

1. *Radix rehmanniae* polysaccharide can promote the tumor suppressor gene p53 expression.
2. *Radix rehmanniae* polysaccharide can induce tumor apoptosis through inhibiting the Akt and Bcl-2 genes expression, increasing the Bax gene expression.
3. *Radix rehmanniae* polysaccharide can start the mitochondria inducing apoptosis pathway through promoting the Cyt-C and Caspase-3 genes expression.
4. *Radix rehmanniae* polysaccharide can enhance the mice immune function by promoting the cytokine TNF- $\alpha$ , IL-2 and IFN- $\gamma$  expression.
5. *Radix rehmanniae* polysaccharide can enhance the anti-tumor effect of cyclophosphamide.

Key words: *radix rehmanniae* polysaccharide; Akt; Bcl-2; Bax; Cyt-C; Caspase-3

## FRAGMENTATION PATTERN OF SPIROSTANOL STEROIDAL SAPONINS FROM ANEMARRHENA ASPHODELOIDES

Yong-Gang Xia, Feng-Qiu Gong, Xin-Dong Guo, Jun Liang, Hai-Xue Kuang

Heilongjiang University of Chinese Medicine, Heilongjiang, China

**Abstract:** *Anemarrhena asphodeloides* (*A. asphodeloides*) rhizome roots of liliaceous is widely used to clearing heat-fire, nourishing Yin and moistening dryness, and recorded in "Chinese pharmacopoeia" 2015 edition [1]. So far, more than 50 steroid saponins have been isolated from *A. asphodeloides*, which is widely used to decrease blood glucose levels, inhibit platelet activity and carcinoma activity [2-4]. This study applying UPLC-MS/MS methods to analyze *Anemarrhena* spirostanol reference standards. Finally, fragmentation regularities of four spirostanol steroid saponins are summarized. The four reference standards (RSs) are timosaponin A II (1), timosaponin A III (2), dioscin (3), ophiopogonin D' (4) and gracillin (5).

Key words: *Anemarrhena asphodeloides*; spirostanol steroidal saponins; UPLC-MS/MS

**Objective:** To analysis the fragmentation pattern of spirostanol steroidal saponins from *Anemarrhena asphodeloides* with UPLC-MS/MS.

**Materials and methods:** Reference standards were purchased from Chengdu Must Bio-technology Co., which the purity of each standard compound was determined to be over 97% by normalization of the peak areas detected by LC-MS analysis. Separation was achieved on Waters UPLC HSS T3 column (2.1 mm  $\times$  150 mm, 1.8  $\mu$ m) and HSS T3 guard column (2.1 mm  $\times$  5 mm, 1.8  $\mu$ m), in the experiment. A contained 0.6308g/L ammonium formate in water and B was acetonitrile; both of them contained 0.1% formic acid. Sciex Qtrap-4000 detector, [M+NH<sub>4</sub>]<sup>+</sup> data were provided in the positive mode. MIM-IDA-EPI mode were used to scan RSs. In MIM transitions, (Q1) and (Q3) were both [M+NH<sub>4</sub>]<sup>+</sup>. DP values were set 50 eV and CE values were set 5 eV. Ion spray voltage was set at +5500 V, interface heater was on and turbo spray temperature was 400 °C. Both nebulizer gas (gas 1) and heater gas (gas 2) were set at 50 psi. EPI scan was performed at a scan rate of 4000 Da/s. The CE of EPI was set at 8, 15 and 35 eV, respectively.

**Results:** Similar deductive reasoning was applied to spirostane-type RSs 1-5. Two concise and clear [M+NH<sub>4</sub>]<sup>+</sup> and [M+H]<sup>+</sup> ions were obviously observed in the ESI<sup>+</sup>-EPI (CE,8) spectra for RSs 1-5. In the ESI<sup>+</sup>-EPI (CE,15) spectra, successive losses of sugar moieties one-by-one were also generated to provide [Agly+H]<sup>+</sup> (RSs 1-5). In addition, the fragmentations of aglycone ions were readily observed in the ESI<sup>+</sup>-EPI (CE, 35) spectra. Two corresponding ion transitions were involved in [S<sub>4</sub>+H]<sup>+</sup>  $\rightarrow$  [S<sub>4</sub>-C<sub>8</sub>H<sub>16</sub>O<sub>2</sub>+H]<sup>+</sup>  $\rightarrow$  [S<sub>4</sub>-H<sub>2</sub>O-C<sub>8</sub>H<sub>16</sub>O<sub>2</sub>+H]<sup>+</sup> ( $\Delta m$ = 144 and 18 Da) and [S<sub>4</sub>+H]<sup>+</sup>  $\rightarrow$  [S<sub>4</sub>-H<sub>2</sub>O+H]<sup>+</sup>  $\rightarrow$  [S<sub>4</sub>-H<sub>2</sub>O-C<sub>8</sub>H<sub>16</sub>O<sub>2</sub>+H]<sup>+</sup> ( $\Delta m$  = 18 and 144 Da) for RSs 1-5. This could be explained through the elimination of E rings and water molecules. The major MS/MS fragment pathways of spirostane-type RSs 1-3 were summarized.

