· Reviews ·

Recent Advance in Studies on *Angelica sinensis*

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Abstract: *Angelicae Sinensis Radix* (ASR) is the root of *Angelica sinensis* which is a fragrant and perennial herb native to China, Japan, and Korea. In traditional Chinese medicine (TCM), the plant is useful for replenishing and invigorating blood, relieving pain, and moistening the intestines, resulting in its application for the treatment of menstrual disorders, and as an emollient and laxative for chronic constipation of the aged and debilitated. An in-depth review of the literature brings to light a great number of chemical constituents that have been isolated from ASR as well as both preclinical (*in vivo* and *in vitro*) and clinical studies, which over the years, have sought to investigate the medicinal relevance of some of these phytoconstituents and/or extract(s) prepared from ASR. The purpose of this review is therefore to present some major pharmacological and pharmacokinetic research findings on some selected phytoconstituents of ASR with emphasis on the current trends in terms of research techniques or design. This review would also provide a wealth of information for users/practitioners of TCM regarding the use of ASR or its products for maximum efficiency and minimal toxicity or side effects.

Key words: *Angelica sinensis*; ethnopharmacology; pharmacokinetics; phytoconstituents; toxicity
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Introduction

Angelicae Sinensis Radix (ASR), the root of *Angelica sinensis* (Oliv.) Diels (Dang-gui in Chinese), is a fragrant and perennial herb found in China, Japan, and Korea. Other common names for *A. sinensis* include Chinese Angelica, Dong Quai in English, Toki in Japanese, Tanggwi in Korean, and Kinesisk Kvan in Danish (Anyones, 2004). As a member of the Umbelliferae family, angelica produces white flowers that bloom in umbrella-like clusters in June and July. The plant typically grows to a height of approximately 2 m. The dried root is valued for its therapeutic properties. Its flavor is a distinct blend of bitter, sweet, and pungent, and its overall effect is warming in nature (Wei *et al*, 2009). Chinese herbalists have used ASR for thousands of years to strengthen heart, lung, and liver meridians, as well as lubricate the bowel. It is considered a blood tonic, and has been used by generations of women for health concerns such as menstrual pain and regulating the menstrual cycle.

Ethnopharmacology

ASR is the dried roots of *A. sinensis* (Umbelliferae) (Figs. 1 and 2). The source of earliest record was in *Shennong Materia Medica*. The properties and taste are warm, sweet, and pungent. It enters the liver, heart, and spleen meridians. The functions are to replenish blood, invigorate blood, stop pain, and moisten the intestines. It is used for the treatment of menstrual disorders, and as an emollient and laxative for chronic constipation of the

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Fig. 1 *Angelica sinensis* (Oliv.) Diels (from http://16tupian.cn/16tupian/2c/13786.html)

Fig. 2 *Angelicae Sinensis Radix* (from http://zcysc.com/spc=4875)

aged and debilitated (Geng *et al*, 1990; Xie and Huang, 1980; Liu and Xiao, 1993; Liu, Xiao, and Li, 2000).

Major active constituents

ASR contains 0.4%−0.7 % of volatile oil, the key components of which are *n*-butylidenephthalide, ligustilide (3-butylidene-4,5-dihydrophthalide), *n*-butyl-phthalide, ferulic acid (4-hydroxy-3-methoxycinnamic acid), nicotinic acid, and succinic acid (Zhu, 1987; Bone and Mills, 2000; Duke, 1992). Significant amounts of vitamin A and carotenoids (0.675%), vitamin B_{12} (2.5−4.0 μg/kg), vitamin E, ascorbic acid, folinic acid, biotin, phytosterols (e.g., β-sitosterol), calcium, magnesium, and other essential macro-minerals are also found in *A. sinensis* root (Zhu, 1987; Duke, 1992; Huang, 1999). Other constituents include *n*-valerophenone-*O*-carboxylic acid, vanillic acid (4-hydroxy-3-methoxybenzoic acid), Δ-2,4-dihydro-phthalic anhydride, adenine (6-aminopurine), uracil [2,4-(1H,3H)-pyrimidinedione], adenine, carvacrol, safrole, isosafrole, sesquiterpenes, β-cadinene, *n*-dodecanol, *n*-tetradecanol, palmitic acid, angelic acid, myristic acid, sucrose (40%), polysaccharide (Zhu, 1987; Huang, 1999), and senkyunolides G−J (Song *et al*, 2011). Natural coumarin derivatives have been attributed to *A. sinensis* but reports differ regarding which ones are truly present. The coumarin derivatives include angelol, angelicone, bergapten, oxypeucedanin, osthole, psoralen, and 7-desmethyl-suberosin (Zhu, 1987; Bone and Mills, 2000; Duke, 1992; Skidmore-Roth, 2001; Tyler, 1995). Amino acids (*L*-alanine, *L*-arginin, aspartic acid, asparagine, *L*(+)-cysteine, *L*-lysine, methionine, phenylalanine, *DL*-serine, *L*-threonine, *D*(+)-tryptophan, tyrosine, valine), choline hydroxide, α-angelicalactone, κ-carrageenan, and phosphatide are presented in *A. sinensis* root. The chemical structures of major active constituents of angelica are shown in Fig. 3.

Pharmacological actions

Effects on cardio- and cerebro-vascular systems

The pharmacological activities of extracts from *A. sinensis* and its active compounds on cardio- and cerebro-vascular systems have been introduced in *Modern Research and Application of Chinese Medicinal Plants* published in 2000 (Liu, Xiao, and Li, 2000).

In anti-arrhythmic test, the alcohol extract of the roots of *A. sinensis* and sodium ferulate showed anti-arrhythmic activity, upon iv injection in rats (Cha, Chen, and Li, 1981). The water extract of the roots of *A. sinensis* and ferulic acid (FA) was able to inhibit rat platelet aggregation induced by adenosine diphosphate (ADP) and collagen *in vitro*. At the dose of 0.4−0.6 mg/kg (iv), sodium ferulate inhibited aggregation induced by ADP and collagen in rats. The effect suggests that the constituent may be related to the therapeutic effect on cerebrovascular diseases (Yin, Zhang, and Xu, 1980). Neither platelet aggregation nor arterial PGI2-like substance release was observed following ig administration of sodium ferulate at a dose of 300 mg/kg. In combined sodium ferulate and acethylsalicylic acid regimen, platelet aggregation and TXA2-like substance were inhibited by 65.5% and 84%, respectively, while the production of arterial PGI2-like

