J. Verbr. Lebensm. (2011) 6:191–195
DOI 10.1007/s00003-010-0627-6

Journal für Verbraucherschutz und Lebensmittelsicherheit
Journal of Consumer Protection and Food Safety

GENERELLE ASPEKTE

Evaluation of α-amylase inhibitory activities of selected antidiabetic medicinal plants

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Received: 27 May 2010 / Accepted: 10 June 2010 / Published online: 29 June 2010
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Abstract Inhibitors of carbohydrate hydrolyzing enzymes such as α-amylase play an important role for the control of diabetes mellitus especially in patients with type 2 diabetes. In this study we selected ten antidiabetic medicinal plants, because they have been recommended to treat diabetes in traditional Iranian medicine, and screened them for α-amylase inhibitory activities. Among the tested samples, *Camellia sinensis* (Theaceae) leaf (IC_{50} = 1.54 mg/mL), *Trigonella foenum-graecum* (Leguminosae) seed (IC_{50} = 1.87 mg/mL) and leaf (IC_{50} = 1.92 mg/mL), and *Urtica dioica* (Urticaceae) leaf (IC_{50} = 1.89 mg/mL) revealed appreciable α-amylase inhibitory activities in a concentration-dependent manner. Furthermore, the most active sample, *Camellia sinensis* leaf, was partitioned by stepwise solvent–solvent extraction process and the inhibitory effect of each fraction on the α-amylase was tested. According to the results, the ethyl acetate fraction (IC_{50} = 0.53 mg/mL) and the residue (IC_{50} = 0.52 mg/mL) had the highest α-amylase inhibitory activities.

Keywords α-Amylase inhibitors · Antidiabetic medicinal plants · *Camellia sinensis* · Diabetes mellitus

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1 Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels. There are two forms of diabetes, type 1 and type 2. At the present time it is estimated that 150 million people worldwide have diabetes and that this will increase to 220 million by 2010 and 300 million by 2025. Globally, the percentage of type 2 diabetes is greater than 90% (Funke and Melzig 2006; Li et al. 2005; Shim et al. 2003). Therefore, it is necessary to find new approaches to managing this health challenge. One goal of therapy for diabetic patients, especially type 2, is the maintenance of normal blood glucose levels after a meal. Postprandial hyperglycemia plays an important role in the development of type 2 diabetes and complications associated with the disease, such as micro- and macro-vascular diseases (Li et al. 2005; Mai and Chuyen 2007).

One of the therapeutic approaches for decreasing of blood glucose rise after a meal is to retard the absorption glucose by inhibition of carbohydrate hydrolyzing enzymes, such as α-amylase and α-glucosidases. Carbohydrates are the major constituents of the human diet that mainly play a role in the energy supply. The complex components of dietary carbohydrates should be broken down to monosaccharides by the α-amylase and glucosidases since only monosaccharides can be absorbed from intestinal lumen and transported into blood circulation (Dewi et al. 2007; Kwon et al. 2006).

It is now believed that inhibition of these enzymes can significantly prolong overall carbohydrate digestion time and decrease the postprandial increase of blood glucose level after a mixed

carbohydrate diet and therefore can be an important strategy in the management of postprandial blood glucose level linked to type 2 diabetes (Ali et al. 2006). One group of drugs introduced in the management of type 2 diabetes is represented by the enzyme inhibitors. They have received considerable attention in the past two decades as they are potential therapeutic agents for the treatment of diabetes. The examples of such inhibitors which are in clinical use are acarbose, miglitol and voglibose (Abesundara et al. 2004; Funke and Melzig 2006).

In recent years, research on traditional medicinal plants for the management of diabetes has attracted the interest of scientists. Grover et al. (2002) reported that more than 1,100 plant species have been used ethnopharmacologically or experimentally to treat diabetes mellitus. A number of plants are known to exert their antihyperglycemic activity via the inhibition of carbohydrate hydrolyzing enzymes. Therefore, natural inhibitors from plant sources can offer an attractive strategy for the effective control of postprandial hyperglycemia (Ali et al. 2006; Mai and Chuyen 2007).

In this investigation, we have chosen frequently prescribed medicinal plants for the treatment of diabetes in Traditional Iranian Medicine and studied the in vitro ability of the plants to inhibit the activity of pancreatic α-amylase. However, these plants are not much explored for this bioactivity.

