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Original Article

Inhibitory Effects of Six *Allium* Species on α-Amylase Enzyme Activity

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Abstract

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia. The management of the blood glucose level is a critical strategy in the control of diabetes complications. Inhibitors of carbohydrate hydrolyzing enzymes have been useful as oral hypoglycemic drugs for the control of hyperglycemia especially in patients with type II disables mellitus. The goal of the present study was to investigate the inhibitory effects of six selected *Allium* species (*A. akaka*, *A. ampeloprasum* subsp. *iranicum*, *A. cepa*, *A. hirtifolium*, *A. porrum* and *A. sativum*) on α-amylase enzyme using an in vitro model. According to the results, ethanol extracts of *A. akaka*, *A. sativum*, *A. porrum* and *A. cepa* were found to have a favorable α-amylase inhibitory activity with IC_{50} values of 16.74, 17.95, 15.73 and 16.36 mg/ml, respectively and they did not reveal any significant differences in their IC_{50} values ($p > 0.05$). However, the two other *Allium* species tested (*A. ampeloprasum* subsp. *iranicum* and *A. hirtifolium*) did not show valuable inhibitory activity.

Keywords: *Allium* species; α-Amylase inhibitory activity; Antihyperglycemic activity; Antidiabetic activity.

Introduction

Carbohydrates are the major constituents of human diet and polysaccharides are one of the main components of carbohydrates that mainly play a role in the energy supply. The dietary carbohydrates should first be broken down to monosaccharides by some gastrointestinal enzymes, since only manosaccharides can be absorbed from intestinal lumen (1, 2). α-Glucosidase and α-amylase are the key enzymes involved in the digestion of carbohydrates (3). α-Amylase degrades complex dietary carbohydrates to oligosaccharides and disaccharides that are ultimately converted into monosaccharides by α-glucosidase. Liberated glucose is then absorbed by the gut and results in postprandial hyperglycemia (4, 5). The inhibition of enzymes involved in the digestion of carbohydrates can significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet by delaying the process of carbohydrate hydrolysis and absorption (2, 6). The control of postprandial hyperglycemia is an important strategy in the management of diabetes mellitus, especially type II diabetes, and reducing chronic complications associated with the disease (3, 7). Therefore, such enzyme inhibitors can be useful in the treatment of type II diabetes (8). There are several reports of established enzyme inhibitors such as acarbose, miglitol, voglibose,

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nojirimycin and 1-deoxynojirimycin and their favorable effects on blood glucose levels after food uptake (6). On the other hand, enzyme inhibitors may also act as effective antiobesity agents (9).

Plants have long been used for the ethnomedical treatment of diabetes in various systems of medicine and accepted as an alternative for diabetes treatment. In recent years, research on medicinal plants for the management of diabetes has attracted the interest of scientists (3, 11). A number of plants are known to exert their antihyperglycemic activity via inhibition of carbohydrate hydrolyzing enzymes. That is why natural enzyme inhibitors from plant sources have offered an attractive strategy for the control of postprandial hyperglycemia (12).

The edible *Allium* species are of major economic and dietary importance all over the world. Garlic (*A. sativum* L.) and common onion (*A. cepa* L.) have a very long history of use as food ingredients and medicine and they are grown, traded and consumed in most countries. Their bulbs and corms (raw or cooked) are wonderfully nutritious and therapeutic. The two plants are samples of natural foods that could prevent the development of the different diseases (13). Investigations conducted on garlic and onion (and some of other *Allium* species) show that the plants have wide and diverse biological activities including antidiebetic, antiatherosclerotic, antithrombotic, antihypertensive, antihyperlipidemic, anti-inflammatory, antioxidant, etc (14).

The antidiabetic property of some *Allium* species has been studied by some scientists (15-20). However, no information is available on α-amylase inhibitory activity of *Allium* species. The aim of this study was to examine the in vitro α-amylase inhibitory potency of six *Allium* species including *A. akaka* Gmel., *A. ampeloprasum* L. subsp. *iranicum*, *A. cepa* L., *A. hirtifolium* Boiss., *A. porrum* L. and *A. sativum* L. and to compare them with each other.

Experimental

Chemicals

All the chemicals were purchased from Sigma-Aldrich Chemie Gmbh (Germany) and Merck (Germany) companies. The chemicals were of analytical grade.

