

## Pancreatic B-Cell Protective Effect of Rutin and Apigenin Isolated from *Teucrium Polium*

Mohammad Ali Esmaeili <sup>1\*</sup> and Heibatollah Sadeghi<sup>2</sup>

<sup>1</sup> Department of Biology, Medicinal Plants and Drug Research Institute, Shahid Beheshti University (G.C), Tehran, Iran

<sup>2</sup> Department of Biochemistry, Faculty of Medicine, Herbal Medicine Research Center, Yasuj University of Medical Science, Yasuj, Iran

### Summary

*Teucrium polium* (Lamiaceae) is used both as a food to prevent health and as a drug to treat hyperglycemia in Iran. In this study, we isolated the high free radical scavenging and anti-glycation compounds from *T. polium* and protective effect of both flavonoid compounds was evaluated on rat isolated islets in streptozotocin induced oxidative stress. Based on our results, rutin (quercetin-3-rutinoside) and apigenin isolated from *T. polium* exhibited high antioxidant activities compared to the other isolated compounds from *T. polium*. Regarding the link between glycation and oxidation, we proposed that the rutin and apigenin might possess significant *in vitro* antiglycation activities as well. These two flavonoids also showed strong inhibitory effects on the production of advanced glycation end products (AGEs) from bovine serum albumin in the presence of glucose in term of PCO formation and thiol oxidation. In addition, rutin as well as apigenin able to prevent oxidative stress condition induced by streptozotocin and increase insulin release in rat islets. It may be concluded that *T. polium* possess potent antiglycation and insulinotropic compounds, which can be of great value in the preventive glycation associated complications in diabetes.

**Keywords:** Advanced glycation end products (AGEs); Apigenin; Free radical scavenging; Insulinotropic; Rutin, *Teucrium polium*

\*Corresponding author:

Mohammad Ali Esmaeili, Department of Biology, Medicinal Plants and Drug Research Institute, Shahid Beheshti University, G.C. Tehran, Iran, Tel: +98-21-29903025, Fax: +98-21-29903023, E-mail: m\_esmaeili@sbu.ac.ir

### Introduction

Reactive oxygen species (ROS) including hydrogen peroxide, singlet oxygen, superoxide radicals, and hydroxyl radicals derived from the metabolism of oxygen in aerobic systems are produced in an imbalance conditions between generation and elimination of ROS (1). ROS provide a wide potential condition for causing damages of cellular components such as DNA, protein and lipid. Accumulation of oxidative damages in intracellular macromolecules is an essential factor in pathogenesis of many diseases such as respiratory distress syndrome, ischemia/reperfusion injury, renal failure, rheumatoid arthritis, local or systemic inflammatory disorders, diabetes, atherosclerosis, cancer and neurodegenerative diseases (2, 3). Several mechanism including autoxidation of glucose, protein glycation and advanced glycation end products (AGEs) formation may to be involved in hyperglycemia mediated oxidative stress (4). Non enzymatic protein glycation initiated by the reversible formation of a Schiff base which leads to formation of heterogeneous and toxic advanced glycation end products (AGEs) (5). The accumulation of the reaction products of protein glycation in living organisms leads to structural and functional modifications of tissue proteins (6). Regarding the significance of oxidative stress to diabetic pathology, numerous natural and synthetic ROS scavengers and antioxidants have been evaluated to inhibit the process of protein modifications against the attack of ROS and/or to suppress the resultant damages (7). Strong evidences from animal studies show that the glycation inhibitor, aminoguanidine, attenuates the development of a range of diabetic vascular complications. However, some toxicity problems have been encountered in clinical trials with aminoguanidine (8) Therefore, there has been an increasing interest in the use of plant compounds as antidiabetic compounds. In an attempt to find new antioxidants agents from plant materials as therapeutic agents, we are interested in the development of antioxidants that have potent anti-glycation activity. *Teucrium polium* was used as hypoglycemic plant in folk medicine in Iran. The biological activities of *T. polium* including anti-inflammatory, anti-nociceptive, anti-bacterial, anti-hypertensive, hypolipidemic, anti-rheumatoid, and hypoglycemic widely reported (9-11). Recently, Esmaeili and Yazdanparast (12), showed the high insulinotropic and anti-hyperglycemic activity of *T. polium* crude extract using both animal and/or isolated rat pancreatic islets. There are also some data in literature about antioxidant activity of various crude extract of *T. polium* (13, 14). Based on these data, the present study was hence, undertaken to isolate and structural elucidate the high antioxidant flavonoid compound(s) from *T. polium* on glycation and oxidation dependent damages to albumin induced by glucose. In addition, the protective effect of these major compounds on pancreatic islets in streptozotocin induced oxidative stress was evaluated.

