival and reduce the incidence of adverse reactions in patients [6 - 10]. Here, we review the research progress related to the treatment of different tumors with Tongguanteng (*Marsdenia tenacissima*), its extract, and Xiaoaiping injection to provide a scientific basis for the further development and utilization of Tongguanteng (*Marsdenia tenacissima*).

## Hematological neoplasms

Ye *et al* [11] studied the role and related mechanisms of the ethanolic extract of Tongguanteng (*Marsdenia tenacissima*) on the human Burkitt's lymphoma cell line Raji, human acute promyelocytic leukemia cell line NB4, and human erythroleukemia cell line K562, and found that Tongguanteng (*Marsdenia tenacissima*) inhibited the proliferation of all three tumor cell lines. However, it showed no effects on normal peripheral blood mononuclear cells. Flow cytometry analysis revealed that Tongguanteng (*Marsdenia tenacissima*) induced

significant K562 cells apoptosis, increased the number of cells in the G0/G1 phase, decreased the number of cells in the S phase, arrested cells in the G0/G1 phase, and inhibited colony formation in a dose-dependent manner. The expression of BCL-2-associated x protein (Bax), Caspase-3, and Caspase-9 was up-regulated, while that of B-cell lymphoma 2 (Bcl-2) and Cyclin D1 was down-regulated. Thus, Tongguanteng (*Marsdenia tenacissima*) was demonstrated to exert good inhibitory effects on K562 tumor cells.

Tenacissoside C, a C<sub>21</sub> steroidal saponin, was extracted from the air-dried stems of Tongguanteng (*Marsdenia tenacissima*) and was found to arrest cells in the G0/G1 phase, decrease Cyclin D1 expression, and inhibit cell proliferation [12]. Analysis of apoptosis-related proteins, including Caspase-3, Caspase-8, Caspase-9, Bax, Bcl-2, Bcl-2 antagonist killer, and Bcl-xl (B-cell lymphoma-extra large), revealed no change in Caspase-8 expression, while Caspase-3 and Caspase-9 were up-regulated, indicating that tenacissoside C promoted cell apoptosis through a mitochondrial pathway. The Raji, NB4, and K562 cell lines were also studied by Xue [13] who found that tenacissoside C among the four C<sub>21</sub> steroid saponin monomers had the strongest proliferation inhibition and apoptosis induction effects in Raji cells strains.

In addition to tenacissoside C, medium and high doses of Tongguanteng (*Marsdenia tenacissima*) extract (MTE) and two derivatives (tenacissoside A and 11 $\alpha$ -O-benzoyl-12 $\beta$ -O-acetyltencigenin B) of tenacigenin B, a type of C<sub>21</sub> steroidal aglycone, from Tongguanteng (*Marsdenia tenacissima*) significantly inhibited Raji cells proliferation [14]. Wang *et al* [15] showed that MTE reduces the viability of the human T-cell acute lymphoblastic leukemia cell line Jurkat and arrests these cells in the S phase in a dose-dependent manner. Mechanistic studies indicated that MTE induces cell apoptosis by promoting the expression of phosphatases and tensin homolog to inhibit the PI3K/Akt/mTOR signaling pathway. However, human multiple myeloma cells RPMI8226 were not sensitive to MTE [16]. Ceng [17] found that a Tongguanteng (*Marsdenia tenacissima*) water extract, containing dextran and heteropolysaccharides as the main components, affected the proliferation rate of K562 cells and altered cell morphology. After 24 h of culturing the cells with 10, 30, and 50  $\mu$ L/mL of the water extract, the growth inhibition rates were 45%, 73%, and 84%, respectively, with an IC<sub>50</sub> value of 11  $\mu$ L/mL. Total aglycones of Tongguanteng (*Marsdenia tenacissima*) Caulis improved the sensitivity of K562 cells to paclitaxel, showing anti-tumor effects [18]. However, the inhibitory effects of high, medium, and low doses of total saponins from Tongguanteng (*Marsdenia tenacissima*) on the mouse lymphocytic leukemia cell line p388 were weaker than those of cyclophosphamide, these results are not ideal [19].

Xiaoaiping injection reduces the mitochondrial trans-membrane potential of the human leukemia cell strains U937, HL60, and K562 and promotes apoptosis



[20, 21]. Using a gene chip to detect the gene expression spectrum in U937 cells, it was found that Tonggulanteng (*Marsdenia tenacissima*) up-regulates multiple apoptosis-inducing genes, including the tumor necrosis factor (TNF) ligand and receptor family, Caspase family, Caspase recruitment domain family, and p53 family of genes [22]. However, it does not induce apoptosis in human erythroleukemia cells [23]. Table 1 summarizes data on the four major hematological tumor cell lines mentioned above.

In a clinical trial of 131 patients with non-Hodgkin's lymphoma, the use of a standard CHOP regimen [Cyclophosphamide 750 mg/m<sup>2</sup>, intravenous injection (i.v.), day 1; Epirubicin 40 mg/m<sup>2</sup>, i.v., day 1; Vinblastine 1.4 mg/m<sup>2</sup>, i.v., day 1; Pred 100 mg/m<sup>2</sup>, taken orally, days 1 to 4, 21 days/cycle] combined with Xiaoaiping injection had an efficacy rate of 71.00%, which was higher than that (58.00%) observed in the control group. Thus, this treatment can improve the quality of life of patients and reduce adverse reactions to chemotherapy [24].

**Table 1 Anti-cancer effect of Tonggulanteng (*Marsdenia tenacissima*) on four kinds of hematological tumor cell**

Tumor cell lines	Drugs	Cell cycle arrest	Protein or/and mRNA expressions	The ways or/and effects on tumor cells	References
Raji	CME/MTE/C <sub>21</sub> steroidal aglycones	G0/G1	Bcl-2, Bcl-x1, Ki-67↓ BIRC2/3 unaffected BID↑	Induced apoptosis, Anti-angiogenic, Growth inhibition.	[11], [13], [14], [16]
K562	CME/ETA/Tenacissoside C	G0/G1	Bcl-2, Bcl-x1, Cyclin-D1↓ Caspase-8 unchanged Bax, Caspase-3/9, Bak↑	Induced apoptosis, Anti-angiogenic, Growth inhibition, Increased antitumor efficacy of anticancer drugs.	[11-13], [17-18], [21]
Jurkat	MTE	S	Bcl-2, p-Akt/Akt↓ p-mTOR/mTOR↓ c-Caspase-3, c-PARP↑, Bax, PTEN↑	Suppressed proliferation, promoted apoptosis by PI3K/Akt/mTOR signaling pathway	[15 - 16]
U937	Xiaoaiping injection	/	Bcl-2, BIK, BNIP3, TNFRSF10A/21, CIDEB, IGF1R, PYCARD↓ c-Caspase-3, PARP, TNF, TNFRSF1B, Caspase3/4/6/9, Bcl-2L10/2A1, BAK1, BIRC2/4, CARD6, CFLAR, ABL-1, p53, p73↑	Inhibited proliferation, Induced apoptosis.	[20], [22]

↓ = Protein or/and mRNA expressions decreased; ↑ = Protein or/and mRNA expressions increased; / = No data.

