**Short Communication**

**In vitro antiglycation activity of Eremurus persicus (Jaub. Et Sp.) Boiss**

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Accepted 7 July, 2011

Diabetes mellitus is a common endocrine disorder characterized by hyperglycemia and long-term complications affecting the eyes, nerves, blood vessels, skin and kidneys. Increased glycation of proteins and accumulation of advanced glycation endproducts (AGEPs) have been implicated in the pathogenesis of diabetic complications. Glycation and AGEP formation are also accompanied by the formation of free radicals via autoxidation of glucose and glycated proteins. Since this plant *Eremurus persicus* is used as an antidiabetic agent in Iranian traditional medicine, we were prompted to evaluate the antiglycation activity of this species. Here, we reported the isolation of a known compound, 5,6,7-trimethoxy-coumarin for the first time for the antiglycation properties of this plant.

**Key words:** Antiglycation, *Eremurus persicus*, 5,6,7-trimethoxy-coumarin.

**INTRODUCTION**

Diabetes mellitus is an endocrine disorder characterized by chronic hyperglycemia which results to a deficiency or resistance to insulin. Diabetes affects 1 to 2% of the population, and there are about 100 million people worldwide. This figure is expected to double over the next 10 to 15 years. Individuals affected by diabetes are prone to complications such as retinopathy, cataract, neuropathy, atherosclerosis, nephropathy, embryopathy and wounds (Muhammed and Nessar, 2006).

Non-enzymatic glycosylation (glycation) between reducing sugar and free amino group of proteins, also known as Millard reaction, leads to the formation of glycated protein termed Amadori product. Further rearrangement, oxidation and reduction of the Amadori products result in the formation of several advanced glycation endproducts (AGEs) such as pentosidine, carboxymethyllysine, crossline and pyralline. Some of these products can react with a nearby free amino group and form crosslinking between proteins (Ulrich and Cerami, 2001). The crosslinked protein, such as crosslinked collagen are postulated to confer pathological conditions found in patients with diabetes and aging, such as arterial stiffness and decreased myocardial compliance, resulting from the loss of collagen elasticity (Singh et al., 2001; Aronson, 2003). Thus, agents that inhibit the formation of AGEs are purported to have therapeutic potentials in patients with diabetes and age-related diseases.

The oxidation process is believed to play an important role in AGEs formation. Further oxidation of Amadori product leads to the formation of intermediate carbonyl compounds that can react with the nearby lysine or arginine residues to form protein crosslink and AGEs. The reactive carbonyl compounds may also be generated from the metal ion-catalyzed autoxidation of glucose (Rahbar and Figarola, 2003; Voziyan et al., 2003). Therefore, agents with antioxidative or metal-chelating property may retard the process of AGEs formation by preventing further oxidation of Amadori product and metal-catalyzed glucose oxidation (Jedsadayanmata, 2005).

*Eremurus persicus* (Liliaceae) locally called "Serish", is widely distributed in south, east and west of Iran. It is traditionally used for the treatment of liver and stomach disorders, constipation and diabetes. Since this plant is traditionally used in patients with diabetes, we were prompted to investigate the antiglycation activity of *E.*
persicus. Here, we reported 5,6,7-trimethoxy-coumarin (Figure 1) as the active compound for the antiglycation property of this plant.

MATERIALS AND METHODS

Chemicals and general experimental procedure

BSA (bovine serum albumin) was purchased from the Research Organics Cleveland, USA, while other chemicals: glucose anhydrous, trichloroacetic acid (TCA), sodium azide (NaN₃), dimethyl sulfoxide (DMSO), sodium dihydrogen phosphate (Na₂HPO₄), potassium chloride (KCl), potassium dihydrogen phosphate (KH₂PO₄), sodium hydroxide (NaOH) were purchased from Sigma Aldrich. Sodium phosphate buffer (pH 7.4) was prepared by mixing Na₂HPO₄ and NaH₂PO₄ (67 mM) containing sodium azide (3 mM), potassium chloride (8.1 mM) + KCl (2.68 mM) + KH₂PO₄ (1.47 mM) at pH 10 and was adjusted with NaOH (0.25 mM), while BSA (10 mg/ml) and glucose anhydrous (50 mg/ml) solutions were prepared in sodium phosphate buffer.