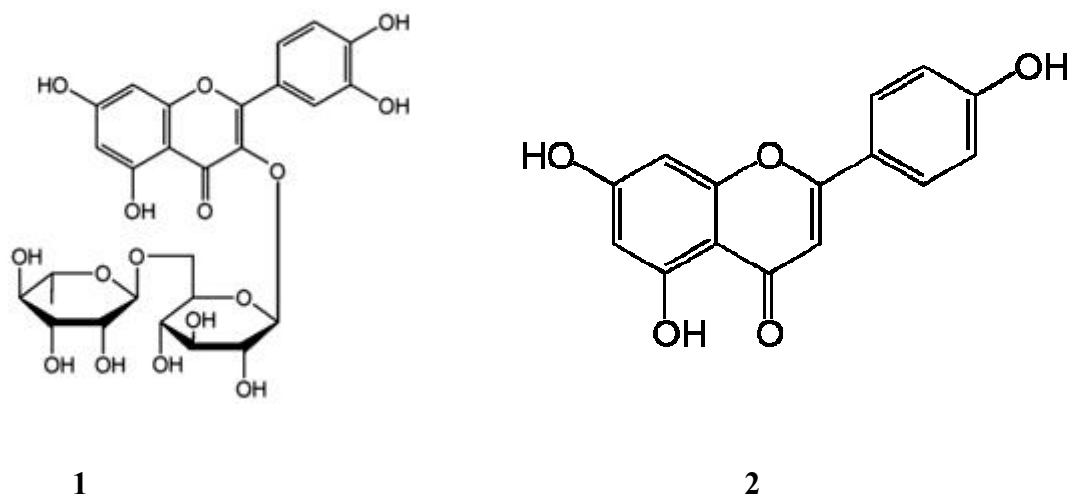
### Material and methods

**Plant material.** Aerial parts of *Teucrium polium* L. was collected from Sepidan area of Fars province, Iran during May 2004 and identified by Dr A. Sonboli, Medicinal Plants and Drug Research Institute, Shahid Beheshti University. A voucher herbarium specimen (No. 570) was deposited at the Herbarium of faculty of pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran. The plant aerial parts were air-dried and grounded in to powder. The powder plant material (250 g) was extracted with methanol three times at room temperature. The plant extract was concentrated under reduced pressure on a rotary evaporator and fractionated by column chromatography on silica gel 60 (70–230 mesh, Merck; Darmstadt, Germany), using petroleum ether and petroleum ether: chloroform (9:1, 8:2, 5:5, v/v), chloroform, and chloroform: ethyl-acetate (9:1, 8:2, 5:5, v/v), ethyl-acetate and ethyl-acetate: methanol (9:1, 8:2, 5:5, v/v) as eluents to afford nine fractions (F1-9). All the fractions obtained were used in antioxidant measurements. The Fraction 1 which showed the most antioxidant activity was purified by TLC on silica gel 230–400 mesh and eluted with ethyl-acetate: water: formic acid: glacial acetic acid (100:26:11:11, v/v/v/v) to obtain four fractions (1–3, 4–8, 9–13, and 14–18). The combined fractions of 14–18 and 9–13 (the most antioxidant activity) were purified on Sephadex LH-20 with methanol to obtain four compounds. Two compounds (Compound 1 and 2) which possess high antioxidant activity were performed for further studies. By comparing the obtained spectral data (IR, NMR and UV) with literature, these two purified compounds were identified as rutin (quercetin-3-rutinoside) and apigenin (Fig. 1).

Rutin (Quercetin-3-rutinoside) (1): yellow-green amorphous powder. m.p. 208-212 °C; UV-vis (nm):  $\lambda_{\max}$  = 257, 351 nm. IR (KBr,  $\text{cm}^{-1}$ ): 3600-3200 (OH), 2920-2700 (CH), 1648 (C=O), 1608-1550 (aromatic rings), 1435, 1321, 1294, 1265. <sup>1</sup>H NMR (300.1 MHz, CDCl<sub>3</sub>): 6.21(1H, d, J=2, C6-H), 6.40 (1H, d, J=2, C8-H), 7.55 (1H, d, J=2.1, C2'-H), 6.86 (1H, d, J=9, C5'-H), 7.56 (1H, dd, J=9, 2.1, C6'-H), 9.71 (1H, s, C4'-OH), 9.21 (1H, s, C3'-OH), 12.62 (1H, s, C5-OH), 10.86 (1H, s, C7-OH), 5.35 (1H, d, J=7.4, H1-G), 5.12 (1H, d, J=1.9, H1-R), 1.00 (3H, d, J=6.1, CH3-R); <sup>13</sup>C NMR (75.47 MHz, CDCl<sub>3</sub>) 157.3 (C-2), 134.1 (C-3), 178.2 (C-4), 157.5 (C-5), 99.5 (C-6), 164.9 (C-7), 94.5 (C-8), 162.1 (C-9), 104.8 (C-10), 122.5 (C-1'), 116.1 (C-2'), 145.6 (C-3'), 149.3 (C-4'), 117.1 (C-5'), 122.0 (C-6'), 101.6 (C1-G), 74.9 (C2-G), 77.3 (C3-G), 72.7 (C4-G), 76.7 (C5-G), 67.9 (C6-G), 102.2 (C1-R), 70.8 (C2-R), 71.2 (C3-R), 71.4 (C4-R), 69.1 (C5-rhamnose), 18.6 (C6-glucose)