MTE, Tonggulanteng (*Marsdenia tenacissima*) extract; CME, Ethanolic extract of Tonggulanteng (*Marsdenia tenacissima*); ETA, Total aglycones of Tonggulanteng (*Marsdenia tenacissima*); Bcl-2, B-cell lymphoma 2; Bcl-x1, B-cell lymphoma-extra large; BIRC, Baculoviral inhibitors of apoptosis repeat containing; Bax, Bcl-2-associated x protein; Bak, Bcl-2 homologous antagonist/killer; Akt, Protein kinase B; mTOR, Mammalian target of rapamycin; PTEN, Phosphatase and tensin homolog deleted on chromosome ten; BIK, BCL2 interacting killer; BNIP3, BCL2/adenovirus E1B 19 kDa protein-interacting protein 3; TNFRSF, Tumor necrosis factor receptor super family member; CIDEB, Cell death-inducing DFFA-like effector b; IGF1R, Insulin-like growth factor 1 receptor; PYCARD, Apoptosis-associated speck-like protein containing a CARD; PARP, Procylic acidic repetitive proteins; TNF, Tumor necrosis factor; CARD, Caspase activation and recruitment domains; CFLAR, CASP8 and FADD-like apoptosis regulator; ABL1, Abelson murine leukemia viral oncogene homolog 1; BID, BH3 interacting-domain death agonist.



## Lung cancer

Han *et al* [25] showed that combining low-dose MTE (8 mg/mL) with gefitinib had synergistic effects in the human non-small-cell lung cancer cell line HCC827 harboring the epidermal growth factor receptor (EGFR) mutation and in wild-type human non-small-cell lung cancer H292 cells. Comparing three different methods of drug administration, MTE addition to the medium and incubating the cells for 12 h followed by gefitinib addition and incubation for another 72 h (MTE → MTE + gefitinib) was the most potent method of inhibiting tumor cell growth. MTE enhanced gefitinib-induced G2/M cell cycle arrest in H292 cells, and HCC827 cells were arrested in the S phase. At the molecular level, MTE → MTE + gefitinib further enhanced the effects on the extracellular signal-regulated kinase (ERK)1/2 and PI3K/Akt/mTOR signaling pathway, indicating cross-activation of EGFR and a hepatic growth factor receptor (c-Met). The combination suppressed the phosphorylation of components downstream of EGFR and suppressed c-Met and tyrosine-protein kinase receptor activation in human lung adenocarcinoma HCC827/ER cells [26]. A similar approach [27] also showed that MTE reverses gefitinib resistance in the human non-small-cell lung cancer cell lines H460 and H1975 and exerts anti-tumor effects. In a study evaluating whether Tonggulanteng (*Marsdenia tenacissima*) can be used to overcome drug resistance in lung cancer cell lines, Yao *et al* [28] found that tenacigenin D, a polyoxyypregnane aglycone isolated from Tonggulanteng (*Marsdenia tenacissima*), suppressed P-glycoprotein (P-gp) transportation of drugs out of cancer cells and circumvented the multidrug resistance mediated by P-gp. Analysis of tenacigenin D treatment combined with erlotinib and gefitinib treatment in H292, H460, and H460/Vbl cells revealed that tenacigenin D potentiates the activity of erlotinib and gefitinib in drug-resistant cells.

Furthermore, from the perspective of the immune system, tenacissoside H, a C<sub>21</sub> steroidal saponin extracted and purified from Tonggulanteng (*Marsdenia tenacissima*), inhibited the growth of Lewis lung tumor xenografts, decreased lung metastases, increased interleukin (IL)-2 levels, attenuated IL-10 levels, restored the T helper 1/T helper 2 balance, and enhanced the immune function in mice to achieve an anti-tumor effect [29]. Additionally, Lin *et al* [30] conducted an MTT assay, a wound healing assay, and a cell invasion assay and found that at increasing injection doses of Xiaoaiping, fewer human lung cancer cells (A549 cells) showed metastasis, growth inhibition increased, the chemokine receptor (CCR) 5 protein levels and C-C chemokine ligand 5 (CCL5) secretion decreased, and CCR9 and CCR4 protein levels were unchanged. Accordingly, the phosphorylation levels of focal adhesion kinase (FAK), activator of transcription 3, ERK, p38, and Ras homolog (Rho) in the CCR5-CCL5 biological axis were decreased. These results indicate that Xiaoaiping attenuated the migration and invasion of A549 cells by down-regulating the Rho C, FAK, and

CCR5-CCL5 biological axis. In the human lung adenocarcinoma ASTC-a-1 cells, Xiaoaiping treatment for 72 h decreased cell proliferation and increased Caspase-3 levels, but Caspase-3 level was not significantly changed after culturing with Xiaoaiping for 24 h; inhibitory effects were not evident [31].

In clinical trials, patients with advanced non-small-cell lung cancer were treated with NP chemotherapy regimen (vinorelbine and cisplatin) [32], TP chemotherapy regimen (paclitaxel and cisplatin) [33], and docetaxel [34]. After combined treatment with Xiaoaiping injection, higher scores for the quality of life were observed than those after treatment without Xiaoaiping [35]. Furthermore, myelosuppression and gastrointestinal reactions were alleviated; however, individual patients experienced discomforts, such as low fever, excessive sweating, migratory muscles, and joint pain, during the injection of Xiaoaiping.

## Gastrointestinal tumor

### Esophageal cancer

Fan *et al* [36] conducted MTT and BrdU incorporation immunofluorescence assays and found that MTE inhibits the growth and proliferation of human esophageal cancer cells KYSE150 and Eca-109 in a time- and dose-dependent manner and arrests cells in the G0/G1 phase. Expression of cell cycle regulatory proteins Cyclin-dependent kinase (CDK)2/4/6, Cyclin-D1/2/3, Cyclin-E1, and retinoblastoma protein decreased (Table 2), while CDK inhibitors did not affect the expression. After MTE administration, the PI3K/Akt signaling pathway was not affected, while the phosphorylation of proteins involved in the ERK, c-Jun N-terminal kinase, and p38 signaling pathways decreased. In the human esophageal squamous cell carcinoma HKESC-1 cells and its cisplatin-resistant subline HKESC-1cisR, MTE up-regulated p21 expression and restored the effects of cisplatin and caused G2/M phase retardation, thereby reversing chemotherapeutic drug resistance in cancer cells [37] (Table 2).