Plant materials

The bulbs of *A. cepa*, leaves of *A. ampeloprasum* subsp. *iranicum* and corms of *A. sativum* were collected in Masal, in Gilan province, Iran in May 2007. The bulbs of *A. hirtifolium* and leaves of *A. akaka* and *A. porrum* were purchased from local markets in Tehran, Iran. Voucher specimens were confirmed and deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University (M. C.), Tehran, Iran.

Extraction

The dried and fine plant parts (100 g) were extracted with ethanol 90% through maceration (48 h × 3 times). The crude extracts were filtered and concentrated under reduced pressure at approximately 40 °C.

α-Amylase inhibition test

The α-amylase inhibitory activity for each extract was determined based on the colorimetric assay using acarbose as the reference compound (21). The starch solution (0.5% w/v) was obtained by stirring and boiling 0.25 g of soluble potato starch in 50 ml of deionized water for 15 min. The enzyme solution (0.5 unit/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 were dissolved in DMSO to give concentrations from 11.8 to 36.0 mg/ml (11.8, 14.7, 18.4, 23.0, 28.8, 36.0 mg/ml). 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).

One ml of each plant extract and 1 ml enzyme 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 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 the water bath, cooled and diluted

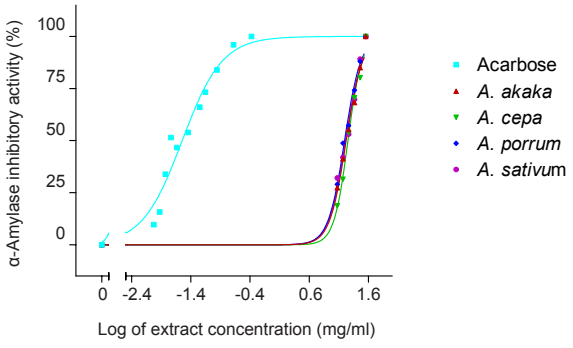

Figure 1. α-Amylase inhibitory activities of the studied *Allium* species. Each point represents the mean of five experiments and the vertical bars represent the SEM.

with 9 ml distilled water and the absorbance value 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 the plant extracts with 1 ml DMSO. Acarbose solution (at the concentrations of 0.0094, 0.0118, 0.0147, 0.0184, 0.023, 0.036, 0.056, 0.07, 0.11, 0.21, 0.42 µg/ml) was used as positive control. The inhibition percentage of α-amylase was assessed by the following formula:

$$I_{\alpha\text{-amylase}}\% = 100 \times (\Delta A_{Control} - \Delta A_{Sample}) / \Delta A_{Control}$$

$$\Delta A_{Control} = A_{Test} - A_{Blank}$$

$$\Delta A_{Sample} = A_{Test} - A_{Blank}$$

The $I_{\alpha\text{-amylase}}$% was plotted against the sample concentration and a logarithmic regression curve established in order to calculate the IC_{50} value (inhibitory concentration). This would represent the concentration of sample (mg/ml) necessary to decrease the absorbance of α-amylase by 50%.

Statistical analysis

The data were expressed as mean±SEM for five experiments in each group. The IC_{50} values were estimated by non linear curve-fitting and presented as their respective 95% confidence limits. One-way analysis of variance (ANOVA) followed by Tukey's post test was used to assess the significant differences ($p<0.05$) between the extracts. All the statistical analyses were accomplished using the computer software GraphPad Prism 3.02 for Windows (GraphPad Software, USA).

Results and Discussion

Although there are citations of antihyperglycemic and antidiabetic activity of some *Allium* species (15-20), there are no previous reports, at least to our knowledge, on the activity of the genus on in vitro α-amylase activity. In the present study, of six *Allium* species tested, four species including *A. akaka*, *A. cepa*, *A. porrum* and *A. sativum* were found to possess favorable inhibitory effects on starch break down in vitro. Incubation of graded concentrations of the extracts (11.8-36.0 mg/ml) with α-amylase and starch in vitro resulted in a noticeable decrease in the enzyme activity (%) from 18.49±0.48 to 67.48±0.26 for *A. akaka*, from 10.27±0.48 to 54.96±0.40 for *A. sativum*, from 20.94±0.48 to 72.19±0.35 for *A. porrum* and from 25.96±0.25 to 80.94±0.34 for *A. cepa*. The IC_{50} values for *A. akaka*, *A. sativum*, *A. porrum* and *A. cepa* extracts were 16.74 (16.30-17.18) mg/ml, 17.95 (17.57-18.33) mg/ml, 15.73 (15.33-16.14) mg/ml and 16.36 (15.84-16.91) mg/ml, respectively (Table 1 and Figure 1). The mentioned extracts exhibited concentration-dependent effects and did not show any significant differences in their IC_{50} values ($p>0.05$). However, *A. ampeloprasum* subsp. *iranicum* and *A. hirtifolium* extracts produced a week enzyme inhibition and they did not achieve 50% inhibition of the enzyme activity. The maximum inhibition (%) was 48.36±0.35 for *A. ampeloprasum* subsp. *iranicum* and 33.29±0.35 for *A. hirtifolium* at a concentration of 36.0 mg/ml (Table 1).