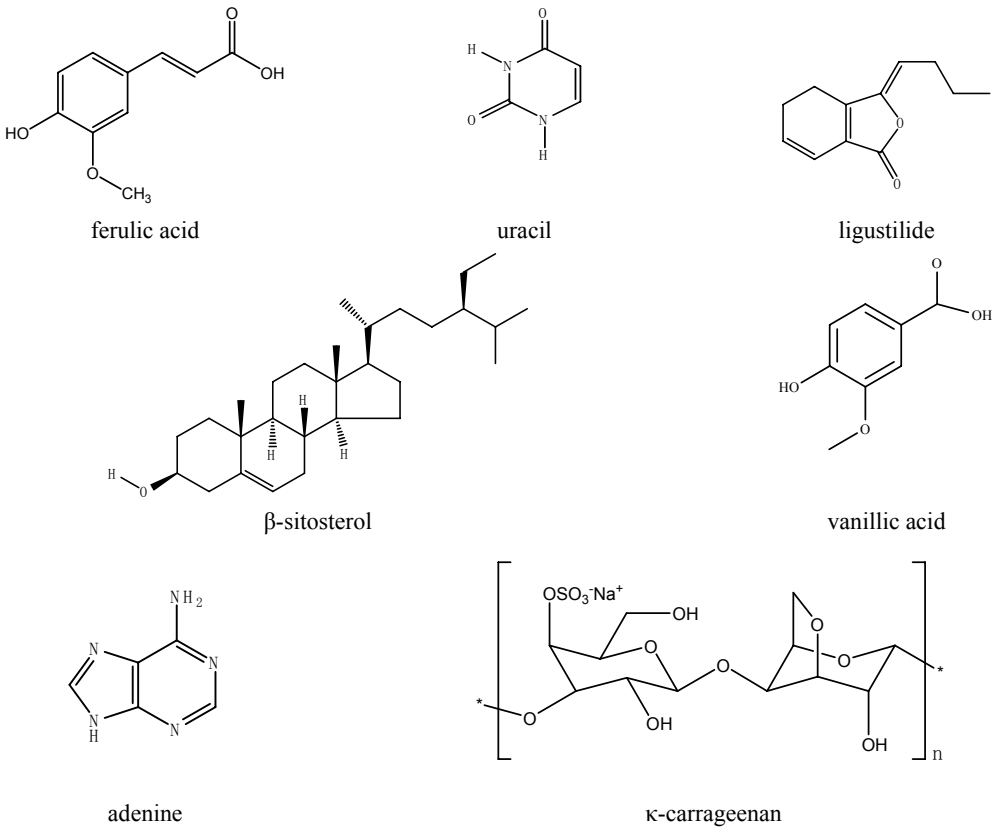

Fig. 3 Major active constituents of *A. sinensis*

substance remained unchanged. The combined treatment using sodium ferulate and acetylsalicylic acid could potentiate the antiplatelet action without inhibiting arterial PGI2-like substance release, suggesting that they may be valuable for the treatment of thromboembolic diseases (Xu, Wang, and Xu, 1985).

Coumarin and its derivatives, natural anti-coagulants in *Angelica* spp., have been associated with both the bioactivity and toxicity of the plants, although *A. sinensis* contains a lower coumarin content compared to other closely related species (DerMarderosian and Beutler, 2004).

FA, one of the constituents of *A. sinensis*, could inhibit the polymerization of platelets in blood circulation. It retards platelet release of 5-hydroxy-tryptamine (5-HT) and ADP (Zhu, 1987). Both FA and an aqueous extract of *A. sinensis* were found to inhibit platelet aggregation and serotonin release (Bone and Mills, 2000).

Due to the untold number of constituents, several pharmacological actions might be attributed to *A. sinensis.* Such characteristics include anticoagulation and antiplatelet activities (Zhu, 1987; Bone and Mills, 2000), as well as hematopoiesis (Huang, 1999), immune support (Wilasrusmee *et al*, 2002a; 2002b), and uterine tonicity (Zhu, 1987; Bone and Mills, 2000; Huang, 1999; Chang and But, 1987).

Anti-inflammatory effect

In 1986, Li (1986) found that sodium ferulate could regulate PGI2/TAX ratio by inhibiting TAX2 activity without affecting PGI2. Ligustilide (LIG) showed a concentration-dependent anti-inflammatory effect in LPS-activated microglia, without causing cytotoxicity. Pretreatment with LIG at 2.5, 5, 10, and 20 μmol/L decreased LPS-induced NO production to 75.9%, 54.4%, 43.1%, and 47.6%; TNF-α content to 86.2%, 68.3%, 40.1%, and 39.9%; Interleukin-1β (IL-1β) content to 31.5%, 27.7%, 0.6%, and 0 ($P < 0.01$); and MCP-1 content to 84.4%, 50.3%, 45.1%, and 42.2%, respectively, compared with LPS treatment alone. LIG (10 μmol/L) significantly inhibited LPS-stimulated immunoreactivity of activated nuclear factor κB (NF-κB), COX-2, and iNOS. LIG exerted a potent anti-inflammatory effect on microglia through inhibition of NF-κB pathway. The data provide direct evidence of the neuroprotective effects of LIG and the

potential application of LIG for the treatment of the neuroinflammatory diseases characterized by excessive microglial activation (Wang *et al*, 2010).

Weiqi Decoction (WQD), also known as Weiqiyin Drink or Stomach Fortune Drink, comprises of Danggui (ASR), Huangqi (*Astragali Radix*, AR, astragalus in English), Dangshen (*Codonopsis Radix*, CR), Zhiqiao (*Aurantii Submatures Fructus*, ASF), Ezhu (*Curcumae Phaeocaulis Rhizoma*, CPR), Bayuezha (*Akebiae Australis Fructus*, AAF), and so on. The essential oil contains some effective ingredients in WQD for treating chronic atrophic gastritis and functional dyspepsia. The essential oil inhibited cell proliferation in a dose- and time-dependent manner, and blocked cell cycle progression at G_2/M stage. At the concentrations that resulted in significant inhibition of cell proliferation and cell cycle arrest, essential oil induced both apoptosis and necrosis. The results suggest that essential oil in WQD contains some effective ingredients for treating chronic atrophic gastritis and functional dyspepsia, and also has an antiproliferative effect on AGS cells through cell cycle arrest and apoptosis promotion *in vitro*. Therefore, essential oil should be retained as much as possible during stewing this decoction (Tan *et al*, 2011).

