Recent Studies and Progression of Yin Chen Hao (茵陳蒿 Yīn Chén Hāo), a Long-term Used Traditional Chinese Medicine

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ABSTRACT

Yin Chen Hao (*Artemisia capillaris* Thunb; 茵陳蒿 Yīn Chén Hāo) is a traditional Chinese medicine for treating hepatic disorders. This review provides recent pharmacological studies of Yin Chen Hao as well as some chemical constituents isolated from Yin Chen.

Key words: *Artemisia capillaris*, Scoparone, Yin Chen

Yin Chen Hao (*Artemisia capillaris* Thunb, 茵陳蒿, Yīn Chén Hāo), also known as Yin Chen, Capillary or Oriental Wormwood belonging to Asteracea family, is a traditional Chinese medicine. Yin Chen Hao was first documented in The Divine Husbandman's Herbal Foundation Canon (神農本草經 shén nóng běn cǎo jīng) for treating hepatic diseases. According to Chinese Pharmacopoeia (中華藥典 Zhōng Huá Yào Diǎn), Yin Chen can be referred to two kinds of herbs: one is *Artemisia scoparia* Waldst et Kit and the other is *Artemisia Capillaris*. Yin Chen must be harvested for its aerial part in spring to exhibit pharmacologic effects. An old saying, "Yin Chen can be used as Yin Chen for treating diseases in February, but can only be used as lumber for burning in May (二月茵陳三月蒿,五月茵陳當柴燒)", indicates huge difference in bioactive component contents of Yin Chen Hao in different seasons.

Hepatic disorders

Yin Chen Hao traditionally was used to treat liver and choleretic disorders. Recently, Yin Chen was found to exhibit hepatoprotective effect by ameliorating murine concanavalin A (con A)-induced hepatitis via suppression of interferon (IFN)-g and interleukin (IL)-12 production.[1] In the following study, orally administrated Artemisiae Capillaris (AC) group (500, 1000, or 2000 mg/10 ml/kg) can decrease serum transaminases activities and IFN-g concentration *in vivo*.[1] Capillarisin, a flavonoid constituent of Yin Chen, is contained in the fraction and has potent hepatoprotective activity *in vivo*. *In vitro* IFN-g production was significantly suppressed by capillarisin in con A-stimulated splenocyte culture and nitrite release from IFN-g-stimulated macrophages was also decreased. Another study also showed that Yin Chen may prevent the EtOH-induced cytotoxicity on human hepatoma cell line and Hep G2 cell.[2] Aqueous extract of AC (0.5–5 μg/mL) inhibited the secretion of EtOH-induced interluekin-1α (IL-1α) and tumor necrosis factor-α (TNF-α). AC also inhibited the EtOH-, IL-1α, and TNF-α-induced cytotoxicities. Furthermore, AC was found to inhibit the EtOH-induced apoptosis of Hep G2 cells. Water extract of Artemisia capillaries (ACWE) was capable of ameliorating the 2,2¢-azobis(2-amidinopropane) dihydrochloride (AAPH)-induced hepatic injury by catechin antioxidant activ-

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ity *in vivo*.[3] ACWE (7.5 g/kg) was orally administered for 7 days before AAPH treatment (60 mg/kg). The treated group significantly reduced hepatic damage by lowering the levels of enzyme markers, such as glutamic oxaloacetic transaminase and glutamic pyruvic transaminase and attenuating the accumulation of thiobarbituric acid-reactive substances (TBARS) in both plasma and liver tissues compared with AAPH alone. High-performance liquid chromatography results showed that catechin composition in the ACWE are 28% (–)-epigallocatechin gallate, 49% (–)-epigallocatechin, and 23% other catechins. Another study also indicated ethyl acetate fraction of AC (100 mg/ml) protected Chinese hamster lung fibroblasts (V79) cells against oxidative stress and increased cell viability by enhancing the antioxidative activity.[4] The ethyl acetate fraction of AC scavenged intracellular reactive oxygen species (ROS) and increased the activities of cellular antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and glutathione (GSH) content, resulting in preventing lipid peroxidation by inhibiting TBARS formation. Interestingly, the antihepatofibrotic effects of water extract of AC and Artemisia iwayomogi (AI), both long-term been used for hepatotherapeutic medicine in Korea, were comparatively analyzed using a carbon tetrachloride (CCl4)-induced liver fibrosis rat model.[5] The results showed that AI exerts greater hepatoprotective and antifibrotic effects as compared with AC via enhancing antioxidant capacity and down-regulating fibrogentic cytokines. Besides, scientific evidence was found to account for Yin Chen's effect on treating jaundice.[6,7] The constitutive androstane receptor (CAR, NR1I3) was identified as a key regulator of bilirubin clearance in the liver. Treatment of wild-type and humanized CAR transgenic mice with AC for 3 days accelerates the clearance of intravenously infused bilirubin, but this effect is absent in CAR knockout animals. Expression of bilirubin glucuronyl transferase and other components of the bilirubin metabolism pathway was induced by Yin Chen treatment of WT mice or mice expressing only human CAR, but not CAR knockout animals.