Sodium phosphate buffer (pH 7.4) was prepared by mixing Na₂HPO₄ and NaH₂PO₄ (67 mM) containing sodium azide (3 mM), phosphate buffer saline (PBS) (PH 10) was prepared by mixing NaCl (157 mM) + Na₂HPO₄ (8.1 mM) + KCl (2.68 mM) + KH₂PO₄ (1.47 mM) at pH 10 and was adjusted with NaOH (0.25 mM), while BSA (10 mg/ml) and glucose anhydrous (50 mg/ml) solutions were prepared in sodium phosphate buffer.

The ¹H-NMR and ¹³C-NMR were recorded on a Bruker AMX 300 NMR instruments using the UNIX data solvent. ¹H-¹³C HMBC and HMQC were recorded at 500 MHz (proton) and 125 MHz (carbon), respectively.

The plant, E. persicus (Liliaceae) was collected from Golpayegan, Isfahan province, Iran, in May 2010, and identified by Dr. Gh. R. Amin at the Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

A voucher specimen (No. 197) has been deposited in the herbarium of the Department of Pharmacognosy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran.

Extraction

The air dried flowering aerial parts of E. persicus (2 kg) was exhaustively extracted by maceration with methanol (3 x 4 L). The extract was evaporated to yield the residue (240 g) which was partitioned between water (18 g), petroleum ether (52 g), CHCl₃ (95 g) and EtOAc (70 g). The CHCl₃ fraction had significant antiglycation, so we subjected it to silica gel chromatography using petroleum ether with a gradient of CHCl₃ up to 100% and followed by methanol. Six fractions were collected. The fraction no. 3 had antiglycation activity, and contained a major spot (Rf = 0.37) on TLC using solvent system petroleum ether-acetone, 50:50. This spot showed blue-green fluorescence under UV 365 nm light with 5% KOH. We isolated this compound by preparative TLC method and solvent system petroleum ether-acetone (50:50). It was crystallized from methanol (48 mg) and identified as 5,6,7-trimethoxy coumarin (Tables 1 and 2). It exhibited a very good antiglycation activity.

In vitro glycation assay

60 μl of sample was prepared by dissolving in DMSO and the sample mixture (20 μl BSA + 20 μl of glucose anhydrous + 20 μl test sample). The glycated control contained 20 μl BSA + 20 μl glucose + 20 μl sodium phosphate buffer, while blank control contained 20 μl BSA and 40 μl sodium phosphate buffer. After incubation in 96 well plates at 37 °C for 7 days, samples were taken out and cooled at room temperature. After incubation, 60 μl 100% TCA was added to each well and centrifuged (15000 rpm) for 4 min at 4°C. After agitation and centrifugation at 14000 rpm for 4 min, the supernatant containing glucose, inhibitor and interfering substance was removed and pellet contained AGE-BSA which was dissolved in PBS. Assessment of fluorescence spectrum (ex. 370 nm), and change in fluorescence intensity (ex. 370 to 440 nm) based on percentage inhibition was calculated using the following formula:

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\text{Inhibition} = 100 - \frac{OD \text{ (test)}}{OD \text{ (blank)}} \times 100
\]

RESULTS AND DISCUSSION

The active compound isolated had pale yellow crystals and was identified as 5,6,7-trimethoxy-coumarin. Here, we reported the 1H-NMR and 13C-NMR data of this compound. 5,6,7-trimethoxy-coumarin exhibited a good antiglycation activity. It was observed that this compound at 3 mM concentration showed 75% inhibition, while the standard inhibitor, rutin showed 83% inhibition.

Based on the results of this study and since antioxidant
agents may retard the process of AGEs formation by preventing further oxidation of Amadori product and metal-catalyzed glucose oxidation, further in vivo and in vitro tests to investigate the antioxidant properties for 5,6,7-trimethoxy-coumarin are recommended. Also, in vivo confirmatory tests to evaluate the anti hyperglycemic activity of this compound are suggested.

ACKNOWLEDGEMENTS

Financial support from the Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran is gratefully acknowledged. We would like to thank Mr. M. Asoudeh for providing the plant material.

REFERENCES