Su *et al* (2011) sought to determine effects of LIG on lipopolysaccharide (LPS)-induced inflammation in RAW 264.7 macrophages. LIG significantly suppressed the production of nitric oxide (NO), prostaglandin E_2 (PGE_2), and TNF-α. The inhibition of NO was concomitant with a decrease in the protein and mRNA levels of LPS-induced NO synthase (iNOS). Furthermore, activation of activator protein-1 (AP-1) and NF-κB in the nucleus and the cytosolic degradation of IκBα were abrogated by LIG. LIG also inhibited the phosphorylation of IκB kinase (IKK) and mitogen-activated protein kinases (MAPKs), including p38 MAPK, extracellular signal-regulated kinase (ERK1/2) and c-Jun *N*-terminal kinase (JNK). The intracellular reactive oxygen species (iROS) level was also significantly decreased. These results suggest that LIG exhibits anti-inflammatory activities by blocking the activation of MAPKs/IKK and the downstream transcription factors AP-1 and NF-κB, which may result from LIG's down-regulation of iROS production (Su *et al*, 2011). LIG significantly decreased neurological deficit score, infarct volume, and RTP801 expression, increased EPO transcription in I/R rats, and induced a significant increase in cell viability and EPO and a decrease in LDH and RTP801 in I/R neurons. Also, LIG increased ERK phosphorylation (p-ERK) and the positive effects of LIG on p-ERK as well as cell viability and EPO could significantly be blocked by PD98059, but not LY294002 and SB203580. In addition, transfection of SH-SY5Y cells with RTP801 plasmid DNA induced a significant increase in RTP801 as well as LDH release, while LIG significantly inhibited the effects of transfection on RTP801 expression and also increased cell viability. Therefore, it suggests that LIG has a significant neuroprotecting role against I/R injury by promoting EPO transcription via a ERK signaling pathway and inhibiting RTP801 expression and has the potential to be developed into a therapeutic agent in preventing and treating ischemic disorders (Wu *et al*, 2011).

Antifibrotic action

A mixture of angelica and astragalus demonstrated antifibrotic activity in a recent animal study. Rat models with chronic Puromycin-induced nephrosis were treated with either angelica and astragalus mixture (3 mL/d) or Enalapril (10 mg/kg). The normal control group received saline, and another group received Puromycin with no treatment (Wang *et al*, 2004). After 12 weeks the untreated rats showed marked renal fibrosis. However, angelica and astragalus mixture significantly retarded the progression of renal fibrosis and deterioration of renal histological damage, with effects comparable to Enalapril (Wang *et al*, 2004).

In the study, Bao *et al* (2010) discovered that both ethanolic and aqueous extracts from either ASR or AR could significantly decrease both the area of collagen fibers and the extent of alveolus inflammation, as well as the content of Hyp in lung tissue and lung index. It was demonstrated that the two extracts could alleviate the process of rat pulmonary fibrosis induced by Bleomycin, and had a protective effect on pulmonary fibrosis in rats in the early period.

Antispasmodic activity

Methods or techniques of cell culture were used to explore the mechanism of angelica polysaccharide (APS) inhibiting proliferation of HaCaT cells from the angulation of apoptosis. The effect of APS on

proliferation of HaCaT cells was examined by trypan blue staining and flow cytometry. Cell growth curve showed that a dose range of 25－2500 mg/L APS had significant inhibition action on HaCaT cells in a dose-dependent manner. Flow cytometry result showed a decrease in the S phase and G_2/M phase HaCaT cells, while a phemomenal increase in the G_0/G_1 phase HaCaT cells was observed at 250 mg/L APS. This study suggested that APS could significantly inhibit proliferation of HaCaT cells, by impairing the mechanism of DNA synthesis in preventing HaCaT cells from entering the S phase (Yu *et al*, 2010).

Previous studies had found that intrauterin hypoxia could stimulate proliferation of neural stem cells (NSCs) of neonatal rats. The proliferation reached a peak during 6 h hypoxia; Proliferation also expressed in 9 h, but the ability began to decline. However, the NSCs showed necrosis or apoptosis in a 12 h hypoxia (Chen *et al*, 2010). To study the effect of intrauterin hypoxia on the proliferation and differentiation of NSCs from neonatal rats and the protective role of angelica injection on NSCs under hypoxia, Chen *et al* (2010) used immunohistochemistry and image processing system to analyze the expression of glial fibrillary acidic protein (GFAP) and neuron specific enolase (NSE). The following results were obtained from the study: (1) Expression of GFAP-positive cells in the hippocampus of neonatal rats in the hypoxia group was higher than control group; (2) Expression of NSE-positive cells was less in the hypoxia group than in the control group; (3) Expression of GFAP-positive cells in the hippocampus of neonatal rats was less in the angelica group than in the hypoxia group, whereas expression of NSE-positive cells was higher in the angelica group than in the control group. These results indicated that hypoxia could stimulate the proliferation of NSCs of neonatal rats and differentiation of NSCs into glial cells. Meanwhile, the number of neurons in hippocampus CA3 area was decreased. The ability of proliferation and differentiation of NSCs into glial cells after hypoxia was attenuated by angelica injection, which was also effective in relieving neuron decrement. Therefore, it was suggested that angelica injection has a certain protective effect on nervous system of neonatal rats with intrauterine hypoxia.

LIG, butylidenephthalide, and butylphthalide were found to have antispasmodic activity against rat uterine contractions and in other smooth muscle systems. The components were characterized as non-specific anti-spasmodics with a mechanism different from papaverine (DerMarderosian and Beutler, 2004; Ko, 1980).

Anti-oxidant activities

FA has been reported to have an excellent anti-oxidant property (Cheng, Yang, and Lin, 2011). Apart from caffeine, coffee contains other compounds including the phenolic compounds FA, caffeic acid, and the chlorogenic acids, which have purported anti-oxidant properties. The chlorogenic acids are the most abundant family of compounds found in coffee, yet their effects on cognition and mood have not been investigated. Compared with the decaffeinated coffee with regular chlorogenic acid and placebo, caffeinated coffee showed a robust positive effect on higher-level mood and attention processes. To a lesser extent, the decaffeinated coffee high in chlorogenic acid also improved some mood and behavioral measures, relative to regular decaffeinated coffee. The results obtained by Cropley *et al* (2011) suggest that non-caffeine compounds in coffee such as the chlorogenic acids may be capable of exerting some acute behavioral effects, thus warranting further investigation. Anti-oxidant properties of four polyphenols: FA, *p*-coumaric acid (hydroxy-cinnamic acids), quercetin (flavonol), and cyanidin 3-glucoside (anthocyanin) and their influence on cholesterol concentration in hypercholesterolemic and normal erythrocytes were investigated by Duchnowicz *et al* (2011). All investigated compounds decreased lipid peroxidation in whole blood. Cyanidin 3-glucoside and quercetin showed higher anti-oxidant properties than hydroxycinnamic acids (FA and *p*-coumaric acid). After incubation of isolated erythrocytes of hyper-cholesterolemic patients with quercetin and cyaniding 3-glucoside, increase of membrane fluidity was noticed. After incubation of isolated erythrocytes of healthy donors with investigated compounds, no changes in membrane fluidity were observed. Their results indicate that flavonols and anthocyanins have higher anti-oxidant properties and higher influence on cholesterol concentration in erythrocytes membranes than simple hydroxycinnamic acids.

