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The total triterpenoid saponins of Xanthoceras sorbifolia improve learning and memory impairments through against oxidative stress and synaptic damage

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ABSTRACT

Background: *X. sorbifolia* is a widely cultivated ecological crop in the north of China which is used to produce biodiesel fuel. It also possesses special medicinal value and has attracted keen interests of researchers to explore its bioactivity.
Purpose: To extract the total triterpenoid saponins from the husk of *X. sorbifolia* (TSX) and investigate its effects on Alzheimer's disease (AD).
Study design: TSX was prepared *via* modern extraction techniques. Its effects on two AD animal models, as well as the preliminary mechanism were investigated comprehensively.
Methods: The behavioral experiments including Y maze test, Morris water maze test and passive avoidance test were performed to observe the learning and memory abilities of the animals. ELISA assays, transmission electron microscope observation and Western blotting were employed in mechanism study.
Results: TSX, the main composition of *X. sorbifolia*, accounted for 88.77% in the plant material. It could significantly increase the spontaneous alternation in Y maze test ($F(6, 65)=3.209$, $P<0.01$), prolong the swimming time in the fourth quadrant in probe test of Morris water maze test ($F(6, 71)=4.019$, $P<0.01$), and increase the escape latency in passive avoidance test ($F(6, 65)=3.684$, $P<0.01$) in AD model animals. The preliminary mechanism research revealed that TSX could significantly increase the contents of hippocampal Ach and ChAT, and enhance activity of ChAT in hippocampus of quinolinic acid injected rats ($F(5, 61)=3.915$, P 0.01; $F(5, 61)=3.623$, $P<0.01$, $F(5, 61)=4.344$, $P<0.01$, respectively). It could also increase the activities of T-AOC and T-SOD, and decrease the content of MDA in hippocampus of Aβ1-42 injected mice ($F(5, 30)=5.193$, $P<0.01$, $F(5, 30)=2.865$, $P<0.05$, $F(5, 30)=4.735$, $P<0.01$, respectively). Moreover, it significantly increased the expressions of SYP, PSD-95 and GAP-43 in hippocampus ($F(4, 27)=3.495$, $P<0.05$; $F(4, 27)=2.965$, $P<0.05$; $F(4, 27)=4.365$, $P<0.01$, respectively), and improved the synaptic ultra-structure damage in model rats.
Conclusion: TSX could significantly improve the impairments of learning and memory. The preliminary mechanism might associate with its protection effects against oxidative stress damage, cholinergic system deficiency and synaptic damage. TSX are perfectly suitable for AD patients as medicine or functional food, which would be a new candidate to treat AD.

Introduction

AD is a devastating neurodegenerative disorder with a relentless progression of memory dysfunction and cognition impairment (Ballard et al., 2011). It is the most common type of dementia. Pathologically, AD is associated with extracellular amyloid-beta (Aβ) deposits, intracellular neurofibrillary tangles (NFT) and massive loose of neurons, accompanying with oxidative stress, mito-

Abbreviations: TSX, Triterpenoid saponins from the husk of X. Sorbifolia; Aβ, Amyloid-beta; NFT, Neurofibrillary Tangles; Xan, Xanthoceraside; QA, Quinolinic acid; NBM, Nucleus basalis of Meynert; T-AOC, Total antioxidant capacity; T-SOD, Total superoxide dismutase; MDA, Malondialdehyde; Ach, Acetylcholine; ChAT, cholineacetyltransferase; AChE, acetylcholinesterase; SYP, Synapsin; GAP-43, Growth associated protein; PSD-95, Postsynaptic density protein.

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chondrial dysfunction and synaptic damage (Du et al., 2010; Tillement et al., 2011; Skaper, 2012).Oxidative stress has been found to be associated with AD and MCI (mild cognitive impairment). Aβ, especially A$\beta_{1\text{-}42}$, is primarily responsible for the oxidative damage taken place in AD, which is strongly posited by Aβ-induced oxidative stress hypothesis (Swomley and Butterfield, 2015). Meanwhile, the synaptic loss has been believed as a typical pathology in neurodegenerative disorders such as AD. A number of evidences suggested that phytochemicals could exhibit neuroprotective effects to neurodegenerative disease through antioxidant and anti-inflammatory activities, etc. (Orhan et al., 2015). Therefore, our group has been committed to studies on the natural-derived anti-AD agent.

