Effects of Some Boraginaceae Species Extracts on Albumin, Hemoglobin and Crystalline Glycation Reaction

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Faezizadeh, et al.: Antiglycation Effects of Some Boraginaceae Species

Non-enzymatic glycation of proteins is the major cause of diabetic complications and therefore, inhibition of this reaction could reduce the morbidity of this disease. The aim of this study was to evaluate the in vitro effects of aqueous and hydroethanol extracts of Echium italicum L., Anchusa arvensis L. and Trichodesma incanum (Bunge) A. DC. of Boraginaceae on albumin, hemoglobin, and crystalline glycation reaction. The results showed that the hydroethanol extract of Echium italicum in the concentrations of 100, 50 and 10 mg/dl could reduce the rate of albumin glycation to 85.10±1.85, 64.14±1.12 and 40.13±1.17%, respectively, and also in the same conditions, rates of hemoglobin glycation were reduced to 69.20±1.71, 46.13±1.93 and 27.73±0.90%, respectively. Moreover, extracts of three tested plants have antiglycation effects on crystalline glycation reaction. Under the identical conditions, the inhibitory effects of hydroethanol extracts of Echium italicum on the albumin, hemoglobin, and crystalline glycation reaction were higher than other extracts. The findings showed that the extracts of tested species, particularly in the hydroethanol form, could be used to prevent and treat complications of diabetes mellitus.

Key words: Echium italicum L., Anchusa arvensis L., Trichodesma incanum (Bunge) A. DC., albumin, hemoglobin, glycation

Members of the family Boraginaceae include herbs, shrubs and trees totaling about 146 genera and 2000 species in the worldwide\cite{1}. Some of these plants grow in Iran and used widely in Iran's folk medicine\cite{2,3}. It has been reported that Borage (Borago officinalis L.) was useful for its antipyretic, antihypertensive, antispasmodic, aphrodisiac and diuretic properties\cite{4,5}. Previous findings showed that some species of Boraginaceae were used for the treatment of hemorrhoids\cite{6}. Furthermore, it has also been observed that various parts of Echium species could be used for their antiinflammatory, antibacterial, antidepressant, antiproliferative, antiviral, antioxidant, cytotoxic and anxiolytic properties\cite{2,3}. Cyanidin, a natural phenolic compound is present in many kinds of plants, such as grapes, pomegranate and some species of Boraginaceae and according to the previous reports, this effective compound has antioxidant and antidiabetic potential\cite{7-10}. In this disease, the macro and microvascular complications lead to severe effects on the eyes, central nervous systems and kidneys\cite{11}. Evidence from in vitro and in vivo experiments indicated that diabetic hyperglycemia could increase the risk of complications\cite{13,14}. It seems that non-enzymatic glycation plays an important role in progression of diabetic complications\cite{11}. Due to prolonged hyperglycemia, the non-enzymatic glycation of proteins could significantly change their structural and functional properties\cite{12}. Previous studies demonstrated that the protection of proteins from glycation could reduce the diabetic complications as well\cite{15,16}. The use of glycation inhibitors is considered due to their therapeutic potential in patients with diabetes\cite{17,18}. The
investigation of new drugs with antiglycation activity has attracted the interest of various researchers\cite{19,20}. Herbal medicines are nowadays gaining importance for treating diabetes mellitus due to their less toxicity, significant results as compared to allopathic medicine\cite{21,22}. It is reported that some medicinal plants and their derivatives could inhibit the glycation of proteins as well\cite{17,18}.

Several species of Boraginaceae family such as \textit{E. italicum} L. (fig. 1A), \textit{Anchusa arvensis} L. (fig. 1B) and \textit{Trichodesma incanum} (Bunge) A. DC (fig. 1C) are used widely in Iran's folk medicine for their antidiabetic properties. According to our knowledge, there is no scientific research about antidiabetic mechanism of these plants and therefore, in this study, we investigated the antiprotein glycation efficacy of these selected species.

**MATERIALS AND METHODS**

All the chemicals used were of analytical grade obtained from either Merck (Darmstadt, Germany) or Sigma (St. Louis, MO, USA). Human glycated albumin ELISA kit was obtained from Nacalai Tesque (Kyoto, Japan). Human glycated hemoglobin ELISA kit was obtained from MyBioSource, Inc. (San Diego, California, USA).

The three species of Boraginaceae family, including \textit{E. italicum}, \textit{A. arvensis} and \textit{T. incanum} were collected during the spring from different regions of Borujerd (Lorestan Province, Iran). These regions have mild climate with mean temperature between 21-25°C in the spring. Plant samples were identified and confirmed by comparison with voucher specimens deposited at the Department of Biology, Faculty of Basic Sciences, Borujerd Branch, Islamic Azad University, Borujerd, Iran.

**Glycation of proteins:**

The glycated human serum albumin, hemoglobin, and crystalline were prepared according to Gharib \textit{et al.} method\cite{17}. In brief, 50 mg/ml of tested proteins were incubated with 40 mmol glucose, 100 μg/ml penicillin, 100 U/ml streptomycin, 2 mg/ml aprotinin, 5 mmol phenylmethylsulfonyl fluoride and 0.5 mg/ml leupeptin in PBS (10 mmol, pH 7.4) at 37°C for 8 weeks. Subsequently, the sample was dialyzed extensively against PBS at 4°C.