2 Materials and methods

2.1 Chemicals

All the chemicals were purchased from Sigma-aldrich Chemie Gmbh (Germany) and Merck (Germany). The chemicals were of the analytical grades.

2.2 Plant materials

The leaves of *Juglans regia* L. (*Juglandaceae*), *Olea europaea* L. (*Oleaceae*), *Camellia sinensis* (L.) Ktze. (*Theaceae*), *Coriandrum sativum* L. (*Umbelliferae*), *Trigonella foenum-graecum* L. (*Leguminosae*), *Urtica dioica* L. (*Urticaceae*); fruits of *Coriandrum sativum* L. (*Umbelliferae*); seeds of *Urtica pilulifera* L. (*Urticaceae*) and *Trigonella foenum-graecum* L. (*Leguminosae*); roots of *Arctium lappa* L. (*Compositae*) and flowers of *Calendula officinalis* L. (*Compositae*) and *Hibiscus gossypifolius* Mill. (*Malvaceae*) were collected or purchased from different parts of Iran during spring and summer 2007 and authenticated by the Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University of Medical Sciences Tehran, Iran where the voucher specimens have been deposited.

2.3 Extraction and fractionation procedure

The dried and fine plant parts (100 g) were extracted with ethanol (70%, 500 mL) through maceration (48 h × three times). The crude extracts were filtered and concentrated under reduced pressure at approximately 40°C.

The crude hydroethanol extract of *Camellia sinensis* (as the most potent extract) was suspended in a mixture of ethanol–water and partitioned successively with *n*-hexane, dichloromethane and ethyl acetate. Each fraction was then concentrated under reduced pressure at approximately 40°C to obtain *n*-hexane, dichloromethane, ethyl acetate and residual fractions.

2.4 α-Amylase inhibition test

The α-amylase inhibition assay was adopted and modified from Giancarlo et al. (2006). The starch solution (0.5% w/v) was obtained by stirring and boiling 0.25 g of starch potato soluble in 50 mL of deionized water for 15 min. The enzyme solution (0.5 IU/mL) was prepared by mixing 0.001 g of α-amylase (EC 3.2.1.1) in 100 mL of 20 mM sodium phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride. The extracts and/or fractions were dissolved in DMSO to give suitable concentrations (2.304 and 0.640 mg/mL for crude extracts and fractions, respectively) for the assay. The color reagent was a solution containing 96 mM 3,5-dinitrosalicylic acid (20 mL), 5.31 M sodium potassium tartrate in 2 M sodium hydroxide (8 mL) and deionized water (12 mL).

1 mL of the extracts and/or fractions and 1 mL of the α-amylase solution were mixed in a tube and incubated at 25°C for 30 min. To 1 mL of this mixture was added 1 mL of starch solution and the tube was incubated at 25°C for 3 min. Then, 1 mL of the color reagent was added and the closed tube placed into an 85°C water bath. After 15 min, the reaction mixture was removed from water bath and cooled thereafter, diluted with 9 mL distilled water and the absorbance value was determined at 540 nm in a Shimadzu Multispect-1501 spectrophotometer (Kyoto, Japan). Individual blanks were prepared for correcting the background absorbance. In this case, the color reagent solution was added prior to the addition of starch solution and then, the tube placed into

the water bath. The other procedures were carried out as above. Controls were conducted in an identical fashion replacing extracts and/or fractions with 1 mL DMSO. Acarbose was used as positive control. The inhibition percentage of α-amylase was assessed by the following formula:

$$I_{\alpha-\text{amylase}}\% = 100 \times \left(\frac{\Delta A_{\text{control}} - \Delta A_{\text{sample}}}{\Delta A_{\text{control}}}\right)$$

$$\Delta A_{\text{control}} = A_{\text{test}} - A_{\text{blank}}$$

$$\Delta A_{\text{sample}} = A_{\text{test}} - A_{\text{blank}}$$

For the extracts and fractions that were shown to exert a significant inhibition (i.e. the extracts obtained from leaves of *Camellia sinensis*, *Trigonella foenum-graecum* and *Urtica dioica*, and seeds of *Trigonella foenum-graecum* and ethyl acetate and residual fractions derived from the extract of *Camellia sinensis* leaves with a value of $I_{\alpha\text{-amylase}} > 50\%$), dose-dependent inhibitory assays were also performed and a logarithmic regression curve was establish for each of them in order to calculate the IC_{50} values (inhibitory concentration).

