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Akebia saponin D, a saponin component from *Dipsacus asper Wall*, protects PC 12 cells against amyloid-β induced cytotoxicity

Yong-Qiang Zhou [a], Zhong-Lin Yang [a,*], Lei Xu [a], Ping Li [a], Yu-Zhu Hu [b]

[a] *Key Laboratory of Modern Chinese Medicines, China Pharmaceutical University, Ministry of Education, Nanjing 210009, PR China*
[b] *Key Laboratory of Drug Quality Control and Pharmacovigilance, Ministry of Education, Nanjing 210009, PR China*

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Abstract

According to Traditional Chinese Medicine, Alzheimer's disease (AD) is regarded as senile dementia, and the etiopathogenesis lies in kidney deficiency during aging. *Dipsacus asper Wall* (DAW), a well-known traditional Chinese medicine for enhancing kidney activity, may possess the therapeutic effects against AD. Our objectives were to investigate the protective effects of DAW against the amyloid-β peptide (Aβ)-induced cytotoxicity and explore its major active components. Injury of PC 12 cells mediated by $A\beta_{25-35}$ was adopted to assess the cytoprotective effects of DAW aqueous extract and various fractions. Salvianolic acid B, a polyphenol compound isolated from *Salvia miltiorrhiza*, was employed as a positive control agent due to its markedly protective effect against neurotoxicity of amyloid β. Five chemical fractions (i.e. alkaloids, essential oil, saponins, iridoid glucoside and polysaccharides) were prepared for activity test and analyzed by HPLC for active components identification. In addition, Akebia saponin D (the most important compound in DAW saponins) and hederagenin (the mother nucleus of akebia saponin D) were prepared for testing of their activity. DAW water extract, saponins fraction and akebia saponin D had the neuroprotective capacity to antagonize $A\beta_{25-35}$-induced cytotoxicity in PC 12 cells. In contrast, other fractions and hederagenin had no cytoprotective action. This research suggests that DAW may represent a potential treatment strategy for AD and akebia saponin D is one of its active components.

Keywords: *Dipsacus asper Wall*; Akebia Saponin D; Amyloid β; Alzheimer's disease

1. Introduction

Alzheimer's disease is characterized by the presence of two hallmark lesions. One is the formation of neurofibrillary tangles, consisting mainly of aggregated forms of the microtubule-associated protein, tau (Herskovits and Davies, 2006). A second lesion is the presence of senile or neuritic plaques composed of degenerating and dystrophic dendrites or axons in association with extracellular β-amyloid, a fibrillar aggregate of β-amyloid peptide (Andreasen and Blennow, 2002). A number of cell models with different mechanisms have been established to investigate neuroprotective effects of agents, such as PC 12 cells treated with Aβ (Misiti et al., 2005; Sultana et al., 2006), neuronal cells given V6421 APP (Niikura et al., 2000), the differentiated SH-SY5Y cell line with retinoic acid and brain-derived neurotrophic factor (Jamsa et al., 2004), and the transfected COS cell line with tau (Mailliot et al., 1998). Taking into account that amyloid β-peptide is the major protein component of the senile plaques, PC 12 cell injury due to $A\beta_{25-35}$ was adopted to test the neuroprotective effect of drugs.

Dipsacus asperoides, also known as *Dipsacus asper Wall* (DAW), has long been used as an anti-oxidatant, anti-inflammatory, analgesic, estrogen-like, anti-osteoporosis, tonic and anti-aging agent in China for the therapy of low back pain, traumatic hematoma, threaten abortion and bone fractures (Hung et al., 2006). According to the chemical structures

* Corresponding author at: Key Laboratory of Modern Chinese Medicines, China Pharmaceutical University, No. 24 Tongjia Lane, Nan-Jing City 210009, PR China. Tel./fax: +86 25 8337 1694.
E-mail address: yzl1950cpu@163.com (Z.-L. Yang).

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involved (Tomita and Mouri, 1996), the active constituents of *Dipsacus asper Wall* can be classified into 5 major categories – alkaloids, essential oil, saponins, iridoid glucoside and polysaccharides. The representative compound of the DAW saponins is akebia saponin D (Dipsacus saponin C) (Kauno et al., 1990) which possesses the anti-nociceptive properties mediated by multiple receptor systems, including GABA, glutamate, noradrenaline, serotonin and neuropeptides (Suh et al., 2000). Among the pharmacological actions of DAW, anti-oxidation, anti-aging and estrogen-like activity are related to the current therapeutic regimen of AD (Levine and Battista, 2004; Morinaga et al., 2007), implying that DAW may possess the potential therapeutic effect against AD.