A series of assays were used to detect the effects of FA (0.1, 1, and 10 μg/mL) on cell proliferation,

DNA synthesis, cell-cycle distribution, and mRNA expression of cyclin D1 and vascular endothelial growth factor (VEGF), using the Cell Counting Kit-8 (CCK-8), bromodeoxyuridine-enzyme-linked immunosorbent assay, flow cytometry, and reverse transcriptionpolymer-asechain reaction, respectively. The results indicated that FA over a range of concentrations from 0.1 to 10 μg/mL could markedly improve cell proliferation and DNA synthesis in a dose-dependent fashion. Flow cytometry showed a significant decrease in the percentage of cells in the G_0/G_1 phase and a significant increase in the percentage of cells in the S phase. Furthermore, it was found that FA enhanced cyclin D1 and VEGF mRNA expression in ECV304 cells suggesting that FA was capable of promoting ECV304 cells proliferation *in vitro*. This effect might be observed through the modulation of cyclin D1 and VEGF (Wang *et al*, 2011). FA has been reported to have an excellent anti-oxidant property. The results obtained by Cheng *et al* (2011) showed that the release of FA from chitosan/gelatin/glycerol phosphate (C/G/GP) hydrogel could decrease the H_2O_2-induced oxidative stress. Post-treatment of FA-incorporated C/G/GP hydrogel on H_2O_2-induced oxidative stress NP cells showed up-regulation of Aggrecan and type II collagen and down-regulation of MMP-3 in mRNA level. The results of sulfated-glyco-saminoglycans (GAGs) to DNA ratio and alcian blue staining revealed that the GAGs production of H_2O_2-induced oxidative stress NP cells could reach normal level. The results of caspase-3 activity indicated that FA-incorporated C/G/GP hydrogel decreased the apoptosis of H_2O_2-induced oxidative stress NP cells. The results suggested that the C/G/GP hydrogel was very suitable for sustained delivery of FA. The FA-incorporated C/G/GP hydrogel would be used to treat the degenerative disc in the early stage before it developed into the latter irreversible stages.

It has been reported that barley phenolic anti-oxidants change in response to the kilning regimen used in preparing malt. Green malt was kilned using four different regimens. There were no major differences among the finished malts in parameters routinely used by the malting industry, including moisture, color, and diastatic activity. FA esterase activity and free FA were higher in malts subjected to the coolest kilning regimen, but malt ethyl acetate extracts (containing FA) contributed approximately only 5% of the total malt anti-oxidant activity. Finished malt from the hottest kilning regimen possessed the highest anti-oxidant activity, attributed to higher levels of Maillard reaction products. Modifying kilning conditions leads to changes in release of bound FA and anti-oxidant activity with potential benefits on flavor stability in malt and beer (Inns *et al*, 2011).

As shown in Tables 1 and 2, lactones of *A. sinensis* (LAS) had strong scavenging effects on 1,1-diphenyl-2-pocrylhudrazyl (DPPH) and hydroxyl radical, and their IC_{50} were 2.0 and 7.6 mg/mL, respectively. LAS also exhibited a significant concentration-dependent inhibition of lipid peroxidation in the linoleic acid and rat erythrocytes hemolysis induced by H_2O_2 with a strong reduction power. This study suggested that LAS beared significant free radical scavenging and anti-oxidant activities (Long, Du, and Chen, 2010).

Table 1 Scavenging effect of LAS on DPPH (n = 3)

Groups	Dose / ($mg \cdot mL^{-1}$)	A517	Scavenging rate / %
control	—	0.582 ± 0.008	—
LAS	0.2	$0.553 \pm 0.009^{*}$	5.0
	0.4	$0.502 \pm 0.012^{**}$	13.7
	0.8	$0.430 \pm 0.014^{**}$	26.7
	1.6	$0.345 \pm 0.016^{**}$	40.7
	3.2	$0.150 \pm 0.007^{**}$	74.2

$^{*}P < 0.01$ $^{**}P < 0.001$ *vs* control group

Neuroprotective action

LIG has shown to reduce ischemic brain injury via anti-apoptotic pathways. Accordingly, in a study, Chen and workers investigated the neuroprotective potential of LIG after experimental subarachnoid hemorrhage (SAH) in rats. Rats with SAH, induced using the established double hemorrhage model were studied with and without LIG treatment. Mortality, neuro-behavioral evaluation, brain water content, blood-brain barrier (BBB) permeability, and vasospasm assessment of the basilar artery were measured on days 3 and 7 after injury. Additional testing was done to evaluate apoptosis using TdT-mediated dUTP-biotin nick end labeling staining, as well as immunohistochemistry and Western blotting to identify key proapoptotic/survival proteins such as p53, Bax, Bcl-2, and cleaved caspase-3. LIG treatment reduced mortality, neurobehavioral deficits, brain edema, BBB permeability, and cerebral

Table 2 Scavenging effect of LAS on hydroxyl radical and inhibition effect of LAS on linoleic acid oxidation ($\bar{x} \pm s$, $n = 3$)

Groups	Dose / (mg·mL^{-1})	A_{532}	Scavenging rate / %	A_{500}			
				6 h	24 h	48 h	96 h
control	—	0.154 ± 0.002	—	0.025 ± 0.001	0.123 ± 0.007	0.234 ± 0.011	1.792 ± 0.069
LAS	1	0.120 ± 0.001*	22.2	0.025 ± 0.001	0.067 ± 0.002	0.220 ± 0.004	1.306 ± 0.136*
	4	0.091 ± 0.001*	40.9	0.024 ± 0.001	0.029 ± 0.007*	1.164 ± 0.004*	0.806 ± 0.037*
	16	0.051 ± 0.002*	66.9	0.024 ± 0.002	0.027 ± 0.007*	0.138 ± 0.002*	0.563 ± 0.029*

*$P < 0.01$ *vs* control group

vasospasm. In addition, treatment reduced the number of apoptotic cells in the surrounding brain injury site, which accompanied a marked down-regulation of proapoptotic proteins, p53, and cleaved caspase-3. Chen *et al*'s data buttressed previous findings, suggesting that LIG might be an effective therapeutic modality for SAH victims by altering apoptotic mechanisms (Chen *et al*, 2011).