Diabetes

Besides hepatic disorders, AC also showed effect on diabetic studies. ß-Cell destruction by cytokines is important event in insulin-dependent diabetes mellitus.[8] Nitric oxide synthase (iNOS) expression and nitric oxide (NO) production, stimulated by cytokines, lead to insulin insufficiency. ACWE (100–500 mg/ml) completely and dose-dependently protected IL-1ß and IFN-γ-mediated cytotoxicity on RINm5F (RIN) rat insulinoma cells.[9] Reduction of IL-1ß and IFN-γ-induced NO production correlated well with reduced levels of the iNOS mRNA and protein, which molecular mechanism involved the inhibition of nuclear factor kappa B (NF-κB) activation.[9] Another study also reported that AC demonstrated the highest advanced glycation endproducts (AGE) inhibitory activity among several indigenous Artemisia species.[10] Glycation can lead to the onset of diabetic complications due to chronic hyperglycemia. An acylated flavonoid glycoside, along with 11 known flavonoids, 6 coumarins, and 2 phenolic derivatives were obtained from Yin
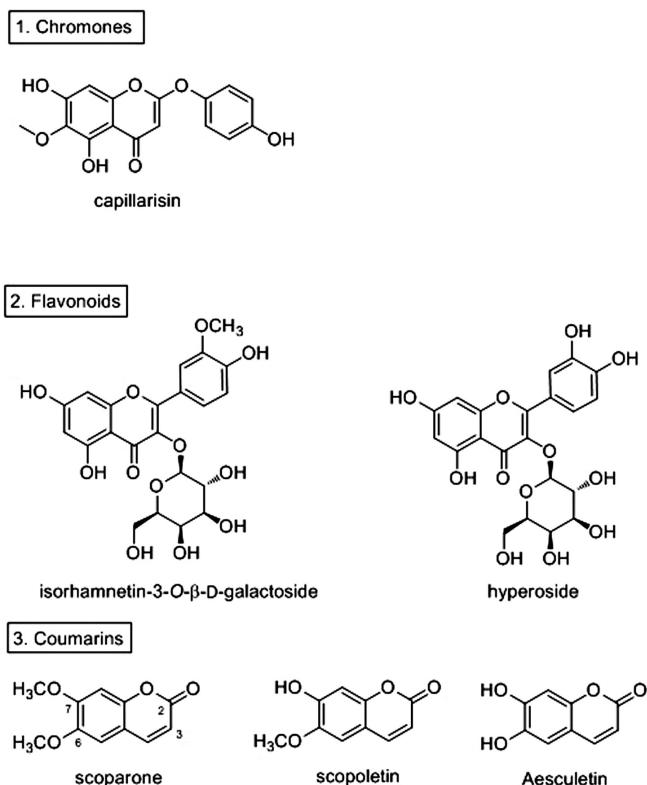

Figure 1. Structures of chemical constituents from *Artemisia capillaries*

Chen and evaluated their AGE inhibitory activity to establish structure-activity relationship (SAR). Presence of hydroxyl group at C-7 and a glucosyl group instead of a methoxyl group at C-6 may play a crucial role in AGE inhibition (coumarin structure, Figure 1).

Lipid metabolism

Lipid metabolism disorders are observed in metabolic syndromes. A study reported the increased lipid metabolism effect of the AC ethyl acetate (ACEA) fraction (0.1 g/kg bw) on high fat diet-induced obesity.[11] *In vitro* the ACEA fraction treatment decreased the leptin level, fat accumulation, and peroxisome proliferator-activated receptor-gamma (PPAR-g) expression in cultured 3T3-L1 adipocytes. Lipid-lowering effect was found in high-fat and ACEA-treated group via increased mitochondrial β-oxidation by increasing the activity of the rate-limiting enzyme, carnitine palmitoyl transferase I. Also, the activity of fatty acid synthase and glycerol-3-phosphate dehydrogenase, related to adipogenic differentiation, were markedly suppressed in the high-fat and ACEA-treated group, as compared with the high-fat group. Moreover, lowered hepatic lipid droplet accumulation and adipose tissue weight and size were seen in the ACEA-treated group.