2.5 Statistical analysis

The data were expressed as mean ± SEM for five experiments in each group. The IC_{50} values were estimated by nonlinear curve-fitting. One-way analysis of variance (ANOVA) followed by Tukey's post test was used to assess the presence of significant differences ($P < 0.05$) between the inhibitory activities. All the statistical analyses were accomplished using the computer software GraphPad Prism 3.02 for Windows (GraphPad Software, USA).

3 Results and discussion

α-Amylase is one of the main enzymes in human that is responsible for the breakdown of starch to more simple sugars thus the inhibitors of this enzyme can delay the carbohydrate digestion and reduce the rate of glucose absorption. Consequently, they can decrease the attenuated postprandial plasma glucose levels and improve the glucose tolerance in diabetic patients (Ali et al. 2006; Kwon et al. 2006). Therefore, in this study, α-amylase inhibitory activity of ten medicinal plants from Iran were studied and compared to each other. These plants have been used frequently to treat patients suffering from diabetes. Although there are scientific reports about antidiabetic activities of some of these plants especially *T. foenum-graecum* (Abdel-Barry et al. 1997; Bordia et al. 1997; Dewi et al. 2007; Jelodar et al. 2005; Kumar et al. 2005; Raju et al. 2001; Vats et al. 2002; Vijayakumar et al. 2005; Xue et al. 2007), *C. sinensis* (Babu et al. 2007; Gomes et al. 1995; Mackenzie et al. 2007; Shoji and Nakashima 2006; Tsuneki et al. 2004), *U. dioica* (Bnouham et al. 2003; Farzami et al. 2003; Onal et al. 2005) and their antihyperglycemic mechanisms, there are no previous studies, at least to our knowledge, on the activity of the plants on α-amylase activity.

Table 1 α-Amylase inhibitory effects of the studied plants

Sample	Part used	Percentage inhibition (at the concentration 2.304 mg/mL)[a]	IC_{50} (mg/mL)[b]
Trigonella foenum-graecum	Leaf	57.27 ± 0.33	1.92 (1.90–1.94)
Trigonella foenum-graecum	Seed	54.34 ± 0.40	1.87 (1.83–1.91)
Camellia sinensis	Leaf	52.26 ± 0.26	1.54 (1.47–1.62)
Urtica dioica	Leaf	51.59 ± 0.22	1.89 (1.83–1.95)
Coriandrum sativum	Fruit	33.59 ± 0.62	–
Coriandrum sativum	Leaf	20.93 ± 0.81	–
Urtica pilulifera	Seed	40.67 ± 0.40	–
Calendula officinalis	Flower	11.13 ± 0.31	–
Juglans regia	Leaf	28.73 ± 0.15	–
Olea europaea	Leaf	15.84 ± 0.26	–
Hibiscus gossypifolius	Flower	25.43 ± 0.40	–
Arctium lappa	Root	35.06 ± 0.38	–
Acarbose (positive control)	–	–	0.025 (0.023–0.026)

[a] α-Amylase inhibitory activities values are means ± SEM ($n = 5$)

[b] The IC_{50} values have been presented along with their respective 95% confidence limits ($n = 5$)

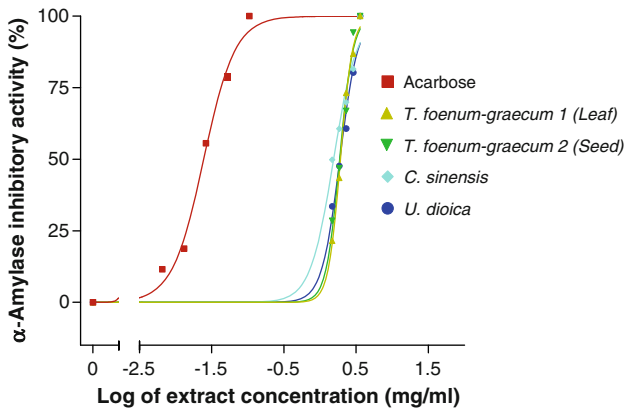

Fig. 1 α-Amylase inhibitory effects of the studied plants. *Each point* represents the mean of five experiments and the *vertical bars* represent the SEM

Table 2 α-Amylase inhibitory effects of the fractions obtained from the extract of *Camellia sinensis*

