EXPERIMENTAL STUDY

Potential for Pakistani traditional medicinal plants to combat diabetes

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Abstract

OBJECTIVE: To investigate the potential of medicinal plants used in traditional healing in Pakistan to inhibit the glycation process. This may be useful in combatting diabetes mellitus and its complications.

METHODS: The in vitro antiglycation activity of 10 medicinal plants was examined by testing the ability of the extracts to inhibit the methyl-glyoxal-mediated development of fluorescence of bovine serum albumin.

RESULTS: Of the tested plants, Persicaria barbata, Geranium collinum and Berberis lycium showed significant inhibition of the formation of advanced glycation end products. The inhibitory capacity of these plants was 68.89%, 62.06% and 54.23%, respectively, compared with the positive control (rutin; 86%). All other plants inhibited AGE formation non-significantly.

CONCLUSION: These findings will be helpful for further research into the use of traditional herbal medicines with antiglycation properties in the treatment of diabetes.

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Key words: Diabetes mellitus; Plants, medicinal; Medicine, traditional; Antiglycation

INTRODUCTION

Diabetes mellitus is a serious metabolic disorder with multiple complications. The role of free radicals in diabetes has been widely discussed, and the involvement of free radicals in diabetes onset and complications has been shown experimentally. Hyperglycemia is the distinguishing feature of diabetes, and persistent conditions in a diabetic patient lead to the formation of oxidative stress for multiple reasons, including the autoxidation of glucose. This autoxidation generates free radicals, including hydrogen peroxide, which is highly reactive after interaction with biomolecules, and accelerates the formation of advanced glycation end products (AGEs). These AGEs accumulate in tissues, and crosslinking with other macromolecules results in abnormalities in cellular and tissue function. At present, the treatment of diabetes involves various therapeutic agents in addition to insulin. However, because of adverse effects and variable efficacy of these agents, there is interest in alternative treatment options. Therefore, plants are considered to be an unexplored and rich source of potential new antidiabetic agents. However, there is a lack of mechanism-based in vitro assays, and only a few plants have been subject to thorough investigation. In recent years, there has been an increasing trend to investigate traditional medicinal plants for their poten-
tial as antidiabetic agents. Various plants have been studied to date include Costus pictus,1 Euphoria ochreata,2 Microcephal laelatata,3 Dendrobium aequum,4 Salvia sahendica,5 Berginia,6 Enicostemma axillare,7 Rhynchosia reniformis,8 and Aralia taibaiensis.9 The objective of the study was to screen plants with antiglycation properties for the treatment of diabetes.

MATERIALS AND METHODS

Plants collection
The plant specimens were collected from Galliyat region, Western Himalaya, Pakistan. Identification of specimens was performed by Plant taxonomy working group at Plant Taxonomy and Biosystematics Laboratory, Department of Plant Sciences, Quaid-i-Azam University (QAU), Islamabad. The plants were Geranium collinum, Persicaria bharbata, Impatiens edgeworthii, Clematis grata, Geranium wallichianum, Boerhavia procumbens, Berberis lyci-um, Artemisia vulgaris, Rubia cordifolia, Boehravha procumbens, and Capsella bursa-pastoris.

Extraction
Fresh plant material of 100 g was collected for each specimen and under the shade dried and then grind to obtain the powdered samples. This powder is then used for the extract preparations. Powder was added with 95% pure methanol (Sigma Co., St. Louis, MO, USA) at room temperature and placed for three days and then whole the process was repeated. The extracts obtained were filtered using Whatman filter paper 1 (Sigma Co., St. Louis, MO, USA) and then filtrates were pooled together. The pooled filtrate was concentrated with rotary evaporator (Laborota 4000, Heidolph, Germany) at 40°C under lowered pressure. Final dried and concentrated extract was weighed. Which were stored in refrigerator for further processing in assay. Rutin (Flex Pharma, Lahore, PK) was used as a positive control.