Skin inflammation

Yin Chen has been reported to treat skin inflammatory conditions in traditional Chinese medicine. Since several allergic and skin inflammatory disorders are involved 5-LOX products, ethanol extract (70%) of the aerial parts of AC was prepared

(3–200 mg/ml) and its 5-lipoxygenase (5-LOX) inhibitory action was studied.[12] Potent inhibitory activity (IC50 < 1.0 μg/mL) against 5- LOX-catalyzed leukotriene production by AC extract was seen in ionophore-induced rat basophilic leukemia-1 cells. Among several constituents isolated from AC, esculetin (ESC) and quercetin were potent inhibitors, with IC50 values of 6.6 and 0.7 μM, respectively. Moreover, AC and ESC strongly inhibited edematic response *in vivo* against arachidonic acid-induced ear edema in mice.

Anticonvulsion

The anticonvulsant effects of AC and its major constituent, ESC, were studied in locomotion, myorelaxation, motor coordination and electroshock seizure experiments in mice.[13] The ethanol extract of AC (50–400 mg/kg) was orally administered to mice 30 min prior to testing and ECT (1–5 mg/kg) was intraperitoneally injected. Locomotor activities and activities on the rota-rod did not change in treated group, suggesting no sedative and myorelaxation effect. However, increased threshold of convulsion induced by electroshock as well as by pentylenetetrazole was observed in AC and ECT-treated group. Moreover, AC and ECT treatment increased the chloride influx into the intracellular area in a dose-dependent manner, which can be inhibited by bicuculline, a GABA antagonist. These results indicate that GABAergic neuron was involved in anticonvulsive effect of AC extract or ECT.

Chemical constituents of AC were extensively studied and they can be divided into several categories such as essential oil, chromones, flavonoids, phenylalkynes, coumarins, benzoids, and lignans. Major and extensively investigated components of AC are coumarin-type compounds, especially scoparone. Several pharmacological studies regarding scoparone were listed below. The structures of some constituents are listed in Figure 1.

Scoparone

Scoparone has been investigated for its bilirubin enhancing effect by up-regulating bilirubin excretion enzymes, such as sulfotransferase or UDP-glucuronosyltransferase 1A1.[6,14] Hepatic cytosolic sulfotransferase activity was increased after treatment with scoparone (0–10 mg/kg) in a dose and time-dependent manner *in vivo*. However, the hepatic cytosolic sulfotransferase was not changed by the addition of scoparone *in vitro*, and was strongly inhibited by the addition of metabolites of scoparone, suggesting the increase of the enzyme activity may involve induction of enzyme proteins by the metabolites of scoparone. Scoparone also reported to activate CAR in primary hepatocytes from both WT and humanized CAR mice and accelerates bilirubin clearance *in vivo*.[7]

Besides liver disorders, scoparone also has been studied in antiinflammatory activities and antidiabetic studies. Reduced the releases of NO and prostaglandin E2 (PGE2) upon stimulation by IFN-g,/LPS or LPS were observed in unstimulated macrophages, but no cytotoxic effect.[15] The inhibitory effect was found to be related with the suppression of iNOS and cyclooxygenase-2 (COX-2) and attenuated the production of TNF-α, interleukin (IL)-113 and IL-6 in IFN-g/LPS stimulated

RAW 264.7 cells. ACWE was found to protect cytokine-induced β-cell destruction.[9] Similarly, scoparone, the major coumarin of AC, also showed protective effect against IL-1β and IFN-g-mediated cytotoxicity of RINm5F and preserved glucose-stimulated insulin secretion in rat pancreatic islets.[16] Inhibition of NF-κB activation was identified as the molecular mechanism of reduced level of iNOS mRNA and protein. Another study also reported that NF-κB activation was inhibited by scoparone in U937 human monocytes activated with phorbol 12-myristate 13-acetate (PMA).[17] Scoparone (5–100 mM) had no cytotoxic effect in unstimulated cells, concentration-dependently reversed PMA-induced toxicity and concentration-dependently reduced the release of IL-8 and MCP-1 protein and expression of IL-8 and MCP-1 mRNA levels induced by PMA. Moreover, scoparone inhibited the level of NF-κB–DNA complex, which is linked to inhibition of NF-κB subunits (NF-κB1 p50, RelA p65, and c-Rel p75) translocation via suppression of IκBa phosphorylation.

Oral administration of scoparone has shown to attenuate IgE-mediated allergic response in mast cells and inhibit passive cutaneous anaphylaxis in rats.[18] Reduced histamine release from rat peritoneal mast cells (RPMC) stimulated by antidinitrophenyl IgE and deduction of the expression and secretion of proinflammatory cytokines, such as TNF-α and IL-6 in RPMC were observed in the presence of scoparone, which may be due to reduced calcium uptake as well as the suppressed activity of p38 MAPK and NF-κB.