Apigenin (2): yellow amorphous powder. m.p. 345-349 °C; UV-vis (nm):  $\lambda_{\max}$  = 265, 297 nm. IR (KBr,  $\text{cm}^{-1}$ ): 3297-3095 (OH), 2925-2617 (CH), 1655 (C=O), 1608-1520 (aromatic rings), 1445, 1354, 1298, 1267. <sup>1</sup>H NMR (300.1 MHz, CDCl<sub>3</sub>): 6.81(1H, s, C2-H), 6.21(1H, d, J=2.1, C6-H), 6.50 (1H, d, J=2.1, C8-H), 7.95 (2H, d, J=8.8, C2', 6'-H), 6.94 (2H, d, J=8.8, C3', 5'-H), 10.39 (1H, s, C4'-OH), 12.94 (1H, s, C5-OH), 10.80 (1H, s, C7-OH); <sup>13</sup>C NMR (75.47 MHz, CDCl<sub>3</sub>) 165.0 (C-2), 103.7 (C-3), 182.6 (C-4), 158.2 (C-5), 99.7 (C-6), 164.6 (C-7), 94.8 (C-8), 162.3 (C-9), 104.6 (C-10), 122.0 (C-1'), 129.4 (C-2'), 116.8 (C-3'), 162.0 (C-4'), 116.8 (C5'), 129.4 (C-6').

Fig.1. Chemical Structures of Compounds 1 (rutin) and 2 (apigenin).



**DPPH free radicals scavenging activity assay.** Radical scavenging capacity was determined according to the technique reported by Blois (15). An aliquot of 1.5 ml of 0.25 mM DPPH solution in ethanol and 1.5 ml of isolated compounds at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm with a Varian spectrophotometer. The DPPH radicals scavenging activity was calculated according to the following equation:

$$\text{Scavenging activity} = [(A_0 - A_1 / A_0) \times 100]$$

Where  $A_0$  is the absorbance of the control (blank, without extract) and  $A_1$  is the absorbance in the presence of the extract or standard sample.

**Trolox equivalent antioxidant capacity (TEAC) assay.** A solution of  $\text{ABTS}^+$  was prepared in 100 mM phosphate buffer saline (pH 7.4, 0.15 M sodium chloride) (PBS) (7 mM) and oxidized using potassium persulfate (2.45 mM) for at least 12 h in dark. The  $\text{ABTS}^+$  solution was diluted to an absorbance of  $0.7 \pm 0.05$  at 734 nm with PBS, pH 7.4. The  $\text{ABTS}^+$  solution was used within 2 h because the absorbance of the radical itself depletes with time. For measuring antioxidant capacity, 10  $\mu\text{l}$  of the sample was mixed with 990  $\mu\text{l}$  of  $\text{ABTS}^+$  solution. Absorbance of the above mixture was measured at 734 nm after 6 min. The decrease in absorption at 734 nm was used for calculating TEAC values. A standard curve was prepared by measuring the reduction in absorbance of  $\text{ABTS}^+$  solution at different concentrations of Trolox (16).

**Evaluation of various stages of protein glycation.** BSA (50 mg/ml) was incubated with glucose (50 mM) and sodium azide (0.02%) with or without CuSO<sub>4</sub> (100 μM) in 0.2 M phosphate buffer (pH 7.4). The rutin and/or apigenin were added to the reaction mixture, and the reaction mixture was incubated for 21 d at 37 °C (17). AG (10 mM) was used as a positive control. Fluorescence intensity of all samples was measured at the excitation and emission maxima of 370 and 440 nm, respectively.

**Evaluation the protein carbonyl and thiol groups level.** For determination of protein carbonyl content, 1 ml of DNPH (10 mM) was added to samples and after incubation for 30 min at room temperature, 1 ml of cold TCA (10 %, w/v) was added to the samples and centrifuged at 3000 ×g for 10 min. The protein pellet was washed three times with 2 ml of ethanol/ethyl acetate (1:1, v/v) and dissolved in 1 ml of guanidine hydrochloride (6 M, pH 2.3). The absorbance of the sample was read at 370 nm. The data were expressed as nmol/mg protein. Thiol groups of native and glycated protein under effect of rutin were measured according to the Ellman's assay (18).

**Rat pancreatic islets isolation.** To evaluate the effect of isolated compounds, pancreatic islets were isolated from 5 male Wistar Albino rats (body weight, 200 g purchased from Institute Pasteur, Tehran, Iran) using a collagenase digestion methods (19). Briefly, under ether anesthesia, the common bile duct was clamped at the opening to the duodenum and cannulated. The pancreas was distended by the infusion of 10 ml of Hank balanced salt solution containing 1 mg/ml collagenase (type V). The distended pancreas was taken out and incubated in 37 °C water bath for 50 minutes and the reaction was stopped by adding cold Hank balanced salt solution with 5 % fetal bovine serum. The mixture of islets was centrifuged at 1000× g to obtained individual islets. After centrifugation, the remainder of pellets was suspended in Hank balanced salt solution three times to remove collagenase and connective tissues. The isolated islets were cultured for 24 h in RPMI 1640 medium (Gibco Life Technologies), containing 5 mM glucose supplemented with 10 % FBS, 100 IU/ml penicillin, and 0.1 mg/ml streptomycin at a CO<sub>2</sub> incubator (95 % air: 5 % CO<sub>2</sub>). After overnight recovery in RPMI 1640 media, islets were perincubated for 30 min at 37 °C in Krebs-Ringer Bicarbonate (KRB) buffer containing 5 mM glucose and/or STZ (5 mM) to evaluate insulin concentration.