Materials
The materials used were as follows: Bovine serum albumin (BSA; Merck Co., Darmstadt, Germany), 96 well microtiter plates (EMD Millipore Corporation, Billerica, MA, USA), glucose anhydrous (Sigma Co., St. Louis, MO, USA), magnesium oxide (Merck Co., Darmstadt, Germany), sodium phosphate buffer (Merck Co., Darmstadt, Germany), NaN3 (Wako Co., Osaka, Japan), trichloroacetic acid (TCA; Sigma Co., Germany), and a spectrophotometer (Shimadzu Japan).

Experimental procedure
The in vitro antiglycation activity of the medicinal plants was examined by testing the ability of the extracts to inhibit the methyl-glyoxal-mediated development of the fluorescence of BSA.10 All the assays were performed in 96-well microtiter plate. The reaction mixture was prepared and 60 µL placed in each well. The reaction mixture comprised of three components: 20 µL of 50 mg/mL glucose and 14 mM magnesium oxide, 20 µL of 10 mg/mL BSA, and 20 µL of the plant extract. Blanks comprised of 60 µL with 40 µL of sodium phosphate buffer and 20 µL of BSA, and the negative control contained 20 µL of 0.1 M sodium phosphate buffer, 20 µL of BSA and 20 µL of 30 mM NaN3. The plate was incubated for 9 days at 37°C. Following incubation, 60 µL of 100% TCA was added to each well, followed by centrifugation at 15 000 rpm at 4°C for 4 min. Next, 5% TCA was used to wash the pellets, containing the AGEs bound with BSA, after that it was dissolved in phosphate buffer solution (60 µL). The supernatant, which included extraneous substances, glucose and inhibitors, was discarded. AGE formation was visualized fluorescence intensity excitation (370 nm) and emission (440 nm) by using spectrophotometer. The percentage inhibition of AGEs was determined as follows:

\[ \text{Percentage inhibition} = \frac{1 - (\text{absorbance extract/absorbance control})}{100} \]

The results were interpreted according to the percent inhibition of AGE’s, Values more than 80% were considered as very significant, between 50%-80% were significant, 30%-50% were less significant and below that were non-significant.

RESULTS
The antiglycation capabilities of the medicinal plants were investigated for their potential to inhibit of the formation of AGEs. Rutin, which was used as a reference drug, showed 86% inhibition. The degree of antiglycation activity varied considerably from plant-to-plant. Persicaria bharbata, Geranium collinum and Berberis lyciium showed significant inhibition of AGE formation. The inhibitory capacity for these plants was 68.89%, 62.06% and 54.23%, respectively. All other plants inhibited AGE formation non-significantly. These included Impatiens edgeworthii (17.15%), Clematis grata (9.43%), Geranium wallichianum (3.17%), Artemisia vulgaris (13.9%), Rubia cordifolia (17.74%), Boehravha procumbens (9.52%), and Capsella bursa-pastoris (5.87%).

DISCUSSION
Of all plants tested in our study, three showed significant inhibition of AGE formation. These were Persicarabartata, Geranium collunum and Berberislycium. No previous reports were found on the antiglycation activities of the plants used in our study in the literature. While the methanolic extract of Microcephala lamella showed antiglycation activity 62.84% and 50% of AGE’s inhibition was shown by Salvia officina-
lis at 0.62 mg/mL concentration, Petroselinum crispum at 0.42 mg/mL, Coriandrum sativum at 0.42 mg/mL, Chenopodium ambrosioides at 0.41 mg/mL and Persea americana at 1.3 mg/mL.\textsuperscript{19} and leaf methanolic extract of Costus pictus showed 53.6%.

Diabetes is one of the leading health problems. And herbal remedies with potential to combat the diabetes will provide a very good alternative to current chemotherapeutics. The current study showed good results in term of AGE’s inhibition potential. Hence, this study will be helpful in identifying new herbal medicines to combat diabetes.

REFERENCES