Numerous clinical trials have shown that Xiaoaiping injection with irinotecan [38, 39], paclitaxel [40, 41], cisplatin [40 - 42], tegafur [43], oxaliplatin [43], docetaxel, or raltitrexed [42] significantly improves the clinical efficacy and progression-free survival as well as reduces diarrhea, oral mucositis, hand-foot syndrome, nausea, leukopenia, and neutropenia in late esophageal carcinoma patients.

### Gastric cancer

Li *et al* [44] showed that Tonggulanteng (*Marsdenia tenacissima*) down-regulates Bcl-2 and Bcl-xl in the human gastric adenocarcinoma cell line BGC-823 in a concentration-dependent manner, but observed no obvious changes in Bax and Bcl-2 antagonist of cell death protein levels. The metadherin gene is a new specific marker of gastric cancer and target for clinical treatment; since Tonggulanteng (*Marsdenia tenacissima*) also significantly reduced its mRNA expression, it could be considered as a new therapeutic strategy for gastric cancer.



Additionally, treatment with the water extract of Tonggulanteng (*Marsdenia tenacissima*) prevented body weight loss and reduce tumor size in nude mice xenografted with MKN-45 human gastric cancer cells. [45]. Compared to the celecoxib group, liver and mesenteric node metastasis in Tonggulanteng (*Marsdenia tenacissima*) group was obviously reduced and cyclooxygenase 2 protein expression decreased in the gastric mucosa [46] (Table 2). Kaempferol [47], a flavonol-like substance and total saponins [19] identified and isolated from Tonggulanteng (*Marsdenia tenacissima*), presented the same inhibitory effects on the growth of the human gastric cancer cell line SGC-7901. Moreover, kaempferol showed stronger effects in the MKN-45 cell line with an IC<sub>50</sub> of 48.87 µg/mL, which differed from the previous studies in which the main anti-tumor substances in Tonggulanteng (*Marsdenia tenacissima*) were C<sub>21</sub> steroidal saponins [47]. The studies described above suggest that Tonggulanteng (*Marsdenia tenacissima*) has good therapeutic effects on gastric cancer. Randomized controlled trials indicated that Xiaoaiping injection + XELOX regimen [Capecitabine 1000 mg/m<sup>2</sup>, po, twice per day (b.i.d), days 1-14; Oxaliplatin 130 mg/m<sup>2</sup>, i.v, day 1, 21 days/cycle] obviously relieved leukopenia compared to the XELOX regimen alone [48], while a meta-analysis [49] indicated that Xiaoaiping injection + FOLFOX (Oxaliplatin + Calcium folinate + 5-fluorouracil) regimen was less effective in relieving leucopenia and gastrointestinal reactions.

### Other gastrointestinal tumors

Yao *et al* [37] treated the SW620 human colon cancer cells and its P-gp-overexpressing subline SW620 Ad300 with polyoxypregnane steroids with an open-chain sugar moiety of Tonggulanteng (*Marsdenia tenacissima*) and MTE coupled with chemotherapy drugs. The IC<sub>50</sub> value of combined therapy was remarkably reduced compared with each compound tested alone, drug transporter activity was suppressed, anti-cancer drug concentration in cancer cells was increased, and tumor suppression effect was more apparent. When administered alone, MTE directly inhibited the growth and proliferation of the human colorectal cancer cells line Lovo in a concentration-dependent manner [50]. Wang *et al* [51] selected the human adenoid cystic carcinoma cell line SACC83 as a target to study the anti-tumor effect and relative mechanisms of Xiaoaiping injection and 17-β-tenacigenin B, which is a C<sub>21</sub> steroidal glycoside extracted from Tonggulanteng (*Marsdenia tenacissima*). They found that 17-β-tenacigenin B had no prominent effects on SACC83 cells, whereas Xiaoaiping repressed SACC83 cell growth by down-regulating Bcl-2 and up-regulating Bax expression (Table 2).

### Hepatic carcinoma

Zhang [52] studied the cytotoxicity of liposoluble constituents extracted from Tonggulanteng (*Marsdenia tenacissima*) on the human hepatocellular carcinoma cell line HepG2 in an MTT assay. After 72 h of liposoluble

**Table 2 Summarized of Tonggulanteng (*Marsdenia tenacissima*) against different gastrointestinal tumor cell stains and relative molecular mechanisms**

Tumor cell lines	Drugs	Cell cycle arrest	Protein or/and mRNA expressions	The ways or/and effects on tumor cells	References
KYSE150 Eca-109	MTE	G0/G1	Cyclin-D1/D2/D3/E1↓ CDK2/4/6, p-ERK, p-JNK↓ p-p38↓ p18, p21, p27 unaffected	Decreased proliferation by inhibiting MAPK signaling pathway.	[36]
HKESC-1	MTE	G2/M	/	Growth inhibition, Reversed chemoresistance	[37]
HKESC-1 cisR	MTE	G2/M	p21↑	Growth inhibition, Reversed chemoresistance	[37]
BGC-823	MTE	/	Bcl-2, Bcl-xl, <i>metadherin</i> ↓ Bax, Bad unchanged Caspase-3↑	Suppressed proliferation, Induced apoptosis	[44]
MKN-45	MTE/kaempferol	/	COX-2↓	Restrained tumor growth	[45-46], [47]
SACC83	Xiaoaiping injection	/	Bcl-2↓, Bax↑	Inhibited cancer growth	[51]

↓ = Protein or/and mRNA expressions decreased; ↑ = Protein or/and mRNA expressions increased; / = No data.

MTE, Tonggulanteng (*Marsdenia tenacissima*) extract; CDK, Cyclin-dependent kinase; ERK, Extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; Bcl-2, B-cell lymphoma 2; Bcl-xl, B-cell lymphoma-extra large; Bax, Bcl-2-associated x protein; Bad, Bcl-2-associated death promoter; COX-2, Cyclooxygenase-2.



component administration, cell proliferation was decreased to different degrees in a time- and dose-dependent manner with good inhibitory effects observed at concentrations of 50-200 mg/L. Huang *et al* [53] incubated HepG2 cells with MTE for 24 h and found that the expression of vascular endothelial growth factor (VEGF)-2 and VEGF-A was decreased according to RT-PCR and enzyme-linked immunosorbent assay results. VEGF is closely related to the formation of tumor microvasculature, indicating that MTE exerts its anti-tumor effects by inhibiting tumor angiogenesis (Table 3). Furthermore, total aglycones of Tonggulanteng (*Marsdenia tenacissima*) caulis decreased the growth of SMMC-7721 human hepatocellular carcinoma cells, increased the thymus index of H<sub>22</sub>-bearing mice [54], and enhanced the sensitivity of HepG2 cells to paclitaxel.