**Flavonoids treatments.** To investigation whether isolated compound exerts direct effect on the insulin release batches of 100 islets were established as followed: control, flavonoid treated, STZ treated and flavonoid and STZ treated islets. Each group was incubated in KBR buffer containing 5 or 11.1 mM glucose for 1 hour at CO<sub>2</sub> incubator to maintain constant pH and oxygenation. The concentration of isolated compounds was (0.5-8 mM). For comparison, batches of islets were also incubated with a standard stimulating agent of insulin release, 0.15 mM tolbutamide. The concentration of STZ used in this experiment was 5 mM.

**Measurements of insulin release and protein carbonyl formation.** Aliquots of the medium were analyzed for insulin content by an enzyme-linked immunosorbent assay using a commercial kit (Rat insulin ELISA; DRG Instruments GmbH, Germany), based on direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule.

The amount of carbonyl group present in isolate rat islets proteins after treatment with STZ and/or flavonoids was determined calorimetrically. The detection of PCO involved derivation of the carbonyl group with DNPH, which lead to formation of stable 2, 4-dinitrophenyl (DNP) hydrazone product as described by Levine *et al* (20).

**Statistical analysis.** The results have been expressed as mean  $\pm$  standard error of mean (S.E.M). Difference in means were compared using one way analysis of variance (ANOVA) followed by Tukey Kramer's post hoc test .  $P < 0.05$  were considered statistically significant.

## Results

### Antioxidant activity of isolated compounds from *T. polium* using different in vitro assay systems

Regarding the antioxidant and free radical scavenging activity of *T. polium*, in this study we aim to isolate and evaluate the high antioxidant and anti-glycation compound(s) from methanol extract of *T. polium*. Fractionation of the methanol extract yielded four major flavonoids. The isolated compounds were screened for their antioxidant and free radical scavenging activities using two different antioxidant approaches including DPPH radical scavenging and TEAC methods. A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. It has also been used to quantify antioxidants in complex biological systems in recent years. The IC<sub>50</sub> values (defined as the concentration of test compound required to produce 50% inhibition) for DPPH scavenging by various isolated compound(s) from *T. polium* was shown in Table 1. According to the findings presented in the table 1, the highest scavenging activity was found for isolated compound 1 of ( $22.6 \pm 1.05 \mu\text{g/ml}$ ), followed by isolated compound 2 ( $26.14 \pm 1.52 \mu\text{g/ml}$ ) and compound 3 ( $33.20 \pm 2.01 \mu\text{g/ml}$ ). Compound 4 exhibited the weakest antioxidant activity in this test system of which IC<sub>50</sub> value is  $\sim 38.11 \pm 2.90 \mu\text{g/ml}$ . In addition, the antioxidant ability of each compounds to scavenge the blue-green colored ABTS<sup>+</sup> radical cation was measured relative to the radical scavenging ability of Trolox. All compounds showed free radical scavenging activity with different potencies. The highest activity was observed for the compound 1, with a TEAC value of 210 mg Trolox equivalents/g compound, followed by the compound 2, compound 3 and compound 4 with the TEAC values of 180, 102 and 87 Trolox equivalents/g compound, respectively (Table 1). Based on these cumulative data, it is clear that the compound 1 and compound 2 isolated from *T. polium* possess high antioxidant activities. Thus these two flavonoid compounds were used in further examinations in this study.

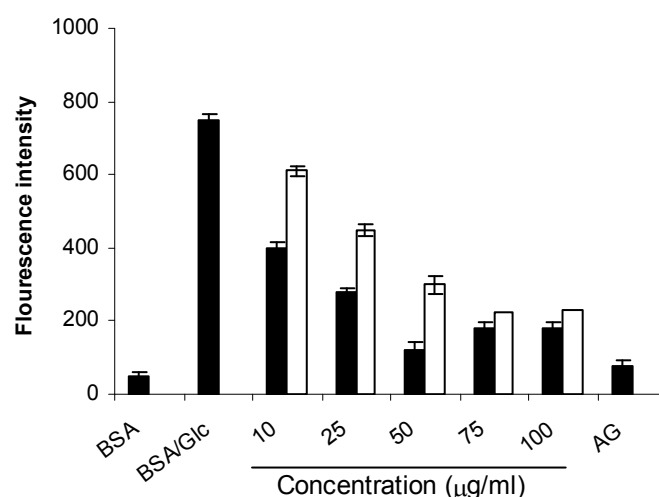
Table. 1 Antioxidant activity determined by the scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH), and Trolox equivalent antioxidant capacity (TEAC) of different isolated compounds from *T. polium*

| Sample             | DPPH (IC <sub>50</sub> ) | TEAC          |
|--------------------|--------------------------|---------------|
| Methanolic extract | 20.54 ± 1.62             | 250.25 ± 8.40 |
| Compound 1         | 22.60 ± 1.05             | 210.23 ± 5.40 |
| Compound 2         | 26.14 ± 1.52             | 180.51 ± 2.80 |
| Compound 3         | 33.20 ± 2.01             | 102.30 ± 4.30 |
| Compound 4         | 38.11 ± 2.90             | 87.10 ± 3.40  |
| BHT                | 18.42 ± 1.21             | -             |