Sample	Percentage inhibition (at the concentration 0.640 mg/mL)[a]	IC_{50} (mg/mL)[b]
Residual fraction	57.65 ± 0.69	0.52 (0.51–0.53)
Ethyl acetate fraction	52.26 ± 0.26	0.53 (0.52–0.54)
Dichloromethane fraction	20.72 ± 0.54	–
n-Hexane fraction	12.60 ± 0.60	–
Acarbose (Positive control)	–	0.025 (0.023–0.026)

[a] α-Amylase inhibitory activities values are means ± SEM ($n = 5$)

[b] The IC_{50} values have been presented along with their respective 95% confidence limits ($n = 5$)

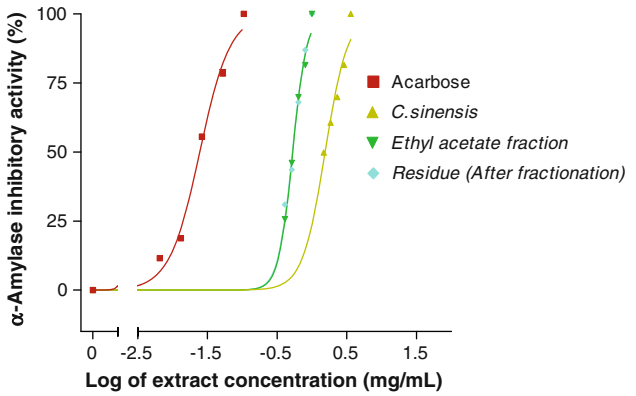

Fig. 2 α-Amylase inhibitory effects of the fractions obtained from the extract of *Camellia sinensis. Each point* represents the mean of five experiments and the *vertical bars* represent the SEM

The α-amylase inhibitory activity varied among the tested plants. The most potent inhibition appeared to be present in the extracts of leaves of *Camellia sinensis*, *Trigonella foenum-graecum* and *Urtica dioica*, and seeds of *Trigonella foenum-graecum*. They strongly inhibited the α-amylase activity ($I_{\alpha\text{-amylase}} > 50\%$) at the concentration of 2.304 mg/mL (Table 1). Therefore, the dose dependent α-amylase inhibitory activities of these plants were further studied and their IC_{50} values calculated. All of them demonstrated a significant dose-dependent reduction in the α-amylase activity and *C. sinensis* extract exhibited the highest inhibitory effect ($P < 0.001$) with an $IC_{50} = 1.54$ (1.47–1.62) mg/mL (Fig. 1 and Table 1).

Since *C. sinensis* extract displayed a favorable inhibitory activity on α-amylase, we focused on the four fractions obtained from the extract by the stepwise solvent–solvent extraction process. The results obtained indicate that the ethyl acetate and residual fractions (at the concentration 0.64 mg/mL) were able notably to inhibit the α-amylase activity ($I_{\alpha\text{-amylase}} > 50\%$) (Table 2). Furthermore, the incubation of graded concentrations of the two fractions (ethyl acetate and residual fractions) with the α-amylase and starch resulted in a noticeable and concentration dependent reduction in the enzyme activity and starch breakdown [$IC_{50} = 0.53$ (0.52–0.54) mg/mL and $IC_{50} = 0.52$ (0.51–0.53) mg/mL, respectively] (Fig. 2 and Table 2). However, no significant differences were observed between their activities ($P > 0.05$).

Preliminary phytochemical screening of the two fractions revealed that they were rich in phenolic compounds especially flavonoids. Flavonoids are a group of polyphenolic compounds which have been reported to possess inhibitory activities on α–glycosidase and amylase (Kim et al. 2000; Mcdougall and Stewart 2009). Hence, the presence of phenolic and flavonoid content in the fractions would have contributed toward α-amylase inhibition.

The traditional Iranian medicine has several reports on antidiabetic plants. However, many of these plants are used for the treatment of diabetes mellitus with no mechanistic basis known of their functioning. The present investigation shows that the antidiabetic property of some of the plants (especially leaves of *Camellia sinensis*), least in part, can be related with their α-amylase inhibitory effects. These results also indicate that *C. sinensis* leaves might possess a poetically therapeutic effect in the type 2 diabetes mellitus therefore it could be considered as a candidate for further studies to isolate carbohydrate α-amylase inhibitors.

Acknowledgments We would like to thank Shahid Beheshti University of Medical Sciences for financial support of this project.

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