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Original article

Inhibitory effect of eleven herbal extracts on advanced glycation end-products formation and aldose reductase activity

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ABSTRACT

The formation of advanced glycation end-products (AGEs) and aldose reductase (AR) activity have been implicated in the development of diabetic complications. Our study sought to characterize the capacities of eleven herbal extracts against the formation of AGEs and the AR activity. An ultrahigh performance liquid chromatography and tandem mass spectrometry (UPLC–MS/MS) method was used for the detection of AR activity and the screening of AR inhibitors in this research. The amount of sorbitol from each analyte was directly detected using the multiple reaction monitoring mode and the sorbitol level could be reduced *via* the addition of an inhibitor. Moreover, the BSA/glucose (fructose) system was applied to investigate their inhibitory activities of AGEs formation in glycation model reactions. Compared with other screened herbs used in our study, *Flos Sophorae Immaturus* and *Radix Scutellariae* seemed to be more effective on inhibiting the formation of AGEs and AR activity. The inhibiting capacities of herbal extracts against AR activity and AGEs formation may be correlated with the bioactive components of the herbal extracts. The differences were correlated with the amount of polyphenol and flavonoid components. In the study, we have investigated the potential anti-hyperglycemic bioactivity of eleven herbal extracts *in vitro*, which could provide a reference for further *in vivo* research in the prevention and treatment of diabetic complications.

1. Introduction

Type 2 diabetes mellitus is a metabolic disorder and chronic disease that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency. Diabetic patients normally suffer from complications, such as nephropathy, neuropathy, cataract formation, retinopathy, atherosclerosis, cardiovascular disease, and cerebrovascular pathologies [1–3]. Long-term secondary complications are the main cause of morbidity and mortality of diabetic patients [4]. Thus preventing, or delaying the onset and progression of diabetic complications has become one of the key issues in medical research [5,6]. Several mechanisms have been proposed for these complications, some evidence suggests that the polyol pathway, formation of advanced glycation end-products (AGEs), and oxidative stress are associated with the etiology of these chronic diseases [7,8]. Aldose reductase (AR, EC 1.1.1.21), which is the key rate-limiting enzyme in the polyol pathway, catalyzes the reduction of glucose to sorbitol in the presence of NADPH [9]. The development of diabetic complications could be controlled by retarding AR activity and also by inhibiting the formation of AGEs [7].

At present, available drugs for type 2 diabetes mellitus show a number of limitations, such as side effects and high rates of secondary failure. The diabetes control and complications trial had demonstrated that even an optimal control of blood glucose could not prevent complications, suggesting that alternative treatment strategies are needed [10]. As a complementary/alternative approach, medicinal herbs with anti-hyperglycemic activities are increasingly administered to diabetic patients by healthcare professionals. Furthermore, traditional Chinese medicines (TCMs), with a long history and unique theory system and a variety of herb remedies, have been attracting more and more attention for their complementary therapeutic effects to western medicines [11–13].

The aim of this investigation was to evaluate the activities of eleven natural product extracts to inhibit AR and AGEs formation. An ultrahigh performance liquid chromatography and tandem

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mass spectrometry (UPLC–MS/MS) method was used to determine the AR inhibitory activities of herbal extracts. Additionally, the fluorometric method was applied to evaluate the inhibitory activities of herbal extracts on AGEs formation in glycation model reactions. The results obtained in this study could provide a reference for clinical studies in managing diabetic complications.

2. Experimental

2.1. Reagents and chemicals

Sorbitol, xylitol, ammonium acetate (NH_4Ac) and DL-glyceraldehyde were purchased from Sigma Chem. Co., (St. Louis, MO, USA). β-NADPH was obtained from F. Hoffmann-La Roche Ltd. (Basel, Switzerland). β-Mercaptoethanol, NaN_3 and bovine serum albumin (BSA) were purchased from Dingguo Biotec. Co., (Beijing, China). Amino guanidine (hemi-sulfate salt) was supplied from Aladdin (Shanghai, China). Epalrestat was obtained from the Yangtze River Pharmaceutical Group (Taizhou, China). Methanol, HPLC grade, was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Deionized water was prepared using a Milli-Q water purification apparatus (Bedford, MA, USA). All other chemicals and reagents were of analytical grade.

2.2. Preparation of herbal extracts

The natural products were purchased from Tongrentang Pharmacy (Changchun, China) and authenticated by Professor Shumin Wang (Changchun University of Traditional Chinese Medicine, China). The plant samples were ground into uniform powder using a high-speed grinder. The powder, after passing a 100 mesh sieve, was weighed accurately, and then a 60% ethanol extraction was carried out at 30 °C for 40 min using ultrasound-assisted extraction process. These mixtures were centrifuged at 4000 rpm for 10 min and the supernatants were filtered through a 0.22 μm membrane filter. Afterward, the filtrates were quantified with the same solvent and the obtained stock solutions of 200 mg/mL were stored at 4 °C.