Xanthoceras sorbifolia Bunge, a shrub belonging to the family of Sapindaceae, is mainly cultivated in the north of China including Inner Mongolia, Gansu, Liaoning, Hebei, and Shanxi provinces, etc. (Flora Republicae Popularis Sinicae, 1985). It is an important crop in agriculture to produce biodiesel fuel. Moreover, its seeds not only are served as nutritious nuts, but also utilized for extracting oil for edible purposes (Flora Republicae Popularis Sinicae, 1985). More importantly, as a medicinal plant, the barks of stem and branch *X. sorbifolia*, which are called "Wen Guan Mu" by locals, are decocted to treat arthritis in the Inner Mongolian. The crude extracts of its seeds are used clinically for the treatment of the enuresis of children in the form of capsule (Chinese Food and Drug Administration, Approval number: Z20040007), which indicated its potential possibility to pass through the blood brain barrier and affect brain diseases such as Alzheimer's disease (AD). Previous phytochemical studies on this plant showed the presences of saponins, flavonoids and sterols (Zhang and Bao, 2000). Our previous study had reported the neuroprotective effects of the husk extracts from *X. Sorbifolia* in AD animal models (Ji et al., 2007), however, the underlying mechanism involved and the possible bioactive ingredients were still unknown. In the meantime, the effects of xanthoceraside (Xan), a triterpenoid from the husks *X. Sorbifolia*, on learning and memory impairment in several AD animal models (Liu et al., 2013; Ji et al., 2014; Jin et al., 2014) have been confirmed and our recently published studies have also revealed the ameliorative effects of *X. Sorbifolia* extracts on dendritic spine deficiency (Li et al., 2016), which indicated its potential contribution on AD. Therefore, in present study, the total triterpenoid saponins of *X. sorbifolia* (TSX) from the husk of *X. sorbifolia* were prepared by solvent extraction and chromatographic isolation. Its effects on two AD animal models were then evaluated, and the mechanisms against oxidative stress and synaptic damage in AD animal models were further explored.

Materials and methods

Plants and chemicals

The husks of *X. sorbifolia* were collected in Oct, 2011, in Chifeng city, Inner Mongolia, and were identified by Pro. Jincai Lu, School of Traditional Chinese Material Medica, Shenyang Pharmaceutical University. A voucher specimen (NO.WGG-1110) was deposited in the Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang, China. 2, 3-Pyridinedicarboxylic acid (Quinolinic Acid, QA) and Amyloid β Protein Fragment 1-42 (Aβ1-42) were purchased from Sigma-Aldrich Co.(St. Louis, MO, USA). Memantine Hydrochloride Tablets was purchased from H. Lundbeck A/S (Denmark). The T-AOC, T-SOD and MDA assay kits were obtained from Jiancheng Reagent Co. (Nanjing, China). Ach, ChAT and AChE assay ELISA kits were purchased from Bio-Swamp Immunoassay R&D Center (Shanghai, China). Primary antibodies against SYP and GAP-43 were from Santa Cruz Biotechnology, and the primary antibody against PSD-95 was purchased from Abcam (Hong Kong) Ltd. All chemicals and solvents used in this study were analytical grade.

Preparation of the extracts

The crushed husks (7.0 kg) were extracted three times with 70% aqueous EtOH (140 l) until reflux maintaining at 90 °C for 3 h, 2 h and 1 h, respectively. After evaporation of the combined ethanol extracts *in vacuo*, the resultant aqueous residues were dissolved in water and then subjected to column chromatography on macroporous resin (HPD-100, 6 kg), eluted successively with H_2O, 70% and 95% aqueous EtOH. The evaporation of the 70% EtOH eluates *in vacuo* afforded the total saponins (TSX) as pale powder (360.0 g), which were dissolved in 0.5% CMC to prepare different concentrations of samples for the oral administrations of the experimental animals.

Content determination

Xan (3.2 mg), which was used as reference substance, was dissolved in methanol (100 ml) to prepare the standard solution (0.32 μg/ml), and 70% EtOH eluate (28 mg) was dissolved in 25 ml of methanol to obtain the test sample solution (1120 μg/ml).

The different volumes of standard solution (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, and 3.0 ml) were heated into dryness and then continued to be heated for an extra 30 min in water bath (80 °C) after adding 0.2 ml of 0.5% vanilling-glacial acetic acid and 1.0 ml of perchloric acid. After cooled into room temperature, 4.8 ml of glacial acetic acid was added and the absorbance values were then determined at 542 nm via the ultraviolet spectrophotometer. The standard curve of Xan was then plotted by the amount of Xan on the horizontal axis and the absorbance on the vertical. 0.1 ml of test sample solutions were accurately measured and then processed by the same procedures as described previously. The absorbance values (A) were recorded on ultraviolet spectrophotometer at 542 nm and the contents of TSX in the sample (Mx) were then calculated according to the standard curve equation, $C = Mx/(0.1\ ml * 1120\ \mu g/ml) * 100\%$.

Compounds identification

The total saponins (TSX) was applied on a semi-preparative liquid chromatograph (YMC 250 × 10 mml. D., 5 μm, SHIMADZU LC-20AR) with acetonitrile-water (9:91, v/v) as mobile phase (flow velocity: 6 ml/min; detection wavelength: 210 nm) and the characteristic compounds were then prepared.

Animals

Male Kunming mice (18–22 g body weight) and Sprague-Dawley rats (180–220 g body weight) were obtained from Changsheng Biotechnology Co., Ltd (Liaoning, China). The mice or rats were housed and kept in a regulated environment (23 ± 2 °C, 50 ± 5% humidity) with a 12/12-h light/dark cycle. Food and water were provided ad libitum. All the animal studies were performed in strict accordance with the guidelines established by the Institute for Experimental Animals of Shenyang Pharmaceutical University and the ethical approval number for the animal study was SYPU-IACUA-2015-06.312-201.

Groups

In A$\beta_{1\text{-}42}$ injection experiment, the mice were divided into 7 groups including sham-operated group, model group, TSX-treated groups (1.33, 4, or 12 mg/kg), Xan-treated group (0.32 mg/kg), and positive control group (memantine 2.6 mg/kg), respectively. In QA injection experiment, the rats were divided into 7 groups which