**Discussion** Whatever spirostanol SSs, all were characterized by preferential loss of a NH<sub>3</sub> (17 Da) from an ammoniated precursor ion in the ESI<sup>+</sup>-EPI (CE, 8) spectra and then C-3 sugar moiety to afford corresponding protonated aglycones in the ESI<sup>+</sup>-EPI (CE, 15) spectra. As expected in ESI<sup>+</sup>-EPI (CE, 35) spectra, diagnostic loss of 144 Da from protonated spirostane-type aglycones was attributed to the absence of an oxygen substitution at the F ring while a neutral loss of 160 Da from protonated aglycones could be ascribed to the presence of an OH group (often seen at C-23) in the F ring. The foregoing deduction processes repeated, neutral losses of 142 and 158 Da ions were readily inferred from corresponding protonated spirostanol aglycones for *A. asphodeloides* SSs. The neutral loss of 142 Da may be explained by the presence of an additional double bond in the F ring (often seen at  $\Delta 25(27)^-$ ), while a diagnostic loss of 158 Da could be interpreted by the presence of both a double bond (often seen at  $\Delta 25(27)^-$ ) and an OH group in the F ring (often seen at C-23).

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## IN THE PREVENTION AND TREATMENT OF CEREBROVASCULAR DISEASE IN CHINESE MEDICINE

Yuanyuan Gu, Donghua Yu, Yanqi Shang, Hui Liang, Zhongguang Zhou\*.

(Heilongjiang University of Traditional Chinese Medicine, Harbin 150040)

**Abstract:** In recent years, Chinese medicine in cardiovascular and cerebrovascular disease prevention and treatment more and more clinical attention. Studies have shown that traditional Chinese medicine and its chemical composition has the effect of improving cardiovascular and cerebrovascular diseases, including blood lipid, prevention and treatment of fatty liver, anti-thrombosis, anti-atherosclerosis, heart and brain protection, so as to achieve the prevention and treatment of cardiovascular and cerebrovascular diseases. In this paper, the progress of research on prevention and treatment of cardiovascular and cerebrovascular diseases is reviewed and discussed.

**Key words:** Cardiovascular diseases; Cerebrovascular disease; Chinese medicine; Summary

Cardiovascular diseases (CVD), is a disease involving the circulatory system, Chinese medicine in the prevention and treatment of cardiovascular and cerebrovascular disease occupies an important position.

### 1. Fall hematic fat

Hyperlipidemia is closely associated with CVD. Serum cholesterol levels were positively correlated with CVD. Danhong injection can significantly reduce the triglyceride, total cholesterol, low density lipoprotein and As index, the prevention and treatment of hyperlipidemia. The results showed that monascus extract could significantly reduce serum TC, TG and LDL-C levels in hyperlipidemia rats, The contents of total cholesterol (TC), triglyceride (TG) and low density lipoprotein cholesterol (LDL-C) in the serum of model rats were significantly decreased, and the levels of serum high density lipoprotein cholesterol (LDL-C) (HDL-C) level, with good blood lipid effect.

### 2. Antithrombotic

The results showed that *Eupolyphaga sinensis*, grubs and earthworms could significantly dissolve rabbit ear vein thrombosis, which significantly enhanced the fibrinolytic activity in rabbits. Proliferation is widely used in the Agkistrodon antithrombotic enzyme by activating the body fibrinolytic system, reduce plasma viscosity, speed up blood flow.

### 3. Anti-atherosclerosis

Atherosclerosis (AS) is the arterial wall of various inflammatory damage to the inflammatory proliferation reaction, overreaction is developed as a plaque. MacKinnon et al [1] found that citrus pectin inhibited galactoside-3 (gal-3), reduced ApoE and gal-3 double knockout mice late atherosclerotic plaque, and reduce atherosclerosis hardening.

### 4. Myocardial protection

Chenpi has anti-myocardial ischemia, myocardial infarction, myocardial injury and so on. Hesperidin, Hesperidin and its derivatives can be anti-myocardial apoptosis, inhibition of coronary angiogenesis and migration in myocardial injury, cardiac remodeling, myocardial ischemia, myocardial infarction have shown a good effect. Hesperidin reduces the apoptotic cell ratio, caspase-3 and caspase-9 activity, to achieve anti-cardiomyocyte apoptosis and cardiomyocyte injury protection [2].

### 5. Brain protection

Dried tangerine peel in the brain, such as brain protection, cerebral ischemia - reperfusion injury protection and improve motor awareness and other effects [3]. Experiments show that puerarin, salvia injection, Chuanxiong injection can increase the blood circulation of brain microcirculation, of which the most obvious effect of Salvia injection.

### 6. Anti-inflammatory

The main active ingredient of the anti-inflammatory effect of the tangerine peel is the flavonoids, including the dihydrogen flavonoids and the polyoxoflavones. Daidzein protects against focal cerebral ischemia-reperfusion injury by reducing the expression of inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$ .

### 7. Anti-oxidation and scavenging free radicals

The hydrolyzate residue of the tangerine peel has an antioxidant effect and is positively correlated with total phenolics, flavonoids.

### 8. Analysis and outlook

Traditional Chinese medicine is widely distributed, rich in resources, low side effects, great potential for development and utilization, and thus its anti-cardiovascular and cerebrovascular effects in-depth study has a very important theoretical and practical value. In addition to the prevention and of cerebrovascular disease in Chinese medicine, through the compatibility of the compound can also play a synergistic effect.