Similar to AC extract, the pharmacological effects of scoparone in a hyperlipidaemic diabetic rabbit model were investigated. Scoparone (5 mg/kg/day, s.c.) can reduce plasma lipid and lipoprotein cholesterol levels, and maintain vascular morphology and vascular reactivity in the hyperlipidaemic diabetic rabbit. These protective effects of scoparone may be partly related to its free radical scavenging property.[19]

In addition, scoparone and scopoletin exhibit a potent inhibitory effect on rabbit platelet aggregation induced by four types of agents, adenosine diphosphate (ADP), platelet activating factor (PAF), sodium arachidonate, and/or collagen.[20] Capillarisin exhibits a potent inhibitory effect on bovinelens aldose reductase (LAR). Finally, scoparone (50–200 μM) was reported to increased dopamine release into the culture medium in PC12 cells by synapsin I phosphorylation via activation of PKA and CaMK II mediated by cyclic AMP levels and Ca2+ influx.[21]

Besides scoparone, other constituents also have been reported to exert pharmacological effects in different fields, such as liver protection and antioxidation.

Esculetin

ESC was shown to reduce CCl4-induced hepatic apoptosis in rats.[22] ESC (100, 500 mg/kg) significantly reduced the elevated activities of serum alanine aminotransferase (ALT) and AST caused by CCl4 and significantly increased the activities of catalase, glutathione peroxidase (GPx), and SOD. Furthermore, ESC (100, 500 mg/kg) decreased the levels of the proapoptotic proteins (t-Bid, Bak, and Bad) and increased the levels of the antiapoptotic proteins (Bcl-2 and Bcl-xL). ESC inhibited the

release of cytochrome c from mitochondria. In addition, the levels of activated caspase-9 and activated caspase-3 were significantly decreased in rats treated with ESC than those in rats treated with CCl4 alone.

Capillarisin

The effect and mechanism of capillarisin from AC on rabbit penile corpus cavernosum (PCC) was evaluated.[23] Capillarisin (10-7, 10-6, 10-5, and 10-4 M) induced precontracted New Zealand White rabbit (2.5–3.0 kg) penile relaxation and enhanced PDE5Is-induced relaxation. Capillarisin increased cGMP and cAMP in the perfusate. Significant inhibition of the relaxation was found in the application of capillarisin on PCC pretreated with Nv nitro-L-arginine-methyl ester (L-NAME, 10-3 M) and 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 10-5 M), which were used to block NO synthase and guanylate cyclase, respectively. Capillarisin exerts the relaxing effect on PCC by activating the NO cGMP and adenylyl cAMP signaling pathways.

In addition, capillarisin (0.01± 1.00 mg/ml) has shown inhibitory effects against t-butyl-hydroperoxide (t-BHP)-caused cytotoxicity and genotoxicity in rat primary hepatocyte cultures at least via two distinct pathways, stabilizing the GSH system and quenching free radicals of 1,1-diphenyl-2-pi-crylhydrazyl (DPPH).[24]

Hyperoside (3-O-galactoside of quercetin)

Hyperoside (50, 100, and 200 mg/kg, i.p.) has found to exert protective effects against CCl4-induced acute liver injury in mice, likely due to enhancement of the antioxidative defense system and suppression of the inflammatory response.[25] The CCl4-caused elevation of serum aminotransferases, lipid peroxidation, and glutathione content were attenuated by hyperoside. Prevention of portal inflammation, centrizonal necrosis, and Kupffer cell hyperplasia were observed in histological analysis. In addition, the CCl4-caused increase of protein and mRNA expression of TNF-α, iNOS, COX-2, and were suppressed and heme oxygenase-1 (HO-1) and nuclear protein expression of nuclear factor erythroid 2-related factor 2 (Nrf2) were augmented.

Isorhamnetin 3-O-galactoside

Isorhamnetin-3-O-galactoside (50, 100, and 200 mg/kg, i.p.) ameliorated CCl4-induced hepatic injury in mice by enhancing the antioxidative defense system and reducing the inflammatory signaling pathways.[26] Isorhamnetin-3-O-galactoside significantly decreased serum aminotransferase activities, hepatic level of malondialdehyde, and serum tumor necrosis factor-α level. The levels of HO-1 protein and mRNA expression were augmented by isorhamnetin-3-O-galactoside, while isorhamnetin-3-O-galactoside attenuated the increase of iNOS and COX-2 protein and mRNA expression levels. In addition, isorhamnetin-3-O-galactoside reduced the increase of NF-κB and c-Jun nuclear translocation, but augmented the nuclear level of Nrf2.

Essential oil

Essential oil from AC not only has insecticide effect but also exerts some pharmacological effects. A study reported that the oil inhibited the LPS-induced expression and production of inflammatory mediators, such as NO and prostaglandin E2 (PGE2), by blocking the MAPK-mediated pathways and inhibiting the activation of NF-kB and AP-1.[27]

Yin Chen Hao is a long-used traditional Chinese medicine. In recent studies, it was proved to prevent CCl4-induced liver injury as well as other pharmacological effects such as anitinflammatory and antiobesity. This review shed some light on discovery and development of traditional Chinese medicine, Yin Chen Hao.

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