Drugs that inhibit carbohydrate hydrolyzing enzymes have been demonstrated to decrease postprandial hyperglycemia and improve impaired glucose metabolism without promoting

Table 1. α-Amylase inhibitory activities and IC_{50} values of the studied *Allium* species extracts.

Plant species	Concentration (mg/ml)	Inhibition (%)[a]	IC_{50} (mg/ml)[b]
A. akaka	36.0	67.48±0.26	16.74 (16.30-17.18)
	28.8	57.41±0.35	
	23.0	46.08±0.39	
	18.4	37.30±0.39	
	14.7	27.81±0.35	
	11.8	18.49±0.48	
A. ampeloprasum subsp. *iranicum*	36.0	48.36±0.35	-
	28.8	41.23±0.35	
	23.0	34.46±0.30	
	18.4	27.81±0.35	
	14.7	20.68±0.35	
	11.8	13.93±0.40	
A. sativum	36.0	54.96±0.40	17.95 (17.57-18.33)
	28.8	44.11±0.35	
	23.0	38.78±0.48	
	18.4	29.53±0.47	
	14.7	17.28±0.35	
	11.8	10.27±0.48	
A. hirtifolium	36.0	33.29±0.35	-
	28.8	24.76±0.35	
	23.0	17.10±0.26	
	18.4	11.62±0.39	
	14.7	3.26±0.25	
	11.8	0.00±0.00	
A. porrum	36.0	72.19±0.35	15.73 (15.33-16.14)
	28.8	63.53±0.35	
	23.0	53.56±0.40	
	18.4	41.31±0.30	
	14.7	35.23±0.40	
	11.8	20.94±0.48	
A. cepa	36.0	80.94±0.34	16.36 (15.84-16.91)
	28.8	72.11±0.34	
	23.0	56.53±0.25	
	18.4	43.03±0.34	
	14.7	33.95±0.34	
	11.8	25.96±0.25	

*Note: The IC_{50} value of the positive control, acarbose, was measured as 0.028 (0.026–0.031) µg/ml.
[a] α-Amylase inhibitory activities values are means±SEM (n=5).
[b] The IC_{50} values are presented with their respective 95% confidence limits (n=5).

insulin secretion in NIDDM patients. These medications are most useful for people who have just been diagnosed with type II diabetes. They also are useful for people taking oral antidiabetic agents who need an additional medication to keep their blood glucose levels within a safe range (2, 3, 6-8). Our in vitro studies demonstrate an appreciable α-amylase

inhibitory activity of some *Allium* species, especially *A. akaka*, *A. cepa*, *A. porrum* and *A. sativum* so, they are good candidates for further studies to isolate carbohydrate hydrolyzing enzyme inhibitors.

References

(1) Abesundara KJ, Matsui T and Matsumoto K. alpha-Glucosidase inhibitory activity of some Sri Lanka plant extracts, one of which, *Cassia auriculata*, exerts a strong antihyperglycemic effect in rats comparable to the therapeutic drug acarbose. *J. Agric. Food Chem.* (2004) 52: 2541-2545

(2) Kwon YI, Jang HD and Shetty K. Evaluation of *Rhodiola crenulata* and *Rhodiola rosea* for management of type II diabetes and hypertension. *Asia. Pac. J. Clin. Nutr*. (2006) 15: 425-432

(3) Ali H, Houghton PJ and Soumyanath A. alpha-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus. J. Ethnopharmacol.* (2006) 107: 449-455