#### Effect of rutin and apigenin on fluorescence intensity of glycated BSA in terms of total AGEs.

The formation of total AGEs was assessed by monitoring the production of fluorescent products at excitation and emission maxima of 370 and 440 nm, respectively, as previously explained (17). The fluorescence intensity of this glycoprotein which is characteristic of AGEs was highly increased through incubation of BSA with glucose. Fig. 2 shows the effect of glucose on the total AGEs formation during 21 days of BSA incubation at 37 °C. Compared to the control values, the fluorescence intensity was significantly higher in samples with glucose. As it is evident in Fig. 2 rutin and apigenin at different concentrations (10–100 µg/ml) has significantly quenched the fluorescence. The rutin and apigenin at the concentration of (50 and 75 µg/ml, respectively) exhibited 85 % and 70% of inhibitory activity, respectively. The ability of AG to inhibit AGE generation was about 90 %. These results indicated that rutin are effective in the prevention of high-glucose mediated protein glycation.

Fig. 2. Effect of rutin (a) and apigenin (b) at concentration of (10–100  $\mu\text{g/ml}$ ) isolated from *T. polium* and aminoguanidine (AG, 1 mM) on fluorescent AGEs formation in the BSA/glucose system. The fluorescence intensity was measured at excitation and emission 370 and 440 nm, respectively. Each value represents the mean  $\pm$  SD ( $n = 3$ ). All values statistically different ( $P < 0.05$ ) except those marked with the \*.



### Inhibitory effect of rutin and apigenin on glycation-induced oxidative modification of BSA.

In addition, the present study demonstrated that exposure of BSA to high-glucose level could enhance protein oxidation levels in terms of protein carbonyl (PCO) formation and loss of protein thiols. Rutin and apigenin inhibited high glucose-induced oxidative damages to protein by decreasing PCO formation and preserving protein thiols from oxidation (Table 2). This indicates that rutin and apigenin by decreasing oxidative stress may be effective in preventing oxidative protein damages which are believed to occur under the glycoxidation processes. The determination of free thiol groups in BSA was performed according to Ellman's method (18) (Table 2). The results showed a significant decrease in the number of free thiol groups when BSA is incubate with glucose. The incubation of BSA solution (pH 7.4) at 37 °C for a period of 21 days is sufficient to decrease the number of reduced thiol groups in albumin by 61% in the presence of glucose. However, rutin as well as apigenin in the presence of glucose significantly inhibited the oxidation of these thiol groups (Table 2). In this study, AG, as a positive standard, moderately inhibited PCO formation and the loss of thiol groups.



Table 2. Effect of rutin and apigenin isolated from *T. polium* and AG on protein carbonyl (PCO) formation and thiol groups content of glucose-glycated BSA

| Sample               | PCO (nmol/mg protein) | Thiol group (pmol/mg protein) |
|----------------------|-----------------------|-------------------------------|
| Control <sup>a</sup> | 0.40 ± 0.05           | 11.14 ± 1.01                  |
| Control <sup>b</sup> | ± 0.13 2.98           | 4.45 ± 0.78                   |
| AG                   | 1.18 ± 0.07*          | 7.12 ± 0.46*                  |
| Rutin                | 0.87 ± 0.04*          | 9.71 ± 0.21*                  |
| Apigenin             | 0.78 ± 0.02*          | 9.45 ± 0.35*                  |

Each value represents the mean ± SD (n = 3). Significantly different from control sample contained glucose (\**p* < 0.001).