2.3. Preparation of AR

The preparation of partially purified AR was performed as previously described [14,15] but with minor modifications. The crude AR solution was obtained from bovine lenses by using ammonium sulphate fractionated precipitation (40%–75%) and ultra-filtration method. The crude enzyme was then verified by using SDS-polyacrylamide gel electrophoresis. Afterwards, the activity of AR was evaluated based on DL-glyceraldehyde as a substrate, according to the previous study [15]. The change in the absorbance at 340 nm due to NADPH oxidation was followed in a Tecan GENios Microplate Reader (Männedorf, Switzerland). Assays were carried out at 25 °C with an appropriated blank subtracted from each reaction to correct for nonspecific oxidation of NADPH.

2.4. AR inhibitory activity

Since DL-glyceraldehyde was used as the surrogate substrate of glucose and the traditional spectrometric method for measuring the change in absorbance at 340 nm of NADPH was an indirect manner for the screening of AR inhibitors, the likelihood of false-positive results increased because NADPH was extremely unstable in acidic medium. In view of these facts, an UPLC–MS/MS method was developed to evaluate the activities of the compounds in this experiment. Glucose, which was reduced to sorbitol in the polyol pathway, was chosen as the substrate of the enzymatic reaction *in vitro*. Then sorbitol was directly detected using multiple reaction monitoring (MRM) mode and the AR inhibitory activities of natural products was evaluated. Two precursor/product ion transitions were elected in creating the MS/MS method to collect sufficient data for the integrative research. The ion transition with the highest sensitivity was chosen for the quantitative analysis; while a second ion transition was selected as the qualitative ion pair for the confirmatory analysis. The ion transitions with appropriate instrumental parameters for the MRM detection are listed in Table 1.

Table 1
Ion transitions and instrumental parameters of sorbitol and xylitol for MRM mode.

Compounds	Precursor ion > product ion (*m/z*)	Dwell time (s)	Cone voltage (V)	Collision energy (eV)
Sorbitol	183.13 > 68.94*	0.078	16	10
	183.13 > 147.02	0.078	16	8
Xylitol	153.07 > 56.95*	0.078	16	10
	153.07 > 117.04	0.078	16	9

Ion transitions marked with asterisk (*) were used for the quantitative analysis, while the other ones were qualitative ion transitions for the confirmatory analysis; *m/z* was the mass-to-charge ratio.

The UPLC–MS/MS analyses were performed using ACQUITY™ UPLC system combined with a Xevo™ TQ mass spectrometer (Waters, Milford, MA, USA). A Waters UPLC® BEH Shield RP 18 column (100 mm × 2.1 mm, i.d., 1.7 μm, Milford, MA, USA) was applied in the chromatographic separation. The binary mobile phase consisted of methanol-water (20:80, v/v), the elution was performed at a flow rate of 0.2 mL/min, with 3 μL of sample was injected. The column temperature was set at 35 °C, while the temperature of sample manager was maintained at 4 °C. The column outlet of the UPLC system was connected *via* capillary to the electrospray ionization (ESI) source of the mass spectrometer. The spray voltage was set at 3.0 kV in positive ion mode. The flow rates of cone gas and desolvation gas were set to 60 and 800 L/h, respectively. The ion source temperature and desolvation temperature were maintained at 150 and 350 °C, respectively. The data were acquired *via* MassLynx4.1 software (Waters), and the MassLynx4.1 with TargetLynx was applied to data processing.

2.5. AGEs formation inhibitory activity

The AGEs formation inhibitory effect of herbal extracts was performed as previously described [16], but with some modifications. Because 0.02% sodium azide could prevent the growth of microorganisms and not interact with protein, BSA was dissolved in the phosphate buffer with 0.02% sodium azide to prevent degradation. Fructose and glucose were prepared together in 0.02% sodium azide, while the herbal extracts were freshly prepared. Briefly, the reaction mixture contained 50 mmol/L phosphate buffer (pH 7.4), 10 mg/mL BSA, 200 mmol/L glucose, 200 mmol/L fructose, and 10 mg/mL extracts (diluted with phosphate buffer). The reaction solution (1 mL) was incubated at 37 °C for 14 days, while the negative control was kept at 4 °C. Amino guanidine (2 mg/mL) was used as a positive control in the AGEs inhibition assay. All incubations were done in quadruplicate. The fluorescence intensity of the sample was determined with excitation and emission wavelengths at 370 and 440 nm, respectively, on a Tecan GENios Microplate Reader (Männedorf, Switzerland). The inhibition percentage (%) was calculated according to the following formula:

$$\text{Inhibition percentage } (\%) = \left(1 - \frac{F_1 - F_0}{F_2 - F_0}\right) \times 100\% \quad (1)$$