Hu *et al* [55] studied the effect of a tenacigenin B derivative on reversing the multidrug resistance of human hepatoma HepG2/Dox cell lines overexpressing P-gp and found that doxorubicin, vinblastine, puromycin, and paclitaxel combined with 20 µg/mL tenacigenin B derivatives increased the susceptibility of HepG2/Dox cells by at least six-fold, presumably because the tenacigenin B derivatives interacted with the P-gp substrate site. Han *et al* [56] found that MTE inhibited cytochrome P450 (CYP450) activity in the human liver using the cocktail substrate method. The inhibition rates were in the order of 3A4 > 2C9 > 2C19 > 1A2 > 2D6. The regimen of gefitinib combined with MTE reduced the activities of cytochrome P2D6 and cytochrome P3A4 *in vitro* by 2.6- and 4.0-fold, respectively, with little effect

on other CYP450s. After combined treatment, the mRNA and protein expression levels of cytochrome P2D6 and cytochrome P3A4 in HepG2 cells were clearly decreased. This indicates that MTE inhibited CYP450 activity in HepG2 cells to interfere with the metabolism of gefitinib.

Animal experiments by Jiang *et al* [57] showed that polysaccharides from Tonggulanteng (*Marsdenia tenacissima*) limited tumor growth in H<sub>22</sub> tumor-bearing mice and increased the spleen index, thymus index, and albumin, TNF-α, and IL-2 levels in the serum, increased the activities of glutathione peroxidase, catalase, and superoxide dismutase (SOD) in the liver, and decreased VEGF and malondialdehyde (MDA) levels (Table 3). These results suggest that the Tonggulanteng (*Marsdenia tenacissima*) polysaccharide regulates the immune function of mice, and its anti-tumor activity may be associated with an antioxidant and immune regulatory response.

Qian [58] used the human hepatocellular carcinoma cell lines BEL-7402, SMMC-7721, and HepG2 and H<sub>22</sub> tumor-bearing mice to determine the inhibitory effect of single and combined administration of Xiaoaiping injection and platinum on the different cell lines. Xiaoaiping injection altered the cell morphology and ultrastructure, decreased cell proliferation rate, arrested the cell cycle in the G0/G1 phase, inhibited Ki-67 protein expression, decreased Bcl-2 expression, and increased Bax expression. These trends were more obvious after combination treatment. VEGF and basic fibroblast growth factor expression also decreased in BEL-7402 cells [59], levels of p53 gene products were decreased [60], and

**Table 3 Comparison of anticancer molecular mechanisms of *Marsdenia tenacissima* on different hepatic carcinoma cell lines**

Tumor cell lines	Drugs	Cell cycle arrest	Protein or/and mRNA expressions	The ways or/and effects on tumor cells	References
HepG-2	MTE/MTE + gefitinib	/	VEGF-A/2, VEGF-A/2↓ CYP2D6, CYP3A4, CYP2D6, CYP3A4↓	Suppressed proliferation, Inhibited gefitinib metabolism, Anti-angiogenic	[18], [52-55], [59-60]
SMMC-7721	ETA	G0/G1	/	Prohibited proliferation	[54], [55]
BEL-7402	Xiaoaiping injection	G0/G1	VEGF, bFGF, p53↓ Ki-67, Bcl-2, VEGF↓ Bax, TNF, IL-2, GSH-Px↑ CAT, SOD↑	Inhibited proliferation, Induced apoptosis	[55-57]
H <sub>22</sub>	Polysaccharide	/	/	Regulated immune function, Suppressed tumor growth, Anti-oxidation	[54], [55], [61]
Hepal-6	Xiaoaiping injection	/	Bcl-2↓, Bax↑	Restrained proliferation, Induced apoptosis	[58]
HepG/DOX	TenacigeninB derivatives	G2/M	/	Decreased proliferation, Induced apoptosis	[59]

↓ = Protein or/and mRNA expressions decreased; ↑ = Protein or/and mRNA expressions increased; / = No data.

MTE, Tonggulanteng (*Marsdenia tenacissima*) extract; ETA, Total aglycones of Tonggulanteng (*Marsdenia tenacissima*); VEGF, Vascular endothelial growth factor; CYP, Cytochrome; bFGF, Basic fibroblast growth factor; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated x protein; TNF, Tumor necrosis factor; IL-2, Interleukin-2; GSH-Px, Glutathione peroxidase; CAT, Catalase; SOD, Superoxide Dismutase.



tumor angiogenesis was inhibited (Table 3). After treatment with Xiaoaiping, mouse hepatocellular carcinoma Hepa1-6 cells showed pseudopod contraction, somatic cells became smaller and round, eosin staining was enhanced, nuclei were smaller and fragmented, chromosomes were stained deep blue or dark black, mouse hepatocellular carcinoma Hepa1-6 cells are adherent cells that were observed suspended in culture medium, and apoptosis was increased according to inverted microscopy results [61]. Xiaoaiping injection directly promoted T and B cell proliferation, regulated immune system function [62], and enhanced the quality of life of patients with advanced hepatocellular carcinoma, improving their immunity and extending their progression-free survival [63].

### Cervical cancer and breast cancer

According to Yao [64], the growth rate of tumors in tumor xenograft nude mice was distinctively slowed by the administration of saponins from Tonggulanteng (*Marsdenia tenacissima*), which suppressed cervical cancer cell growth in the tumor-bearing mice. The human cervical cancer cell line HeLa and human breast cancer cell line MCF-7 showed increased mortality with an increasing dose of betulinic acid, a pentacyclic triterpenoid present in Tonggulanteng (*Marsdenia tenacissima*). The effects were similar to those of 5-fluorouracil, but the inhibitory effect of betulinic acid on human breast ductal carcinoma cells ZR was not strong [65]. Similarly, total saponins of Tonggulanteng (*Marsdenia tenacissima*) and ETA weakly inhibited the growth of HeLa cells [54], whereas ETA enhanced the anti-cancer activity of taxol in the human papillomavirus-related endocervical adenocarcinoma cell line KB-3-1 and markedly decreased the IC<sub>50</sub> values than that of taxol alone [18]. Moreover, the combination of Xiaoaiping injection and docetaxel + epirubicin + cyclophosphamide inhibited estrogen receptor ER- $\alpha$ 36 expression in breast cancer tissues and improved overall clinical efficacy, three- and five-year disease-free survival, and overall survival in breast cancer patients [66, 67].