**Measurement of glycated albumin, hemoglobin:**

The rate of glycated albumin was measured using the human glycated albumin ELISA kit (Nacalai Tesque Company), according to the manufacturer’s protocol. In brief, 50 μl of phosphate buffer (10 mmol, pH 7.4) containing 20 μl of the test samples and 0.5 ml/l Tween 20 were added to each well of a 96-well micro titer plate that was coated with antihuman serum albumin antibody. Thereafter, wells were incubated for 20 min and washed three times. Subsequently, the boronate-horseradish peroxidase conjugate in 50 μl of glycine-NaOH (100 mmol, pH 9.0) containing MgCl$_2$ (20 g/l), bovine hemoglobin (3 g/l), and Tween 20 (0.5 ml/l) was added to wells. Again, wells were incubated for
20 min and the reaction was terminated by adding 50 μl of sulfuric acid (2 mol), and the absorbance at 492 nm was measured using a microplate reader (MTP-120; Corona Electronic). Finally, the percentage rate of glycated albumin in the samples was measured. The rate of glycated hemoglobin was measured using the human glycated hemoglobin ELISA kit (MyBioSource, Inc.), according to the manufacturer’s protocol. Furthermore, the rate of crystalline non-enzymatic glycation was measured by the TBA method as described previously[23].

**Preparation of plants extracts:**

The hydroethanol and aqueous extracts of *E. italicum*, *A. arvensis* and *T. incanum* were prepared according to the previously reported method[24]. In brief, the air-dried plants were ground into powder using grinder (Moulinex, France), and then 1 g of sample powders was blended with 60 ml of 45% ethanol or water solvents. Subsequently, the blended samples were mixed on a sample stirrer at 900 rpm for 1 min, and then the liquid volumes were increased to 100 ml by solvents. These mixtures were placed in a bath by stirring and cooled in a refrigerator. Subsequently, the mixtures were centrifuged (Shimadzu, Japan) at 2500 rpm for 10 min and vacuum filtered, and the loss solvents were replaced. Finally, the prepared extracts were stored at 4° until used for analysis.

**In vitro antiglycation studies:**

The percentages of glycated albumin, hemoglobin, and crystalline glycation in the samples were determined after incubation of these tested proteins and glucose in the presence of different concentrations (0, 10, 50, and 100 mg/dl) of each extract for 8 weeks. As positive control, 20 mg/ml aminoguanidine bicarbonate, as an antiglycation drug, was used.

**Statistical analysis:**

The results were expressed as mean±SD. The statistical analysis was performed using one-way ANOVA followed by Tukey’s test and *P*-value less than 0.05 was considered significant. Data were analyzed using SPSS software (version 19.0, SPSS, Inc., IL, USA). All experiments were done in triplicate.

**RESULTS AND DISCUSSION**

In this research, the effects of the various concentrations of tested plant extracts on rates of albumin, hemoglobin, and crystalline glycation were studied. Under the identical conditions, the inhibitory effects of hydroethanol extracts of tested plants on glycation rates of three tested proteins were higher than those of aqueous extracts (Tables 1, 2 and 3). In all conditions, the albumin, hemoglobin, and crystalline glycation rates were significantly inhibited compared to the negative control group. Moreover, treatment with 100 mg/ml hydroethanol extracts of *E. italicum*, *A. arvensis* and *T. incanum* could reduce the rate of hemoglobin glycation to 69.20±1.71 and 41.90±1.25 and 27.85±1.30%, respectively (Table 2). As positive control aminoguanidine bicarbonate could decrease the rate of albumin, hemoglobin, and crystalline glycation to 56.12±1.39, 39.15±0.50 and 34.10±1.35%, respectively. Compared to control group, the rate of albumin glycation with 100 mg/ml hydroethanol extracts *E. italicum*, *A. arvensis* and *T. incanum* were reduced to 85.10±1.85, 53.10±0.69 and 38.13±1.81% at 8 weeks, respectively (Table 1). Under the identical conditions, the inhibitory effects of *E. italicum* extracts on albumin, hemoglobin, and crystalline glycation were significantly higher than those of other tested plants. Furthermore, the antiglycation rates of extracts on crystalline were lower than those of other tested proteins (Table 3).

In diabetes mellitus, the nonenzymatic glycation of proteins occurs in a wide variety of proteins,

<table>
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<tr>
<th>TABLE 1: EFFECT OF HYDROETHANOL AND AQUEOUS EXTRACTS ON IN VITRO ALBUMIN GLYCATION REACTION</th>
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<tr>
<td>Plant scientific name</td>
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<tr>
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<tr>
<td><em>Echium italicum</em> L</td>
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<tr>
<td><em>Anchusa arvensis</em> L</td>
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<tr>
<td><em>Trichodesma incanum</em> (Bunge)</td>
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<td>A. DC.</td>
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Data presented as mean±SD. Values significantly different from negative control (*P*-0.05)
and this phenomenon could enhance diabetic complications\cite{25}. Therefore, in medicine, the inhibition of protein glycation has been proposed as an important strategy for the restriction of diabetic complication development\cite{26}. The use of some medicinal plant extracts to reduce of diabetic complications has been widely investigated\cite{17,27}.