Immune support and hematopoiesis

FA-induced anti-immobility was prevented by pretreatment with PCPA, WAY-100635, ketanserin, sulpiride, SCH233390, haloperidol, and yohimbine, independently. CRH, ACTH, and 5-HT were significantly decreased, but ghrelin was apparently increased compared with vehicle. In summary, FA induced antidepression and prokinetics via inhibiting 5-HT, norepinephrine and dopamine reuptakes, regulating HPA axis, increasing ghrelin and stimulating jejunal contraction simultaneously (Zhang *et al*, 2011).

Lymphocyte proliferation assays indicate that *A. sinensis* consistently exerts an immunostimulatory effect (Wilasrusmee *et al*, 2002a; 2002b). A high molecular weight polysaccharide found in *A. sinensis* has demonstrated immunostimulating activity and a blood tonifying effect by inducing hematopoiesis in the bone marrow. This is accomplished, in part, by either direct or indirect stimulation of macrophages, fibroblasts, erythrocytes, granulocytes, and lymphocytes, and could induce an increased secretion of human growth factors from muscle tissue. The evidence of hematopoiesis is further supported by the presence of significant amounts of vitamin B_{12}, folinic acid, and biotin in *A. sinensis* (Huang, 1999).

Other pharmacological activities

Cheng and his co-workers investigated the effect of *Z*-ligustilide (*Z*-LIG) on Scopolamine-induced memory impairment in ICR mice. *Z*-LIG (2.5－40 mg/kg) or tacrine (10 mg/kg) was ig administrated for 26 d. Behavior was examined in the Morris water maze and Y-maze after Scopolamine administration (2 mg/kg, ip). The central acetylcholinesterase (AChE), and choline acetyltransferase (ChAT) activities were assessed spectrophotometrically. *Z*-LIG significantly improved spatial long-term memory and short-term memory impairment, inhibited AChE activity and increased ChAT activity. Moreover, Z-LIG and tacrine showed comparable efficacy in both neurobehavioral and cholinergic evaluation. Thus, Z-LIG might alleviate memory deficits probably via enhancing cholinergic function (Cheng, Yang, and Lin, 2011).

In another study, Shao *et al* (2011) investigated the protective effects of LIG against lipopolysaccharide (LPS)-induced endotoxic shock in Japanese white rabbits and attempted to elucidate the possible mechanism underlying these effects. Forty-two rabbits were randomly divided into six groups: normal, LPS, Dexamethasone (5 mg/kg), and three LIG groups (20, 40, and 80 mg/kg). After rabbits being received a LPS infusion (0.3 mg/kg), Dexamethasone and LIG were iv injected at the above-mentioned dosages. Heart rate (HR), mean arterial pressure (MAP), and rectal temperature (RT) were recorded throughout the experiment. TNF-α, IL-1β, and NO levels were measured by radioimmuno-assay every 30 min for the first hour and every 60 min thereafter until the end of the experiment. The serum levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT), creatinine kinase (CK), lactate dehydrogenase (LDH), total protein (TP), creatinine (Scr), blood urea nitrogen (BUN), total bilirubin (TBIL), and counts of formed elements of blood were measured at 0, 120, and 300 min after the administration of LPS. Hemorheology was assayed 300 min after the LPS injection. The vital organs were collected and weighed before histopathologic examination. A comparison between LPS and LIG

groups showed that LIG significantly inhibited the decline in MAP and RT and decreased the levels of TNF-α, IL-1β, and NO, but had no apparent effect on HR. LIG also inhibited the increase in the levels of biochemical markers, such as ALT, AST, ALP, GGT, LDH, CK, BUN, and Scr, but showed no apparent effect on TBIL and TP. Furthermore, LIG partly restored the function of injured vital organs including the heart, livers, lungs, and kidneys. Their study indicated that LIG protected the rabbits against LPS-induced endotoxic shock.

Animal experiments *in vivo* have demonstrated increased excitability of the uterus, where the contractive rhythm of uterine smooth muscle changed from fast, weak, and irregular to slower, stronger, and more coordinated (more rhythmic), depending on uterine tone. This is believed to be the pharmacological basis for use of *A. sinensis* during dysmenorrhea (Zhu, 1987). The root does not exert estrogenic activity (Chang and But, 1987). In a recent animal study the astragalus/angelica mixture was found to retard the progression of renal fibrosis and deteriorate the renal function with an effect similar to the drug Enalapril (Wang *et al*, 2004).

The antitumor effect of a chloroform extract of *A. sinensis* (AS-C) on malignant brain tumors was examined by Tsai *et al* (2005). The AS-C displayed potency in suppressing growth of malignant brain tumor cells without cytotoxicity to fibroblasts. Growth suppression of malignant brain tumor cells by AS-C was due to cell cycle arrest and apoptosis. AS-C could up-regulate the expression of cdk inhibitors, including p21, to decrease phosphorylation of Rb proteins, resulting in cell arrest at the G_0/G_1 phase for DBTRG-05MG and RG2 cells. The apoptosis associated proteins were dramatically increased and activated in DBTRG-05MG cells and RG2 cells by AS-C. *In vitro* results revealed that AS-C triggered both p53-dependent and independent pathways for apoptosis. In *in vivo* studies, AS-C did not only suppress growths of malignant brain tumors of rat and human origin but also caused a shrink in the volume of *in situ* GBM, significantly prolonging survival (Fig. 4). Thus, *A. sinensis* might be a good source of potent compound(s) against human GBM tumor (Tsai *et al*, 2005).

Mechanisms of action

Wang *et al* (2010b) used a semi-quantitative RT-PCR and Western blotting assays to study the effects of *A. sinensis* injection on the protein expression and gene of human erythroleukemia multidrug resistance (MDR) K562/A02 cell line. K562/A02 cells were observed to be partly reversed by *A. sinensis* injection, which might be associated with reduction of P-gp expression, resulting from down-regulation of mRNA expression of MDR1 gene and up-regulation of Topo II expression. Furthermore, Zhou *et al* (2010) used three methods namely thiazole blue (MTT colorimetric method, fluorescent quantitation polymerase chain reaction (real time-PCR), and Western-blotting method to investigate the effect of *A. sinensis* Decoction (ASD) for supplementing blood on mesangium cell proliferation, TGF-β1 mRNA and NF-κB. Marked proliferation of *in vitro* cultured mesangium cells was stimulated by high levels of glucose, coupled with increased expressions of TGF-β1 mRNA and NF-κB in mesangium cells ($P < 0.05$). In contrast, ASD significantly suppressed glomerular mesangium cells (GMC) proliferation, expression of TGF-β1 mRNA, and the protein of NF-κB in the GMC ($P < 0.05$, 0.01), in a dose-dependent manner of the expression with glucose