### Others

Huang *et al* [68] found that after incubation with the combination of doxorubicin and MTE, the human osteosarcoma cell line MG63 showed decreased survival, increased apoptosis, altered cell morphology, and up-regulated Fas expression, with significant differences compared to the effects of the two drugs alone, indicating that MTE improves the sensitivity of MG63 cells to doxorubicin.

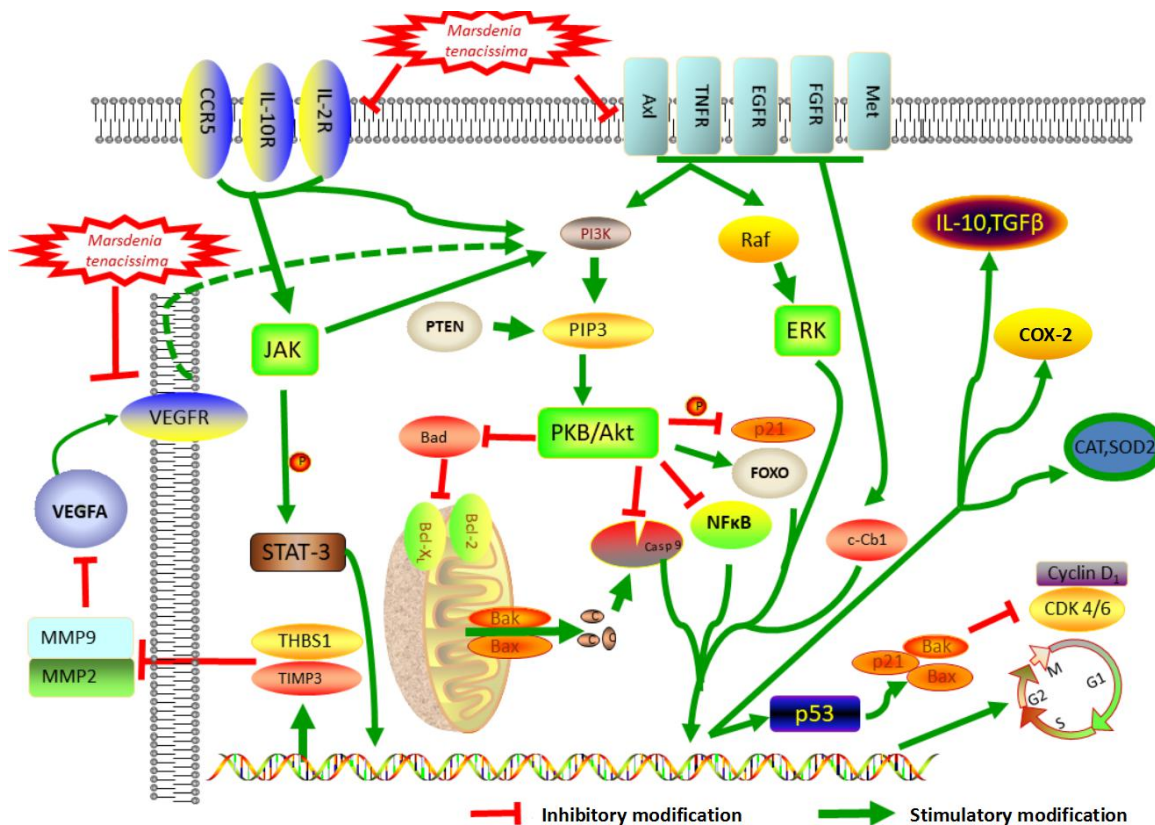
Chen *et al* [69] studied the effects of different concentrations and incubation times of MTE. MTE exerted anti-angiogenic effects and inhibited the proliferation of human umbilical vein endothelial cells by weakening the interaction of VEGF/VEGFR2 mediated by CCL-2 and promoted apoptosis via the p53-dependent mitochondrial pathway induced by PKC $\delta$ . These results

verify the effects of MTE on tumor angiogenesis. Dai *et al*. [70] also found that MTE inhibits tumor growth and angiogenesis in A20 mouse lymphoma and endothelial cells, which play a vital role in the pre-angiogenesis phase. As the MTE concentration was increased, apoptosis also increased. The apoptotic rates in the endothelial cells induced by 0, 6.25, 12.5, and 25  $\mu$ L/mL MTE were 4.8%, 23.3%, 49.8%, and 92.3%, respectively, based on acridine orange/propidium iodide staining and flow cytometry. Peritumoral neovascularization and microvessel density were clearly decreased *in vivo* in A20 solid tumors after administration of 300  $\mu$ L/d MTE for 14 days in BALB/c mice. The expression levels of VEGF, matrix metalloproteinase-2 (MMP-2), and MMP-9 were lower than those in the control group. Additionally, Tonggulanteng (*Marsdenia tenacissima*) showed inhibitory effects toward human subcutaneous glioma U87 cells in nude mice [71] and xenograft mouse sarcoma S180 cells [72].

### Discussion

The chemical composition of Tonggulanteng (*Marsdenia tenacissima*) is complex, and studies have focused on its monomer components, derivatives, preparations, and crude extracts. These components showed anti-cancer effects on various tumor cell lines with different mechanisms that varied by cell type. Tonggulanteng (*Marsdenia tenacissima*) exerts its anti-tumor effects mainly in five ways, as shown in Figure 1. First, Tonggulanteng (*Marsdenia tenacissima*) increases the expression of apoptosis-related proteins, such as Caspase-3, Caspase-9, and p53 while decreases those of Bcl-2, Bcl-xl, to promote apoptosis in tumor cells, inhibits Cyclin D1/2/3, Cyclin E1, CDK2/4/6, and other cell cycle proteins, and arrests tumor cells in the G2/M or S phase to inhibit tumor cell proliferation. Second, the expression of VEGF-2, VEGF-A, MMP-2, MMP-9, and basic fibroblast growth factor is down-regulated to suppress the formation of tumor microvasculature. Third, Tonggulanteng (*Marsdenia tenacissima*) increases the expression of IL-2, TNF- $\alpha$ , glutathione peroxidase, catalase, and SOD and down-regulates IL-10 and MDA expression, thereby enhancing the body's immunity and antioxidant mechanisms. Fourth, the phosphorylation levels of FAK, activator of transcription 3, ERK, p38, and Rho are reduced in the downstream pathway of the CCR5-CCL5 axis to decrease tumor cell migration and invasion. Fifth, EGFR, c-Met, and tyrosine-protein kinase receptor activation is inhibited, which enhances the anti-tumor efficacy of EGFR-TKIs. Additionally, Tonggulanteng (*Marsdenia tenacissima*) inhibits P-gp and CYP450 to enhance the concentration of other anti-tumor drugs in tumor cells, thereby enhancing their anti-tumor effects. Other studies have focused on HIV inhibition [72]. Therefore, Tonggulanteng (*Marsdenia tenacissima*) as an anti-neoplastic medicinal plant, either alone or in combination with conventional clinical anti-cancer drugs, has great therapeutic effects.





