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Short Communication

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

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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 autooxidation 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.*

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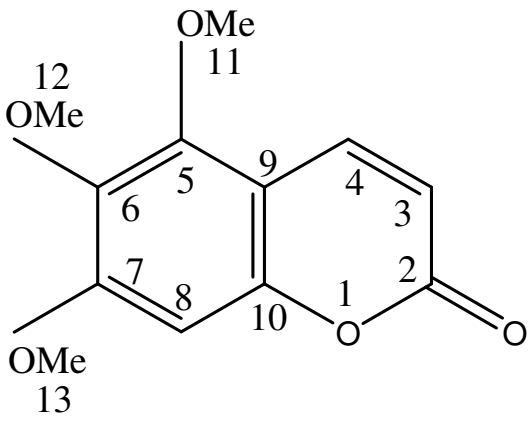

Figure 1. Structure of 5,6,7-trimethoxy coumarin.

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 (NaN_3), dimethyl sulfoxide (DMSO), sodium dihydrogen phosphate (Na_2HPO_4), potassium chloride (KCl), potassium dihydrogen phosphate (KH2PO4) and sodium hydroxide (NaOH) were purchased from Sigma Aldrich.

Sodium phosphate buffer (pH 7.4) was prepared by mixing Na_2HPO_4 and NaH_2PO_4 (67 mM) containing sodium azide (3 mM), phosphate buffer saline (PBS) (PH 10) was prepared by mixing NaCl (137 mM) + Na_2HPO_4 (8.1 mM) + KCl (2.68 mM) + KH_2PO_4 (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 ^{1}H-NMR and ^{13}C-NMR were recorded on a Bruker AMX 300 NMR instruments using the UNIX data solvent. ^{1}H-^{13}C 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), $CHCl_3$ (95 g) and EtOAc (70 g). The $CHCl_3$ fraction had significant antiglycation, so we subjected it to silica gel chromatography using petroleum ether with a gradient of $CHCl_3$ up to 100% and followed by methanol. Six fractions were collected. The fraction no. 3 had antiglycation activity, and contained a major spot (R_f = 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.

Table 1. ^{1}H-NMR (CDCl3) data of 5,6,7-trimethoxy-coumarin.

Position	δ ^{1}H-NMR
C (2)	-
H–C (3)	7.88 (1H, *d,* J=9.6)
H–C (4)	6.18 (1H, *d,* J=9.6)
C (5)	-
C (6)	-
C (7)	-
C (8)	6.57 (1H, *s*)
C (9)	-
C (10)	-
MeO (11)	3.99 (3H, *s*)
MeO (12)	3.82 (3H, *s*)
MeO (13)	3.88 (3H, *s*)

δ is expressed in ppm and *J* is in Hz.

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 AGEs were monitored by using spectrofluorimeter RF-1500 (Shimadzu, Japan). Rutin was used as the standard inhibitor. The comparison of fluorescence intensity at 370 nm excitation and emission at 440 nm was obtained by using spectrofluorimeter. Percentage inhibition was calculated using the following formula:

Inhibition (%) = 100 – [OD (test) / OD (blank)] x 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.

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REFERENCES

Aronson D (2003). Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffness of aging and diabetes. J. Hypertention, 21: 3-12.

Jedsadayanmata A (2005). In vitro antiglycation activity of arbutin, Pharmacol. Res. 13(2): 35-41.

Muhammad Saeed A, Nessar A (2006). Antiglycation properties of aged garlic extract: role in prevention of diabetic complications. J. Nutr., 3: p. 3.

Rahbar S, Figarola JL (2003). Novel inhibitors of advanced glycation endproducts. Arch. Biochem. Biophys. 419: 63-79.

Singh R, Barden A, Mori T, Beilin L (2001). Advanced glycation endproducts: A review, Diabetologia, 44: 129-146.

Ulrich P, Cerami A (2001). Protein glycation, diabetes and aging. Recent Progress Hormone Res. 56: 1-21.

Voziyan PA, Khalifah RG, Thibaudeau C, Yildiz A, Jacob J, Serianni AS, Hudson BG (2003). Modification of proteins *in vitro* by physiological levels of glucose, J. Biol. Chem. 278: 46616-46624.