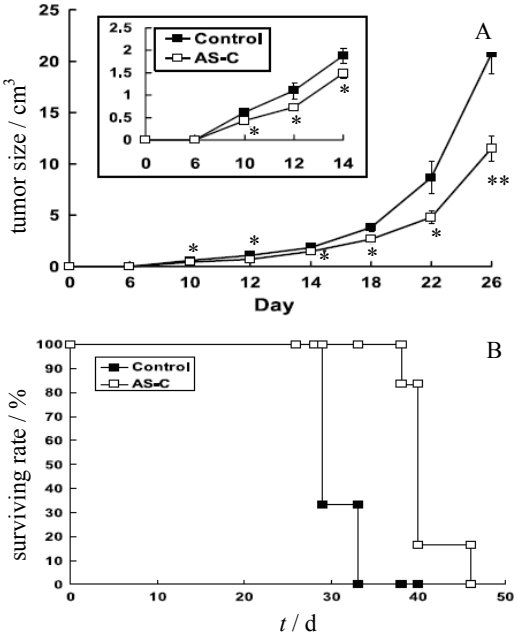

Fig. 4 AS-C inhibition of tumor growth with improvement in survival rate in syngenic rat GBM model

RG2 cells (1×10^6) were sc implanted into the hind flank region of F344 rats

A: tumor size was measured using a caliper ($^*P < 0.05$ $^{**}P < 0.001$)

B: survival was monitored daily ($P < 0.0001$)

Rats were sacrificed when tumor size exceeded 25 cm^3. Tumor sizes are represented as $\bar{x} \pm s$ (Tsai *et al*, 2005)

or medicine concentration augmentation, GMC proliferation, TGF-β1 mRNA and the protein of NF-κB in the GMC expression increased or decreased. It suggested that high glucose could promote GMC proliferation, TGF-β1 mRNA and the protein of NF-κB expressions in GMC, but ASD for supplementing blood could retroconversion those phenomenons. These results thus showed that ASD directly repressed GMC proliferation, TGF-β1 mRNA and the protein of NF-κB expression in GMC under high glucose, thereby preventing and treating glomer- ulosclerosis and interstitial fibrosis, delaying diabetic nephropathy progression (Zhou and Zhang, 2010).

To explore the effect and mechanism of FA on differentiation in bFGF-treated PC12 cells, the length of neurite outgrowth and the percentage of PC12 cells induced in the presence (or absence) bFGF (1 ng/mL) were assayed. Compared with that of control group, FA could enhance the differentiation effect of bFGF treated PC12 cells ($P < 0.01$) and the enhancing effect could be blocked by the specific MAPK inhibitor, PD98059.

FA potentiates neurite outgrowth in bFGF-treated PC12 cells by MAPK-dependent signaling pathway (Lin *et al*, 2011). The study carried out by Wang *et al* (2011) showed that FA, over a range of concentrations from 0.1 to 10 μg/mL could markedly improve cell proliferation and DNA synthesis in a dose-dependent manner. Flow cytometry showed a significant decrease in the percentage of cells in the G_0/G_1 phase and a significant increase in the percentage of cells in the S phase. Additionally, it was discovered that FA enhanced cyclin D1 and VEGF mRNA expression in ECV304 cells. FA was able to promote ECV304 cells proliferation *in vitro*. This effect might be observed through the modulation of cyclin D1 and VEGF.

Decoction of Jingqianfang (JQF), including angelica root, wild peony root, motherwort, and other herbs, is an effective formulation for the treatment of primary dysmenorrhea (PD) in clinic with the therapeutic cure rate as high as 96%. To understand its role in regulating prostaglandin, estrogen and other endocrines in rats with dysmenorrhea, the effect of JQF on the changes of PGF2α, PGE2, TXB2, 6-keto-PGFlα, estrin, and progestogen in the uterine tissue was studied by Fang *et al* (2010), using radioimmunity assay. As depicted in Tables 3, 4, and 5, relative to the control group, PGE2 and progestogen were increased significantly, whereas PGF2α, PGF2α, PGE2, TXB2, TXB2, 6-keto-PGFlα, and estrin decreased in the group with high dose of JQF ($P < 0.01$). Thus, JQF was effective in regulating prostaglandin, estrin, and progestogen in uterus of rats with dysmenorrhea, and helpful in restraining the contraction of smooth muscle in uterus, and reducing the tension of the muscle，which could be the underlying mechanism of action of JQF decoction.

Traditional Chinese medicine (TCM) formulas have been used for promotion of “blood production” for centuries and there is growing interest in developing novel thrombopoietic medicines from these TCMs. The

Table 3 Comparison of PGF2α and PGE2 in rats of each group ($\bar{x} \pm s$, $n = 6$)

Groups	PGF2α / (ng·mL^{-1})	PGE2 / (ng·mL^{-1})	PGF2α/PGE2
normal	209.65 ± 77.51	801.12 ± 111.32	0.27 ± 0.11
model	802.79 ± 153.69**	594.88 ± 137.15**	1.50 ± 0.56**
JQFJQF (2.5 g·kg^{-1})	505.65 ± 98.02$^{\#\#}$	619.65 ± 187.78	0.99 ± 0.39
JQF (5.0 g·kg^{-1})	221.42 ± 140.67$^{\#\#}$	810.35 ± 208.62$^{\#}$	0.27 ± 0.16$^{\#\#}$
Aspirin	488.78 ± 134.22$^{\#\#}$	875.62 ± 244.11$^{\#}$	0.59 ± 0.12$^{\#\#}$

$^{**}P < 0.01$ *vs* normal group; $^{\#\#}P < 0.01$ $^{\#}P < 0.05$ *vs* model group

Table 4 Comparison of TXB2 and 6-keto-PGF1α in rats of each group ($\bar{x} \pm s$, $n = 6$)

Groups	TXB2 / (ng·mL^{-1})	6-keto-PGF1α / (ng·mL^{-1})	TXB2/6-keto-PGF1α
normal	542.73 ± 53.01	914.81 ± 100.64	0.55 ± 0.08
model	722.42 ± 90.54*	1023.63 ± 75.55	0.67 ± 0.09
JQF 2.5 (g·kg^{-1})	582.63 ± 64.11$^{\#}$	1018.74 ± 59.79	0.57 ± 0.05
JQF 5.0 (g·kg^{-1})	520.87 ± 66.27$^{\#}$	1288.60 ± 207.00$^{\#}$	0.40 ± 0.08$^{\#\#}$
Aspirin	398.18 ± 25.38$^{\#\#}$	779.40 ± 65.30$^{\#\#}$	0.52 ± 0.04$^{\#}$

$^{**}P < 0.01$ *vs* normal group; $^{\#\#}P < 0.01$ $^{\#}P < 0.05$ *vs* model group