**Figure 1** Schematic description of the mechanisms of antitumor for Tonggulanteng (*Marsdenia tenacissima*)

Tonggulanteng (*Marsdenia tenacissima*) chiefly through inhibiting three signal pathways, including Raf-ERK pathway, PI3K/PKA/Akt pathway and JAK/STAT pathway, and related proteins' phosphorylation to against cancer. VEGFA, Vascular endothelial growth factor A; VEGFR, Vascular endothelial growth factor receptor; MMP, Matrix metalloproteinase; CCR5, Chemokine receptor 5; IL-2R, Interleukin receptor; IL-10R, Interleukin-10 receptor; Bcl-2, B-cell lymphoma 2; Bcl-xL, B-cell lymphoma-extra large; IL-10, Interleukin-10; COX-2, Cyclooxygenase-2; CDK, Cyclin-dependent kinase; Bax, Bcl-2-associated x protein; Bak, Bcl-2 homologous antagonist/killer; ERK, Extracellular signal-regulated kinase; Akt, Protein kinase B; Bad, Bcl-2-associated death promoter; JNK, c-Jun N-terminal kinase; PTEN, Phosphatase and tensin homolog deleted on chromosome ten; CAT, Catalase; SOD2, Superoxide Dismutase2; JAK, Janus kinase; STAT-3, Signal transducer and activator of transcription 3; PI3K, Phosphoinositide 3-kinase; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; Casp-9, Caspase-9; THBS1, Thrombospondin 1; TIMP3, Metalloproteinase inhibitor 3; PIP3, Phosphatidylinositol (3,4,5)-trisphosphate; FOXO, Forkhead box proteins; c-Cb1, The cellular homolog of the v-cbl oncogene. Axl, tyrosine-protein kinase receptor.

However, the mechanism underlying the anti-tumor activity of Tonggulanteng (*Marsdenia tenacissima*) is not completely understood, and only a few randomized control studies and large multicenter trials have been reported on the clinical efficacy of Xiaoaiping injection for treating tumors. Additionally, its adverse effects and benefits of co-administration with other therapies, for example, co-application with molecular targeted drugs, require further analysis because many reports have documented adverse reactions to Tonggulanteng (*Marsdenia tenacissima*) [73, 74]. Thus, the clinical application of this plant for treating cancer remains limited. Further studies on the anti-cancer mechanism, long-term effect, and toxic components of Tonggulanteng (*Marsdenia tenacissima*) [75] are needed to provide a basis for the development and application of this plant in the clinical treatment of tumors.

## References

1. Xing WX, Chen B, Mi HM, *et al.* Textual research of Wuguteng. *Chin Med Mat* 2003: 524-526.
2. Cai F, Chu ZY, Zhang H. Research progress of traditional Chinese medicine *Marsdenia tenacissima*. *J Liaoning Univ Tradit Chin Med* 2007: 37-39.
3. Han L, Zhang H, Kang TG, *et al.* Research progress of *Marsdeniae Tenacissimae*. *Chin Arch Tradit Chin Med* 2016: 2329-2331.
4. Ye DS, Luan YG, Li HQ. Symposium on the treatment of malignant tumors by *marsdeniae tenacissimae caulis*. *Bulletin Med Res* 1984: 9-10.
5. Ye DS. Anti-tumor effect of Wuguteng preparation through identification. *Bulletin Med Res* 1985: 193.
6. Zhang GZ, Liu SN. The effect of Xiaoaiping injection in patients with advanced esophageal