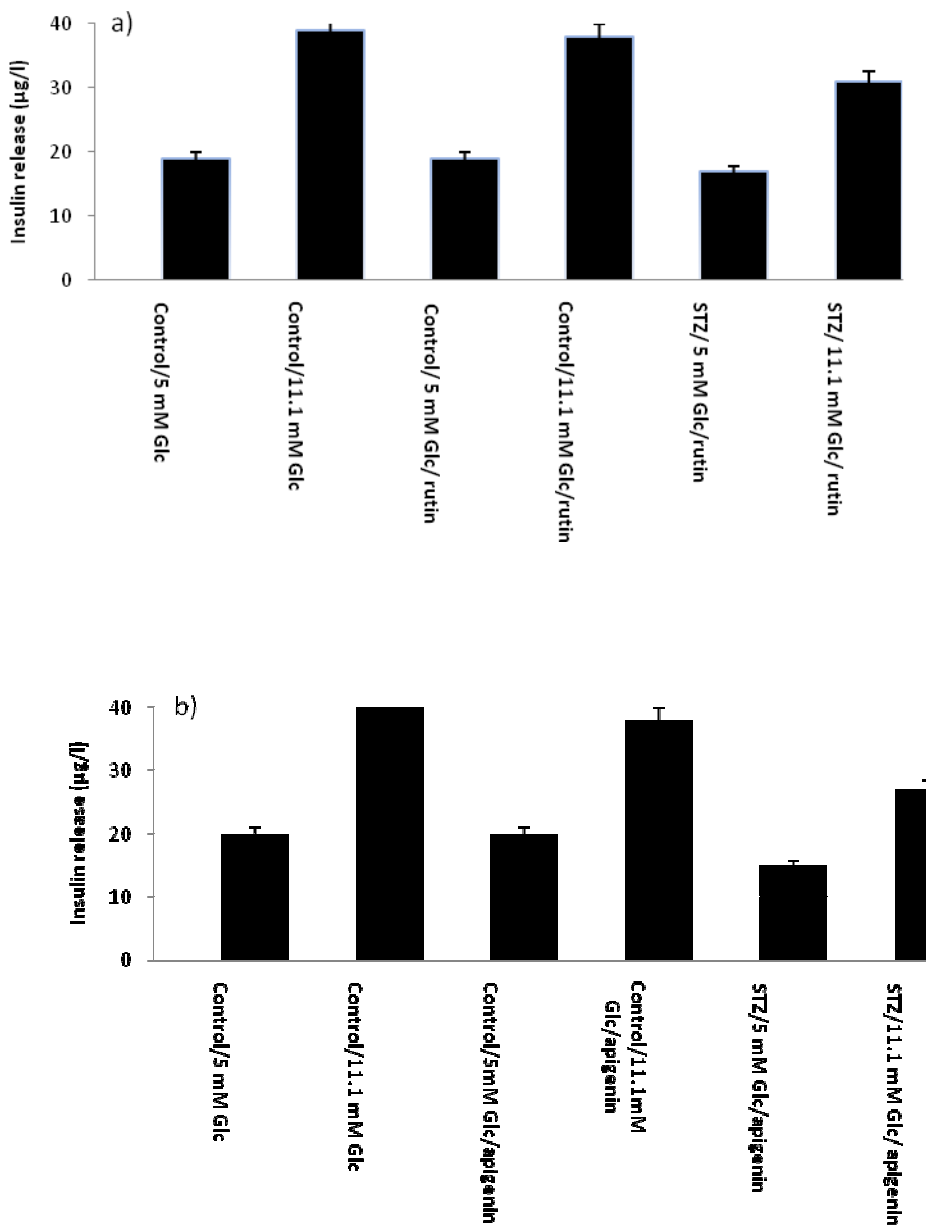
<sup>a</sup> Reaction mixture with out glucose

<sup>b</sup> Reaction mixture in the presence of glucose

### Protective of flavonoids on pancreatic β-cell and increase insulin secretion.

In this assay, rutin and apigenin, alone did not affect insulin release in the presence of either 5 mM and/or 11.1 mM D-glucose compared with that of control tests. However, tolbutamide at 0.15 mM concentration stimulated insulin secretion near 1.5 fold at both used glucose concentrations (5 and 11.1 mM). In addition, in STZ treated rat islets, the insulin release was significantly reduced compared with that of the other groups (data not shown). This phenomenon might be due to the destruction of β-cells by STZ (21). Mean while, in the treated islets with all used compounds before STZ treatment, especially rutin, there was no statistical difference in basal insulin release (5 mM glucose) compared with the normal control (Fig. 3). However, the insulin release in flavonoids and STZ treated islets at high glucose concentration (11.1 mM glucose) was statistically greater than that in STZ-treated islets, although, it did not affect the level of insulin release in normal control islets (Fig. 3). In addition, in the presence of rutin and/or apigenin at concentration of 50 and 75 μg/ml, respectively) a distinct inhibition of carbonyl groups in islets proteins occurred (about 73 and 65 %, respectively) (data not shown). These observations may be related to their protective effect on pancreatic islets.

Figure 3. Stimulation of rat islets release by rutin and apigenin isolated from *T.polium*. Rutin (a), and apigenin (b) induced at 50 and 75  $\mu\text{g/ml}$ , respectively initiation of basal (5 mM glucose) and stimulated (11.1 mM glucose) insulin secretion at various conditions. The insulin released by STZ treated islets with out any additive compound was  $8 \pm 1.2 \mu\text{g/l}$ . Tolbutamide at 0.15 mM concentration stimulated insulin secretion at both used glucose concentrations (5 and 11.1 mM) with 30 and 60  $\mu\text{g/l}$ , respectively. The data present the mean  $\pm$ SD. statistically significances of the observed differences in insulin release compared to the appropriate controls are \* $P < 0.001$ .