Table 5 Effect of JQF on estrin 2 and progestogen in rats with dysmenorrheal ($\bar{x} \pm s$, $n = 6$)

Groups	Estrin 2 / ($ng \cdot mL^{-1}$)	Progestogen / ($ng \cdot mL^{-1}$)
normal	19.07 ± 3.32	20.69 ± 4.87
model	38.75 ± 10.57**	10.44 ± 5.37*
JQF 2.5 ($g \cdot kg^{-1}$)	25.63 ± 3.23$^{\#\#}$	15.54 ± 9.14
JQF 5.0 ($g \cdot kg^{-1}$)	23.63 ± 3.09$^{\#\#}$	20.11 ± 7.52$^{\#}$
Aspirin	25.72 ± 4.708$^{\#}$	21.59 ± 8.78$^{\#}$

$^{**}P < 0.01$ vs normal group; $^{\#\#}P < 0.01$ $^{\#}P < 0.05$ *vs* model group

hematopoietic effects of Dang-gui Bu-xue Tang (DBT), a formula composed of ASR and AR in animal and cellular models has been studied. Yang *et al*'s results (2009) showed that DBT treatment significantly increased the recovery of the megakaryocytic series. DBT significantly enhanced the platelet recovery and colony forming unit-megakaryocytic (CFU-MK) formation *in vivo*. DBT significantly promoted CFU-MK and CFU-F formation. Last, they observed the anti-apoptotic effects of DBT on M-07e cells. DBT might promote haematopoiesis and thrombopoiesis in the mouse model through directly promoting the growth of megakaryocytes and indirectly promoting the growth of bone marrow stromal cells; inhibiting apoptosis of megakaryocytes. Liu *et al* (2010) studied the hematopoietic and thrombopoietic effects of polysaccharide-enriched fractions from the roots of ASR. In animal models, ASR significantly enhanced not only the recovery of platelets, other blood cells and their progenitor cells, but also the formation of colony forming unit (CFU). In M-07e cells, they observed the anti-apoptotic effect of ASR. Treatment by Ly294002 alone increased the percentage of cells undergoing apoptosis, while addition of ASR to Ly294002-treated cells significantly reduced the percentage of cells undergoing apoptosis. This effect likely resulted from the anti-apoptotic activity of *A. sinensis* polysaccharides (ASP), involving the PI3K/AKT pathway. Ding *et al* (2003) used cDNA microarray to study the modulating effect of $GdCl_3$ and ASP on differentially expressed genes in liver of hepatic immunological mice to understand the molecular mechanism of hepatic immunological injury, as well as the intervention of the drug. Consequently, both ASP and $GdCl_3$ decreased the number of the differentially expressed genes in liver tissue of mice with hepatic immunological injury. When ASP pre-treating mice with hepatic immunological injury, the expression of Humnlk up-regulation may show ASP prevent cell apoptosis. It remains to be demonstrated whether the altered genes are target of protective effect of $GdCl_3$ or ASP on the immunological liver injury, and further analysis of the function of these genes is needed.

Drug-drug interactions and pharmacokinetics

A. sinensis may potentiate the therapeutic and/or adverse effects associated with antiplatelet medication. A pharmacokinetic study conducted on rabbits observed the interaction between *A. sinensis* and Warfarin. Single sc dose of Warfarin (2 mg/kg) was administered with or without oral *A. sinensis* extract (2 g/kg, twice daily for 3 d). The *A. sinensis* treatment alone did not affect prothrombin time, but significantly lowered the value 3 d after co-administration with Warfarin. No significant variation in the pharmaco-kinetic parameters of Warfarin was observed after Dong-gui treatment for either single-dose administration or steady-state concentrations of Warfarin (Bone and Mills, 2000; Heck, Dewitt, and Lukes, 2000).

In a case report, a 46-year-old woman, who had been taking 5 mg/d Warfarin for nearly two years and had an international normalized ratio (INR) stabilized at 2－3, experienced an increase in her INR to 4.9 over the course of approximately two months (Page and Lawrence, 1999). Changes in medication regimen, diet, alcohol consumption, or other lifestyle factors that may affect INR were sufficiently ruled out. However, the patient stated that for the past four weeks she had been taking *A. sinensis* for perimenopausal symptoms as recommended by an herbalist, and had forgotten to mention this earlier. The dosage was one 565-mg tablet 1－2 times/d. The patient was instructed to discontinue *A. sinensis* medication, and within four weeks her INR declined to 2.48 in the therapeutic range. In view of this information, caution is advised for patients receiving chronic treatment with Warfarin.

Kimura *et al* (2012) investigated whether the uptake of triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) and dicamba (3,6-dichloro-2-methoxy-benzoic acid) across the apical membrane of Caco-2 cells was mediated via proton-linked monocarboxylic acid transporters (MCTs). The uptake of triclopyr from the apical membranes was fast, pH-, temperature-, and

concentration-dependent, required metabolic energy to proceed, and was competitively inhibited by mono-carboxylic acids such as benzoic acid and FA (substrates of *L*-lactic acid-insensitive MCTs, but not by *L*-lactic acid). Thus, the uptake of triclopyr in Caco-2 cells appears to be mediated mainly via *L*-lactic acid-insensitive MCTs. In contrast, the uptake of dicamba was slow, and it was both pH- and temperature-dependent. Co-incubation with FA did not decrease the uptake of dicamba, although co-incubation with benzoic acid moderately decreased it. The uptake of dicamba appears to be mediated mainly via passive diffusion, which is in contrast to the uptake of benzoic acid via MCTs. Researchers speculate that the substituted groups in dicamba may inhibit uptake via MCTs.

Side effects and toxicity

The oral LD_{50} of a concentrated (8:1 to 16:1) *A. sinensis* extract in rats was measured at 100 g/kg body weight (Bone and Mills, 2000). Iv administration of the essential oil to animals at a dose of 1 mL/kg could cause a drop in blood pressure and depression of respiration (Huang, 1999). *A. sinensis* is contrain-dicated in pregnancy, particularly in the first trimester, due to potential uterine stimulant and relaxant effects (Bone and Mills, 2000; Brinker, 1998). Curcumin and LIG could generate both genotoxic and/or chemo-preventive effects. The biological targets of BRIs formed from botanical dietary supplements and their resulting toxic and/or chemopreventive effects are closely linked to the reactivity of biological reactive intermediates (BRIs) causing toxicity, as well as dose and time of exposure (Dietz and Bolton, 2011).