- cancer. *Oncol Prog* 2017; 670-672.
7. Wang ZL, Fan QX, Fan KS. Clinical study of Xiaoaiping in the treatment of cancer. *J Henan Med Univ* 1994; 79-80.
  8. Zhang J, He W J, Wang JG, *et al.* Clinical observation on Xiao'aiping injection combined with icotinibin treatment of non-small cell lung cancer. *J Hubei Univ Chin Med* 2018; 42-45.
  9. Bai RL. Effect of Xiaoaiping injection combined with chemotherapy in the treatment of advanced non-small cell lung cancer. *Chin J Misdiagn* 2010; 5359-5360.
  10. Wu Y. Comparison and analysis of irinotecan alone or combined with Xiaoaiping in the treatment of elderly patients with advanced gastric cancer. *Chin Pharm* 2016; 657-659.
  11. Ye B, Li J, Li Z, *et al.* Anti-tumor activity and relative mechanism of ethanolic extract of *Marsdenia tenacissima* (Asclepiadaceae) against human hematologic neoplasm in vitro and in vivo. *J Ethnopharmacol* 2014, 153: 258-267.
  12. Ye B, Yang J, Li J, *et al.* In vitro and in vivo antitumor activities of tenacissoside C from *Marsdenia tenacissima*. *Planta Med* 2014, 80: 29-38.
  13. Xue HL, Huang XD, He D, *et al.* Effect of *Marsdenia tenacissima* extract on proliferation and apoptosis of hematological neoplasm cell lines. *J Sichuan Univ* 2012, 43: 174-179.
  14. Li D, Li C, Song Y, *et al.* *Marsdenia tenacissima* extract and its functional components inhibits proliferation and induces apoptosis of human Burkitt leukemia/lymphoma cells in vitro and in vivo. *Leuk Lymphoma* 2015, 1-10.
  15. Wang Y, Chen B, Wang Z, *et al.* *Marsdenia tenacissima* extraction (MTE) inhibits the proliferation and induces the apoptosis of human acute T cell leukemia cells through inactivating PI3K/AKT/mTOR signaling pathway via PTEN enhancement. *Oncotarget* 2016, 7: 82851-82863.
  16. Chen B, Li CP, Chen JH, *et al.* Effect of extract from *Marsdenia tenacissima* on Jurkat, Raji and RPMI8226 cells in vitro. *Chin J Biochem Pharm* 2009, 30: 174-177.
  17. Ceng C, Chen Y, Shan LH, *et al.* Inhibition effect of *Marsdenia tenacissima* on K562 cells proliferation. *Anti-tumor Pharm* 2011, 1: 505-507.
  18. Zhu RJ, Shen XL, Dai LL, *et al.* Total aglycones from *Marsdenia tenacissima* increases antitumor efficacy of paclitaxel in nude mice. *Molecules* 2014, 19: 13965-13975.
  19. Lin YC. The inhibition of total glycosides in glaucescent fisticigma root on SGC-7901, S180 and P388 tumor cells. *J Pharm Res* 2015, 34: 376-378.
  20. Li D, OuYang J, Li CP, *et al.* *Marsdenia tenacissima* Induces apoptosis of human U937, HL60 leukemia cells. *Chin J Biochem Pharma* 2008: 33-37.
  21. Chen B, Li CP, Chen JH, *et al.* Study on the inhibition and apoptosis of leukemia cells induced by *Marsdenia tenacissima* extraction. *J Nanjing TCM Univ* 2009, 25: 233-234.
  22. Li D, OuYang J, Li CP, *et al.* Effect of *Marsdenia tenacissima* on the apoptosis-associated gene expression profile of U937 cells. *Chin J Biochem Pharm* 2008: 240-243.
  23. Fang YQ. Research of Xiaoaiping injection on HEL cell line In vitro. *Med Inf* 2010, 5: 2506-2507.
  24. Wang SG, Feng LF, Li D. One hundred and thirty one cases of non hodgkin lymphoma treated with Xiaoaiping injection in combination with chemotherapy. *Henan Tradit Chin Med* 2017: 1488-1490.
  25. Han SY, Ding HR, Zhao W, *et al.* Enhancement of gefitinib-induced growth inhibition by *Marsdenia tenacissima* extract in non-small cell lung cancer cells expressing wild or mutant EGFR. *BMC Complem Altern M* 2014, 14:165.
  26. Han SY, Zhao W, Han HB, *et al.* *Marsdenia tenacissima* extract overcomes Axl- and Met-mediated erlotinib and gefitinib cross-resistance in non-small cell lung cancer cells. *Oncotarget* 2017, 8: 56893-56905.
  27. Han SY, Zhao MB, Zhuang GB, *et al.* *Marsdenia tenacissima* extract restored gefitinib sensitivity in resistant non-small cell lung cancer cells. *Lung Cancer* 2012, 75: 30-37.
  28. Yao S, To KK, Wang YZ, *et al.* Polyoxypregnane steroids from the stems of *Marsdenia tenacissima*. *J Nat Prod* 2014, 77: 2044-2053.
  29. Wei WJ, Zhang GQ, Liu SJ, *et al.* Anti-tumor effect of Tenacissoside H and Influence on immune function of lewis lung cancer mice. *Chin J Inf Tradit Chin Med* 2014, 2163-66.
  30. Lin SS, Li FF, Sun L, *et al.* *Marsdenia tenacissima* extract suppresses A549 cell migration through regulation of CCR5-CCL5 axis, Rho C, and phosphorylated FAK. *Chin J Nat Med* 2016, 14: 203-209.
  31. Chen TS, Wang XP, Wang ZP, *et al.* Fluorescence emission analysis of caspase-3 activation induced by Xiaoaiping (XAP) inside living human lung adenocarcinoma cells. *Spectrosc Spectr Anal* 2008: 1327-1331.
  32. Bai RL. Effect of Xiaoaiping injection combined with chemotherapy in the treatment of advanced non-small cell lung cancer. *Chin J Misdiagn* 2010: 5359-5360.
  33. Ye L, Fu B. Effect of Xiaoaiping injection on the efficacy of recent TP regimen chemotherapy in patients with advanced non-small cell lung cancer. *Shanxi Med J* 2017: 1189-1191.
  34. Zhang GH. Effect of Xiaoaiping combined with docetaxel on advanced non-small cell lung cancer. *J Huaihai Med* 2017: 479-480.
  35. Zhang H, Zhang J, Ding H, *et al.* Clinical value of Tongguanteng (*Radix seu Herba Marsdeniae Tenacissimae*) extract combined with chemotherapy in the treatment of advanced non-small cell lung cancer: a Meta-analysis. *J Tradit Chin Med* 2016, 36: 261-270.