In folk medicine, Boraginaceae species have been used for the treatment and prevention of many ailments and diseases such as hypertension, diarrhea and diabetes mellitus and have been a tremendous resource for the development of new drugs\cite{28-31}. Many species of Boraginaceae family are grown in Iran and some of them are used in ethnomedicine\cite{4}. However, there were no studies about their antiglycation properties and therefore, in the present study, we investigated the in vitro effects of aqueous and hydroethanol extracts of *E. italicum*, *A. arvensis* and *T. incanum* on albumin, hemoglobin, and crystalline glycation reactions. The results from this study revealed that the all prepared extracts possessed antiprotein glycation. These findings correlated with those reported by other investigators about antiglycation efficacy of some natural plants, such as *Allium cepa* L.\cite{32}, *Curcuma longa* L.\cite{33}, *Rosmarinus officinalis* L.\cite{34}, *Cinnamomum verum*\cite{35} and *Origanum officinalis*\cite{36} under in vitro conditions. Under identical conditions, the antiglycation potential of extracts on albumin was higher than hemoglobin, and crystalline glycation, respectively. According to the previous studies, this difference may contribute to the protein structure\cite{37}. The hydroethanol extracts of each tested plant have more inhibitory effects on protein glycation reaction, compared to aqueous extracts. These results may be related to effects of extracting solvents on the isolation of phytochemical constituents\cite{38}.

Under identical conditions, the extracts of *E. italicum* have more potent antiglycation activity compared to *A. arvensis* and *T. incanum* extracts. Moreover, our data revealed that the antiglycation potential of *E. italicum* was higher than *A. sativum*, *Zingiber officinale*, *A. cepa* and *Thymus vulgaris* that their antiglycation activities were reported previously\cite{33,34}. Thus, the ability of *E. italicum* to modulate glycation might be providing its use as a significant source of the natural product in preventing the diabetic complications.

In summary, the present study was carried out to evaluate the antiglycation potential of *E. italicum*, *A. arvensis* and *T. incanum* extracts on albumin, hemoglobin, and crystalline glycation reaction. Results indicated that the hydroethanol extracts of these plants might be promising antiglycation agents for the prevention of diabetic complications through inhibition of protein

<table>
<thead>
<tr>
<th>Plant scientific name</th>
<th>Extracts</th>
<th>% Inhibition of hemoglobin glycation</th>
<th>Concentrations of extracts (mg/dl)</th>
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<tr>
<td><em>Echium italicum</em> L.</td>
<td>Hydroethanol</td>
<td>27.73±0.90*</td>
<td>69.20±1.71*</td>
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<td>Aqueous</td>
<td>25.28±0.65*</td>
<td>61.93±1.10*</td>
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<tr>
<td><em>Anchusa arvensis</em> L.</td>
<td>Hydroethanol</td>
<td>19.28±0.65*</td>
<td>41.90±1.25*</td>
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<td></td>
<td>Aqueous</td>
<td>17.23±0.51*</td>
<td>32.10±1.13*</td>
</tr>
<tr>
<td><em>Trichodesma incanum</em> (Bunge)</td>
<td>Hydroethanol</td>
<td>15.13±0.54*</td>
<td>27.85±1.30*</td>
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<td></td>
<td>Aqueous</td>
<td>10.38±0.43*</td>
<td>21.15±0.83*</td>
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Data presented as mean±SD. Values significantly different from negative control (*P<0.05)

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<thead>
<tr>
<th>Plant scientific name</th>
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<th>% Inhibition of crystalline glycation</th>
<th>Concentrations of extracts (mg/dl)</th>
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<tr>
<td><em>Echium italicum</em> L.</td>
<td>Hydroethanol</td>
<td>24.13±1.10*</td>
<td>64.75±0.43*</td>
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<tr>
<td></td>
<td>Aqueous</td>
<td>21.35±0.70*</td>
<td>58.77±0.25*</td>
</tr>
<tr>
<td><em>Anchusa arvensis</em> L.</td>
<td>Hydroethanol</td>
<td>15.83±0.53*</td>
<td>39.87±1.45*</td>
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<tr>
<td></td>
<td>Aqueous</td>
<td>13.12±0.70*</td>
<td>26.18±1.20*</td>
</tr>
<tr>
<td><em>Trichodesma incanum</em> (Bunge)</td>
<td>Hydroethanol</td>
<td>15.18±0.84*</td>
<td>23.35±1.10*</td>
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<td>A. DC.</td>
<td>7.18±0.32*</td>
<td>18.13±0.75*</td>
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</table>

Data presented as mean±SD. Values significantly different from negative control (*P<0.05)
glycation. Therefore, the prepared extracts could be considered as a promising strategy for developing the antidiabetic drugs.

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Conflicts of interest:
There are no conflicts of interest.

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