There has been one isolated case of a man who developed gynecomastia (mammary glandular hyper-plasia) after taking *A. sinensis* capsules daily for approximately one month (Goh and Loh, 2001). The patient discontinued the *A. sinensis* pills and his gynecomastia had regressed completely when examined three months later. It is important to note that the pills in question were not properly analyzed to confirm or refute the purity of the product. Consequently, the authors could not rule out presence of a pharma-cologically active contaminant that may have contributed to the patient's condition (Kiong, 2001). Other related species of angelica (e.g., *A. gigas*, *A. dahurica*, and *A. pubescens*) pose a greater risk than *A. sinensis* due to their higher furanocoumarin content (DerMarderosian and Beutler, 2004).

Clinical applications

Angelicae Sinensis Radix is officially listed in the *Chinese Pharmacopoeia*. It is one of the most commonly used Chinese traditional drugs. It has been used in the treatment of menstrual disorders, menorrhagia, and rheumatalgia. It is also used in many tonic preparations and Chinese patent drugs. Angelica root is used to replenish blood, invigorate blood, and stop pain, and to moisten intestines. The head of the roots is more effective for nourishing blood, the tail for moving blood, and the body is used to invigorate and nourish blood (Liu, Xiao, and Li, 2000).

The pathogenesis of plastic anemia is considered to be both deficiency of vital and blood in traditional Chinese medicine. Angelica has been used for raising the vital ("Qi") and nourishing the "blood" of the individual and has shown promising effects on aplastic anemia by stimulating the proliferation of bone marrow cells, anti-apoptosis, modulating immune activities, and improving the hematopoietic micro-environment (Li and Liu, 2010). *A. sinensis* is available in several different forms, and dosages vary accordingly. Typical oral dosages (Bone and Mills, 2000; DerMarderosian and Beutler, 2004; Skidmore-Roth, 2001) are as follows: dried *A. sinensis* root: 3－15 g daily by decoction; powdered root: 1－2 g three times daily; tea: one cup 1－3 times daily (1 g per cup); tincture (1:2): 4－8 mL (1－2 tsp) per day; and capsules/tablets: 500 mg 1－6 times daily (Anyones, 2004).

Cardio-cerebrovascular disease

A. sinensis has demonstrated quinidine-like activity on the heart (Bone and Mills, 2000). It could prolong the refractory period, lower blood pressure, and correct experimental atrial fibrillation induced by atropine, pituitrin, strophanthin, acetylcholine, or electrical stimulation (Chang and But, 1987). *A. sinensis* could dilate the coronary vessels, increase coronary flow, and reduce respiratory rate. An animal study using a water based extract of *A. sinensis* demonstrated a marked protective effect against myocardial dysfunction and myocardial injury induced

by ischemia (Huang, 1999). A recent histological study demonstrated that a preparation of angelica and ligusticum significantly protected human umbilical vein endothelial cells against H_2O_2 damage, primarily by inhibiting reactive oxygen species formation and promoting endothelial NO synthase expression (Hou *et al*, 2004). This might be the mechanism of the above-noted cardio-protective activity. LIG might be considered as a potential complementary drug candidate for treating inflammatory processes associated with cerebrovascular diseases (Or *et al*, 2011).

One hundred and ninety-four cases with cerebral thrombosis received clinical treatment. Ninety-nine cases were as treated group with complex sodium ferulate preparation for four weeks, and 95 as control group with piracetam were used for clinical evaluation in double method. The result showed that total effective rate was 84% for treated group and 28% for control group (Jiang *et al*, 1991).

Menopause

One of the most common applications for *A. sinensis* in the United States is for relief of vasomotor symptoms associated with menopause. Such symptoms include hot flashes, skin flushing, perspiration, and chills. The mechanism of action, however, is still unclear. In a randomized, double-blind, and placebo-controlled clinical trial, postmenopausal women received either *A. sinensis* root (4.5 g) or placebo daily for 24 weeks (Hirata *et al*, 1997). There were no differences in vasomotor symptoms between the two groups, and there appeared to be no estrogen-like effects on vaginal epithelial tissue. The use of *A. sinensis* alone could be critical because traditional Chinese practitioners never prescribe it alone, but rather in combination with several other herbs. The researchers choose to study *A. sinensis* alone because many women in the United States who take it to relieve menopausal symptoms purchase the herb over-the-counter as a single entity. Women should be discouraged from using *A. sinensis* alone for the relief of menopausal complaints.

A herbal mixture containing *Angelicae Sinensis Radix*, *Paeoniae Radix Rubra*, *Chuanxiong Rhizoma*, *Atractylodes Rhizoma*, *Alismatis Rhizoma*, and *Poria* has been reported to reduce menopausal disturbances, including vasomotor symptoms by 70% (Chang and But, 1987; Hirata *et al*, 1997).

Nephrotic syndrome and dysmenorrhea

An herbal preparation of astragalus and angelica has long been used in China to treat nephrotic syndrome, as it was thought to elicit antifibrotic effects. Two general components of *A. sinensis* affect uterine smooth muscle in opposite ways. The antispasmodic component of the herb is attributed to constituents of the volatile oil, such as LIG, butylidenephthalide, and butylphthalide, balanced by the uterine stimulating aspect, which is related to the water-soluble, and nonvolatile constituents of the herb (Zhu, 1987; Bone and Mills, 2000; Huang, 1999; Chang and But, 1987).

Conclusion

ASR is the root of *A. sinensis* which is a fragrant, perennial herb native to China, Japan, and Korea. Chinese herbalists have used ASR for thousands of years to strengthen heart, lung, and liver meridians, as well as lubricate the bowel. It is considered as a blood tonic, and has been used by generations of women for health concerns such as menstrual pain and regulating the menstrual cycle.

ASR contains 0.4%—0.7% of volatile oil, the key components of which are *n*-butylidenephthalide, LIG (3-butylidene-4,5-dihydrophthalide), *n*-butyl-phthalide, ferulic acid (4-hydroxy-3-methoxycinnamic acid), nicotinic acid, and succinic acid. The extracts of *A. sinensis* and its active compounds have wide-range pharmacological activities on cardio- and cerebro-vascular systems, anti-inflammatory effect, immune support and hematopoiesis, and so on. Clinically, ASR is officially listed in the *Chinese Pharmacopoeia*. It is one of the most commonly used traditional Chinese medicines, which has been used in the treatment of menstrual disorders, menorrhagia, and rheumatalgia. It is also used in many tonic preparations and Chinese patent drugs. Angelica root is used to replenish blood, invigorate blood, stop pain, and to moisten intestines. The head of the roots is more effective for nourishing blood, the tail for moving blood, and the body is used to invigorate and nourish blood. *A. sinensis* is used for relief of vasomotor symptoms associated with menopause, such symptoms include hot flashes, skin flushing, perspiration, and chills in the United States.

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