36. Fan W, Sun L, Zhou JQ, *et al.* Marsdenia tenacissima extract induces G0/G1 cell cycle arrest in human esophageal carcinoma cells by inhibiting mitogen-activated protein kinase (MAPK) signaling pathway. *Chin J Nat Med* 2015, 13: 428-437.
37. Yao S, To KK, Ma L, *et al.* Polyoxypregnane steroids with an open-chain sugar moiety from Marsdenia tenacissima and their chemoresistance reversal activity. *Phytochemistry* 2016, 126:47-58.
38. Wu Y. Comparison and analysis of irinotecan alone or combined with Xiaoaiping in the treatment of elderly patients with advanced gastric cancer. *Chin Pharm* 2016: 657-659.
39. Hong HJ, Fang L. Application of Xiaoaiping combined with CPT-11 chemotherapy in elderly patients with advanced gastric cancer. *J Med Forum* 2017: 129-130.
40. Lv XR. Effect of Xiaoaiping injection combined with chemotherapy on advanced esophageal cancer. *World Latest Med Inf* 2017: 102.
41. Sheng YL. Therapeutic value of xiaoaiping combined with TP on advanced esophageal cancer. *Guide Chin Med* 2017: 207-208.
42. Qian XL, Zhou F, Zuo Y, *et al.* Clinical observation on treating TS by chemotherapy plus the Xiaoaiping. *Clin J Chin Med* 2017: 56-58.
43. Liu W. Clinical observation and study of Xiaoaiping injection combined with SOX regimen for advanced gastric cancer. *Shandong Univ* 2017.
44. Li QH, Wang N, Pan SY, *et al.* Effect of extract from Marsdenia Tenacissima on the apoptosis of BGC-823 gastric cancer cell and the expression of MTDH gene. *Chin Arch Tradit Chin Med* 2017, 35: 892-896.
45. Zhou YY, Li YY, Li L, *et al.* The experimental study on the extractive of Glaucous Fissistigma root inhibiting the gastric cancer tumors growth in mouse. *Lab Animal Sci* 2012, 29: 13-15.
46. Chen Y, Chen YY, Lin CH. Effects of acanthopanax obscurus on growth, metastasis and cyclooxygenase-2 expression in MKN-45 human gastric cancer in situ. *Chin J Gerontol* 2015, 35: 2939-2941.
47. Zhang W, Wang ZF, Wang J, *et al.* Chemical constituents from Marsdenia tenacissima and their anti-tumor activities. *Chin Tradit Patent Med* 2017, 39: 334-338.
48. Zhang D, Wu J, Wang K, *et al.* Which are the best Chinese herbal injections combined with XELOX regimen for gastric cancer? A PRISMA-compliant network meta-analysis. *Med* 2018, 97: e0127.
49. Zhang D, Zheng J, Ni M, *et al.* Comparative efficacy and safety of Chinese herbal injections combined with the FOLFOX regimen for treating gastric cancer in China: a network meta-analysis. *Oncotarget* 2017, 8: 68873-68889.
50. Zhu XX, Zhao LH, Yan SH, *et al.* Study of Glaucous Fissistigma Root extractive on multiplication of Lovo cells. *Chin J Clin Pharm Ther* 2007: 1372-1375.
51. Wang Q, Cao J, Wang P, *et al.* Antitumor effect of C21 steroidal glycosides on adenoid cystic carcinoma cell line SACC83. *Clin Lab* 2015, 61: 1553-1560.
52. Zhang H, Pei ZD, Zhang XY, *et al.* Antitumor activities of liposoluble components of caulis Marsdeniae tenocissimae and analysis on its chemical constituents. *Chin J Chin Mat Med* 2010, 35: 3325-3328.
53. Huang Z, Lin H, Wang Y, *et al.* Studies on the anti-angiogenic effect of Marsdenia tenacissima extract in vitro and in vivo. *Oncol Lett* 2013, 5: 917-922.
54. Han L, Leng CY, Li WH, *et al.* Study on in-vitro and in-vivo antitumor activity of extracts from caulis Marsdeniae Tenacissimae. *Tradit Chin Drug Res Clin Pharm* 2017, 28: 51-55.
55. Hu YJ, Shen XL, Lu HL, *et al.* Tenacigenin B derivatives reverse P-glycoprotein-mediated multidrug resistance in HepG2/Dox cells. *J Nat Prod* 2008, 71: 1049-1051.
56. Han SY, Zhao HY, Zhou N, *et al.* Marsdenia tenacissima extract inhibits gefitinib metabolism in vitro by interfering with human hepatic CYP3A4 and CYP2D6 enzymes. *J Ethnopharmacol* 2014, 151: 210-217.
57. Jiang S, Qiu L, Li Y, *et al.* Effects of Marsdenia tenacissima polysaccharide on the immune regulation and tumor growth in H22 tumor-bearing mice. *Carbohydr Polym* 2016, 137: 52-58.
58. Qian J. Experimental study of Marsdenia tenacissima injection combination with platinum in treatment of primary liver cancer. *Nanjing Univ Chin Med* 2009.
59. Zuo XD, Cui YA, Qin SK, *et al.* Expressions of VEGF and bFGF in human hepatocarcinoma cell with glaucous fissistigma root injection. *Chin Clin Oncol* 2010, 15: 1062-1065.
60. Zhang R, Liu J, Liu YC, *et al.* Experimental study on effect of glaucous fissistigma root preparation on Bel-7402 cells apoptosis. *Mod Oncol* 2013, 21: 488-491.
61. Li WJ, Lin CF, Ren YM, *et al.* Experimental study of Xiaoaiping against hepatoma cells growth in vitro. *Chin Remed Clin* 2010, 10: 164-165.
62. Chen B, Li CP, OuYang J, *et al.* Effect of extractive of marsdenia tenacissima on human normal immunocytes and hemopoietic stem cells in vitro. *Chin Clin Oncol* 2010, 15: 887-890.
63. Huang Z, Wang Y, Chen J, *et al.* Effect of Xiaoaiping injection on advanced hepatocellular carcinoma in patients. *J Tradit Chin Med* 2013, 33: 34-38.
64. Yao M, Xiao CM, Li Y, *et al.* Chemical constituents of glycosides from Marsdenia tenacissima and the anticancer activity in vivo. *J Nanchang Univ* 2015, 39: 88-90.
65. Ni CH, Pei ZD, Zhang JX, *et al.* Study on extraction, isolation and antitumor activity of betulinic acid from caulis Marsdeniae tenocissimae. *Chin J Exp*



- Tradit Med Form 2012, 18: 172-175.
66. Ruan LW, Deng YC. Study on effect of Xiaoaiping in enhancing efficacy of neoadjuvant chemotherapy for breast cancer and its mechanism. *Chin J Chin Mat Med* 2015, 40: 749-752.
  67. Li XG, Liu BX. Effect of Xiaoaiping combined with neoadjuvant chemotherapy on the pro-proliferation molecule expression and immune function in patients with breast cancer. *J Hainan Med Univ* 2017: 3096-3099.
  68. Huang T, Gong WH, Zou CP, *et al.* Marsdenia tenacissima extract sensitizes MG63 cells to doxorubicin-induced apoptosis. *Genet Mol Res* 2014, 13: 354-362.
  69. Chen BY, Chen D, Lyu JX, *et al.* Marsdeniae tenacissimae extract (MTE) suppresses cell proliferation by attenuating VEGF/VEGFR2 interactions and promotes apoptosis through regulating PKC pathway in human umbilical vein endothelial cells. *Chin J Nat Med* 2016, 14: 922-930.
  70. Dai X, Ji Y, Jiang P, *et al.* Marsdenia tenacissima extract suppresses tumor growth and angiogenesis in A20 mouse lymphoma. *Oncol Lett* 2017, 13: 2897-2902.
  71. Zhang ZQ, Han F, Xie HT, *et al.* Experimental study on the effect of Marsdenia tenacissima on glioma in nude mice. *J New Chin Med* 2013, 45: 160-161.
  72. Pang X, Kang LP, Fang XM, *et al.* C21 steroid derivatives from the Dai herbal medicine Dai-Bai-Jie, the dried roots of Marsdenia tenacissima, and their screening for anti-HIV activity. *J Nat Med* 2017, 72:166-180
  73. Xu XJ, Li GZ. Xiaoaiping injection caused serious adverse reactions in 1 case. *People's Mil Surg* 2017, 60: 925-926.
  74. Zhang Y. Xiaoaiping injection caused serious adverse reactions in 1 case. *Drug Eval Res* 2013, 36: 488.
  75. Porwal M, Khan NA, Maheshwari KK. Evaluation of acute and subacute oral toxicity induced by ethanolic extract of Marsdenia tenacissima leaves in experimental rats. *Sci Pharm* 2017, 85: pii: E29.