### Discussion

Regarding the presence of free radicals and oxidative steps in the glycoxidation process, supplementation with antioxidants such as lipoic acid, vitamin E or flavonoids could indirectly strengthen the anti-glycation defence system. Natural flavonoids are known for their significant scavenging properties on oxygen radical in both conditions *in vivo* and *in vitro* (7). Fractionation of the methanol extract yielded four major flavonoids. The isolated compounds were screened for their antioxidant and free radical scavenging activities. Our data clearly established that the isolated compound 1 and 2 (rutin and apigenin, respectively) from *T. polium* possesses potent antioxidant properties. The relationship between the chemical structure of flavonoids and their radical-scavenging activities was evaluated. In general, antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups. The essential requirement for effective radical scavenging is the 3', 4'-orthodihydroxy configuration in ring B and 4-carbonyl group in ring C. The presence of 3-OH group or 3- and 5-OH groups, giving a catechol-like structure in ring C, is also beneficial for the antioxidant activity of flavonoids and make them 10-fold more potent than the corresponding catechol and 3-OH free flavonoids (22). The presence of the C2–C3 double bond configured with a 4-keto arrangement is known to be responsible for electron delocalisation from ring B and it increases the radical-scavenging activity. Rutin has a catechol structure in ring B, as well as a 2, 3-double bond in conjunction with a 4-carbonyl group in ring C, allowing for delocalisation of the phenoxyl radical electron to the flavonoid nucleus. Flavonols have a hydroxyl group at position 3, which suggests a structurally important role of the 3-OH group of the chroman ring responsible for enhancement of antioxidant activity. The unsaturation in the C ring of flavonoids allows electron delocalization across the molecule for stabilization of the aryloxyl radical (23).

Regarding the link between glycation and protein oxidation, antioxidant flavonoids might possess anti-glycation properties. In diabetic patients, the concentration of Schiff's base and AGEs is higher than those in normal human (5). Thus, it could be of great interest to propose the usage of natural flavonoids for the prevention of protein glycation. For example, Daidzein (an isoflavonoid) has been shown to interfere with AGEs mediated oxidative DNA damage in hypertensive rats (24). In addition, flavones glycosides isolated from *Zea mays*, showed the glycation inhibitory activity like to that of AG (25). The present study demonstrated that exposure of BSA to high-glucose level could enhance protein oxidation levels in terms of protein carbonyl (PCO) formation and loss of protein thiols. Rutin and/or apigenin inhibited high glucose-induced oxidative damages to protein by decreasing PCO formation and preserving protein thiols from oxidation. This indicates that these flavonoids by decreasing oxidative stress may be effective in preventing oxidative protein damages which are believed to occur under the glycoxidation processes. Our results suggested that the inhibitory activity of flavonoids was not only due to their antioxidant properties, but also some other mechanisms needed to be clarified in future. Since the ability of *T. polium* extract on scavenging of reactive forms of oxygen has been studied (26), it may be concluded that the inhibition of glycation by this plant could also be attributed to its overall antioxidant properties. Our findings clearly support not only the view that oxidative reactions have significant influence on AGEs formation but also on the possibility that antioxidants could be effective AGEs inhibitors.

Previous studies of Esmaeili and Yazdanparast (27), showed that *T. polium* crude extract is able to reduce the blood glucose by increasing the insulin release due to prevent destruction of pancreatic  $\beta$ -cells in a model of streptozotocin (STZ) induced diabetes.

In addition, based on our unpublished data, the flavonoid rich fraction of methanol extract of *T. polium* is able to protect pancreatic  $\beta$ -cells against STZ-induced damages in experimental animals. At a high concentration of (300 mg/kg body weight for one month) this fraction reduced serum glucose levels and increased serum insulin levels in STZ-diabetic rats. At the same time, it improved cellular oxidative damages and decreasing lipid peroxides. There fore, it concluded that some flavonoids in *T. polium* crude extract might prevent  $\beta$ -cell destruction in oxidative stress conditions, and this could be attributed at least in part, to their antioxidant properties. Thus, in our studies, we focused the effects of rutin and apigenin (high antioxidant agents from plant) on insulin release using isolated rat islets. However, the insulin release in flavonoids and STZ treated islets at high glucose concentration was statistically greater than that in STZ-treated islets, although, it did not affect the level of insulin release in normal control islets. These finding are in agreement with Kim *et al* studies (21) which showed that the increased insulin release in epicatechin (a flavonoid isolated from tea) and STZ treated islets, might have resulted from the remaining  $\beta$ -cell mass rich in intact insulin granules and did not form the stimulatory effect of epicatechin on insulin secretion. Our results also confirmed the Kamalakkannan and stanely studies (28) on antioxidant effect of rutin in STZ diabetic rats. This is the first report on the preventive effect of major flavonoids from *T. polium* on  $\beta$ -cells destruction in a model of STZ-induced diabetes (mimicking human type 1 diabetes). In summary, we found that flavonoids are responsible for the insulinotropic and hypoglycemic activity of *T. polium*. However, these compounds and other compounds should be assay in additional biological examination to evaluate their mechanism of action on insulin release and protective pancreatic  $\beta$ -cells from oxidative stress induced by streptozotocin to find therapeutic drugs for diabetes complication and related disease.

### Acknowledgements

The authors wish to thank the research council of Shahid Beheshti University for funding for the research.