To explore the potential neuroprotective effect of DAW, water extract and 5 fractions were tested for their activities to protect PC 12 cells from Aβ cytotoxicity. In addition, akebia saponin D and hederagenin were used to identify the active components of DAW. The findings suggest that saponins fraction and akebia saponin D, the major active constituents in DAW, could protect PC 12 cells from amyloid β-induced cytotoxicity by blocking Aβ-induced Ca^{2+}-intake, LDH release, cells viability decrease and cell apoptosis. Meanwhile, salvianolic acid B was selected as a positive control due to its marked protective effect against neurotoxicity mediated by amyloid β (Lin et al., 2006; Durairajan et al., 2008).

2. Materials and methods

2.1. Materials

Dipsacus asper Wall collected from Lianshan County, Sichuan Province, China was authenticated by Professor Ping Li, Department of Traditional Chinese Medicine, China Pharmaceutical University. Voucher specimens have been deposited in the laboratory of Modern Chinese Medicines, China Pharmaceutical University. Amyloid β-protein Fragment 25–35 ($A\beta_{25-35}$), Bisbenzimide H 33258 (HO33258), Propidium iodide (PI) and Fura 2-AM were purchased from Sigma–Aldrich (St. Louis, MO, USA). Stock $A\beta_{25-35}$ solution was prepared by dissolving the peptide in sterile tri-distilled water at a concentration of 75 μM and stored at −20 °C. RPMI 1640-based culture medium and new-born calf serum (NBCS) were bought from HyClone laboratories, Inc. (Utah, USA). Lactate dehydrogenase (LDH) kit was obtained from Jiancheng Bioengineering Institue (Nanjing, China). The standard substances of Akebia saponin D (Batch No. 111685-200401) and salvianolic acid B (Batch No. 111562-200504) were collected from Chinese National Institute for the control of pharmaceutical and biological products (Beijing, China). HPD300 and AB-8 macroporous adsorption resin were purchased from Bonchem Co. Ltd. (Cangzhou, China).

2.2. Preparation of sample

2.2.1. Water extract of DAW

Sliced (1–2 mm) crude DAW (10 g) was extracted with water (100 ml) for 1 h with 3 refluxes. After concentration under reduced pressure, the extracts were vacuum-dried, yielding 5.2672 g. As described previously (Tomita and Mouri, 1996; Zhang and Xue, 1991), there were many different chemical constituents in DAW extract, including polysaccharides, saponins, tannin and iridoid glucoside. Before administration, the extract was dissolved in water and filtered through a sterile 0.2 μm filter (Acrodisc® Syringe Filter, PALL Life Sciences, Germany). The test solutions of different concentrations were prepared by diluting the stock solution with sterile tri-distilled water and the same amount of RPMI 1640-based culture medium.

2.2.2. Various categories of ingredient fractions in DAW

2.2.2.1. Total alkaloids. Fifty grams of fine powdered DAW were extracted by refluxing in 95% ethanol (250 ml) for 10 h. The extract was diluted with distilled water and adjusted to pH 3.3 with 6 M HCl. After filtering, the filtrate was adjusted to a pH 8.8 with ammoniated water. Crude alkaloids fraction was obtained by extracting the alkaline solution with chloroform, which was suspended in 500 ml distilled water and adjusted to pH 3.3. The acid solution of crude alkaloids was washed with ether, and the water layer was adjusted to pH 10 and extracted with chloroform. After removal of the chloroform under reduced pressure, the alkaloid fraction (0.0152 g) was obtained, which contained mainly gentianine, venoterpine and cantleyine (Yang et al., 1993). Before adding to cultures, the alkaloid fraction was dissolved in 2% DMSO and filtered with a sterile 0.2 μm filter.

2.2.2.2. Total essential oil. Fifty grams powder of DAW was refluxed with ether (200 ml) twice for 5 h. The extracts were combined and the solvent removed. The precipitate was put into a Soxhlet extractor and refluxed with distilled water (200 ml) for 6 h. The collected oil was dissolved in 10 ml ether and the solvent evaporated to give 0.0463 g essential oil fraction, in which there were many phenols and a few terpenoids (Wu et al., 1994). Before adding to cultures, the essential oil fraction was dissolved in 2% DMSO and filtered through a sterile 0.2 μm filter.

2.2.2.3. Total saponins. Ten grams dried powder of DAW was extracted by refluxing in 70% ethanol (100 ml × 3) for 1 h. The ethanol extract was concentrated under reduced pressure at 60 °C. The residue was suspended in 100 ml distilled water and successively partitioned with petroleum ether and n-butyl alcohol saturated by water. After removing the solvent under reduced pressure, the precipitate was dissolved in 200 ml distilled water, and applied to an AB-8 macroporous resin column (R 10 mm × H 200 mm) equilibrated with water. The column was eluted successively with water (200 ml) and 50% ethanol (200 ml). The eluent of 50% ethanol was concentrated and vacuum-dried to yield the saponin fraction (1.2036 g). In the saponin fraction, there were many pentacyclic triterpenoid compounds which had the same mother nucleus, hederagenin (Zhang and Xue, 1991). Before administration, the saponin