(4) Kim JS, Kwon CS and Son KH. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. *Biosci. Biotechnol. Biochem.* (2000) 64: 2458-2461

(5) Shim YJ, Doo HK, Ahn SY, Kim YS, Seong JK, Park IS and Min BH. Inhibitory effect of aqueous extract from the gall of *Rhus chinensis* on alpha-glucosidase activity and postprandial blood glucose. *J. Ethnopharmacol.* (2003) 85: 283-287

(6) Kim YM, Jeong YK, Wang MH, Lee WY and Rhee HI. Inhibitory effect of pine extract on alpha-glucosidase activity and postprandial hyperglycemia. *Nutrition* (2005) 21: 756-761

(7) Subramanian R, Asmawi MZ and Sadikun A. *In vitro* alpha-glucosidase and alpha-amylase enzyme inhibitory effects of *Andrographis paniculata* extract and andrographolide. *Acta Biochim. Pol*. (2008) 55: 391-398

(8) Schäfer A and Högger P. Oligomeric procyanidins of French maritime pine bark extract (Pycnogenol) effectively inhibit alpha-glucosidase. *Diabetes Res. Clin. Pract.* (2007) 77: 41-46

(9) Kotowaroo MI, Mahomoodally MF, Gurib-Fakim A and Subratty AH. Screening of traditional antidiabetic medicinal plants of Mauritius for possible alpha-amylase inhibitory effects *in vitro. Phytother. Res.* (2006) 20: 228-231

(10) Mai TT and Chuyen NV. Anti-hyperglycemic activity of an aqueous extract from flower buds of *Cleistocalyx operculatus* (Roxb.) Merr and Perry. *Biosci. Biotechnol. Biochem*. (2007) 71: 69-76

(11) McCue P, Kwon YI and Shetty K. Anti-diabetic and anti-hypertensive potential of sprouted and solid-state bioprocessed soybean. *Asia. Pac. J. Clin. Nutr.* (2005) 14: 145-152

(12) Onal S, Timur S, Okutucu B and Zihnioğlu F. Inhibition of alpha-glucosidase by aqueous extracts of some potent antidiabetic medicinal herbs. *Prep. Biochem. Biotechnol.* (2005) 35: 29-36

(13) Stajner D, Igić R, Popović BM and Malencić Dj. Comparative study of antioxidant properties of wild growing and cultivated *Allium* species. *Phytother. Res.* (2008) 22: 113-117

(14) Nencini C, Cavallo F, Capasso A, Franchi GG, Giorgio G and Micheli L. Evaluation of antioxidative properties of *Allium* species growing wild in Italy. *Phytother. Res.* (2007) 21: 874-878

(15) Islam MS and Choi H. Comparative effects of dietary ginger (*Zingiber officinale*) and garlic (*Allium sativum*) investigated in a type 2 diabetes model of rats. *J. Med. Food* (2008) 11: 152-159

(16) Eidi A, Eidi M and Esmaeili E. Antidiabetic effect of garlic (*Allium sativum* L.) in normal and streptozotocin-induced diabetic rats. *Phytomedicine* (2006) 13: 624-629

(17) Musabayane CT, Bwititi PT and Ojewole JA. Effects of oral administration of some herbal extracts on food consumption and blood glucose levels in normal and streptozotocin-treated diabetic rats. *Methods Find Exp. Clin. Pharmacol.* (2006) 28: 223-228

(18) Jelodar GA, Maleki M, Motadayen MH and Sirus S. Effect of fenugreek, onion and garlic on blood glucose and histopathology of pancreas of alloxan-induced diabetic rats.*Indian J. Med. Sci.* (2005) 59: 64-69

(19) Campos KE, Diniz YS, Cataneo AC, Faine LA, Alves MJ and Novelli EL. Hypoglycaemic and antioxidant effects of onion, *Allium cepa*: dietary onion addition, antioxidant activity and hypoglycaemic effects on diabetic rats. *Int. J. Food Sci. Nutr.* (2003) 54: 241-246

(20) Roman-Ramos R, Flores-Saenz JL and Alarcon-Aguilar FJ. Anti-hyperglycemic effect of some edible plants. *J. Ethnopharmacol.* (1995) 48: 25-32

(21) Nickavar B, Abolhasani L and Izadpanah H. α-Amylase inhibitory activities of six *Salvia* species. *Iran. J. Pharm. Res.* (2008) 7: 297-303

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