### References

1. Halliwell B, Antioxidant characterization: Methodology and mechanism. *Biochem Pharmacol* 1995; 49:1341–1348.
2. Kokkini S, Karousou R, Vokou D, Pattern of geographic variation of *Origanum vulgare* trichomes and essential oil content in Greece. *Biochem System & Ecology* 1994; 22: 517–528.
3. Pryor WA, The antioxidant nutrients and disease prevention–what do we know and what we need to find out. *American J Clin Nutr* 1991; 53: 391–393.
4. Wolff SP, Dean RT. Glucose autoxidation and protein modification. The potential role of 'autoxidative glycosylation' in diabetes. *Biochem J* 1987; 245: 243–250.
5. Baynes JW, Thorpe SR, Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes* 1999; 48: 1–9.
6. Rahbar S, Figarola JL, Novel inhibitors of advanced glycation endproducts. *Arch Biochem Biophys* 2003; 419: 63–79.
7. Rudnicki M, Oliveira MR, Pereira TV, et al. Antioxidant and antiglycation properties of *Passiflora alata* and *Passiflora edulis* extracts. *Food Chem.*2007; 100: 719-724.

8. Thornalley PJ, Use of aminoguanidine (Pimagedine) to prevent the formation of advanced glycation endproducts. Arch Biochem Biophys 2003; 419: 31-40.
9. Rasekh HR, Khoshnood-Mansourkhani MJ, Kamalinejad M, Hypolipidemic effects of *Teucrium polium* in rats. Fitoterapia 2001; 72: 937-939.
10. Tariq M, Ageel AM, Al-Yahya MA, et al. Anti-inflammatory activity of *Teucrium polium*. Int J Tissue React 1989; 11: 185-188.
11. Munir N, Gharaibeh Hamzeh H, Elayan Abdulazim S, Salhab S, Hypoglycemic effects of *Teucrium polium*. J Ethnopharmacol 1988; 24: 93-99.
12. Esmaeili MA, Yazdanparast R, Hypoglycaemic effects of *Teucrium Polium*: studies. with rat pancreatic islets. J Ethnopharmacol 2004; 95:27-30.
13. Ljubuncic P, Dakwar S, Portnaya I, et al. Aqueous Extracts of *Teucrium polium* Possess Remarkable Antioxidant Activity *In Vitro*. Evid Based Complement Altern Med 2006; 3: 329 - 338.
14. Ardestani A, Yazdanparast R, Inhibitory effects of ethyl acetate extract of *Teucrium polium* on in vitro protein glycooxidation. Food Chem Toxicol 2007; 12: 2402-2411.
15. Blois M.S, Antioxidant determination by the use of a stable free radical. Nature 1958; 181: 1199-1200.
16. Madhujith T, Izydorczyk M, Shahidi F, Antioxidant properties of pearled barley fractions. J Agric Food Chem 2006; 54: 3283-3289.
17. Sharma SD, Pandey BN, Mishra KP, Sivakami S, Amadori product and age formation during nonenzymatic glycosylation of bovine serum albumin *in vitro*. Biochem Mol Biol Biophys. 2002; 6: 233-242.
18. Ellman L, Tissue sulfhydryl groups, Arch Biochem Biophys 1959; 82: 70-77.
19. Kim MJ, Lee KH, Distributional pattern of phospholipase C isozymes in rat pancreas. Pancreas 2001; 22: 47-52.
20. Levin RL, Garland D, Oliver CV, et al. Determination of carbonyl count in oxidative modified proteins. Method Enzymol 1990; 186: 464-478.
21. Kim MJ, Ryu GR, Chung JS, et al. Protective effect of epicatechin against the toxic effect of streptozotocin on rat pancreatic islets: in vivo and in vitro. Pancreas 2003; 26: 292-299
22. Heim KE, Tagliaferro AR, Bobilya DJ, Flavonoid antioxidant: Chemistry, metabolism and structure-activity relationship. J Nutr Biochem 2002; 13: 572-584
23. Rastija V, Medić-Šarić M, QSAR study of antioxidant activity of wine polyphenols. Eur J Med Chem 2009; 44: 400-408
24. Mizutani K, Ikeda K, Nishikata T, et al. 2000; 18: 1833-1840.
25. Suzuki R, Okada Y, Okuyama T, Two flavone C-glycosides from the style of *Zea mays* with glycation inhibitory activity. J Nat Prod 2003; 66: 564-565.
26. Ljubuncic P, Dakwar S, Portnaya I, et al. Aqueous extracts of *Teucrium polium* possess remarkable antioxidant activity *in vitro*. Evid Based Complement Alternat Med 2006; 3: 329-338.
27. Yazdanparast R, Esmaeili MA, Ashrafi JH, *Teucrium polium* extracts pancreatic function of streptozotocin diabetic rats: A histological examination. Iran Biomed J 2005; 9: 81-5.
28. Kamalakkannan N, Stanely Mainzen Prince P, Rutin improves the antioxidant status in streptozotocin induced diabetic rats tissues. Molecul cell Biochem 2006; 293: 211-219.