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Original Article

Influence of Three *Morus* Species Extracts on α-Amylase Activity

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Abstract

Diabetes mellitus is one of the most common endocrine diseases and its type II is the major form of diabetes, accounting for 90% of cases worldwide. The inhibition of carbohydrate hydrolyzing enzymes such as α-amylase can be an important strategy in the control of blood glucose levels in patients with type II diabetes. In this investigation, three *Morus* species, *M. alba*, *M. alba* var. *nigra* and *M. nigra*, were studied for their possible effects on the starch breakdown by α-amylase *in vitro*. *M. alba* var. *nigra* [IC_{50}=13.26 (12.86-13.66) mg/ml] and *M. alba* [IC_{50}=17.60 (17.39-17.80) mg/ml] revealed appreciable α-amylase inhibitory activities in a concentration-dependent manner. Furthermore, the most active extract (i.e. *M. alba* var. *nigra*,) was partitioned by stepwise solvent-solvent extraction process and the inhibitory activities of each of the fractions on the enzyme were studied. According to the results, all the fractions (*n*-hexane, chloroform, ethyl acetate and aqueous fractions) had potent inhibitory effects on the α-amylase activity. However, the lowest inhibitory potency was observed for the aqueous fraction.

Keywords: α-Amylase inhibitory activity; Diabetes mellitus; *Morus* species; Postprandial hyperglycemia.

Introduction

Diabetes mellitus is an endocrinal chronic disease caused by altered carbohydrate metabolism and characterized by elevated blood glucose levels. There are two main types of diabetes, type I and type II that they affect more than 200 million people worldwide. The most prevalent form of diabetes is non-insulin dependent diabetes mellitus (NIDDM/type II) accounting for 90% of cases throughout the world (1-3).

The control of hyperglycemia is critical in the management of diabetes because in long term, acute and chronic complications can occur if blood glucose concentration is not kept in normal levels. One therapeutic approach to decrease the hyperglycemia, especially after a meal, is to retard and reduce the digestion and absorption of ingested carbohydrates through the inhibition of carbohydrate hydrolyzing enzymes (such as α-amylase and/or α-glucosidases) in the digestive organs. As a result, these inhibitors could decrease the postprandial rise in blood glucose concentration. For example, acarbose (an α-amylase inhibitor) could inhibit the action of α-amylase enzyme leading to a reduction in the breakdown of starch into glucose (1, 4, 5). Furthermore, the other putative beneficial effect of the inhibitors is their ability to induce weight loss (6, 7).

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

Plant species	Concentration (mg/mL)	Inhibition (%)[1]	IC_{50} (mg/mL)[2,3]
M. alba	23.0	75.58 ± 1.10	17.60 (17.39 - 17.80)[a]
	18.4	45.91 ± 0.73	
	14.7	9.60 ± 0.35	
	11.8	4.40 ± 0.26	
M. alba var. *nigra*	23.0	73.91 ± 0.35	13.26 (12.86 -13.66)[b]
	18.4	66.07 ± 0.53	
	14.7	40.29 ± 0.36	
	11.8	29.79 ± 0.34	
M. nigra	23.0	20.19 ± 0.36	-
	18.4	25.76 ± 0.82	
	14.7	34.81 ± 0.90	
	11.8	46.56 ± 1.20	

* Note: The IC_{50} value of the positive control, acarbose, was measured as 0.026 (0.024 - 0.029) μg/mL.
[1] α-Amylase inhibitory activities values are means ± SEM (n =5).
[2] The IC_{50} values are presented along with their respective 95% confidence limits (n=5).
[3] Letters (*a*-*b*) denote homogenous subsets at $P < 0.001$ (Tukey's post test).

However, the management of diabetes by chemical drugs without any side effects is still a challenge to the medical system. Therefore, many efforts have been made to identify new antidiabetic agents from different sources, especially medicinal plants because of their effectiveness, fewer side effects and relatively low cost (8, 9). Approximately 800 plants worldwide have been documented to support antidiabetic effects (10) and several plant species have been advocated in Traditional Iranian Medicine for their hypoglycemic effects (11).

The leaves of mulberry trees (*Morus* species, Moraceae) have long been used to control diabetes mellitus all around the world, especially in Iran (11). The hypoglycemic effects of *Morus* species have been supported by various scientific studies and N-containing sugars (such as 1-deoxynojirimycin and its derivatives) have been identified in the leaves of mulberry trees as potent inhibitors of α-glucosidases (12-15). However, at least to our knowledge, there are no previous reports on the α-amylase inhibitory effect of *Morus* species.

The aim of the present study was to examine the *in vitro* α-amylase inhibitory activity of three mulberries [including *M. alba* L. (white mulberry), *M. alba* L. var. *nigra* and *M. nigra* L. (black mulberry)] and to compare the results.

Experimental

Chemicals

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

Plant materials

The leaves of *Morus alba* L., *Morus alba* L. var. *nigra* and *Morus nigra* L. (Moraceae) were collected from Tehran Province, Iran in May 2006. 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 and fractionation procedure

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

The crude ethanol extract of *Morus alba* var. *nigra* (as the most potent extract) was dissolved in a mixture of ethanol-water (20:80 v/v) at room temperature and partitioned successively with *n*-hexane, chloroform and ethyl acetate. An aqueous final fraction was also obtained.

The fractions were concentrated under reduced pressure at 40°C.

Assessment of α-amylase inhibition

The α-amylase inhibition assay was performed according to our previous report (16). 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 and/or fractions were dissolved in DMSO to give suitable concentrations 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).

One ml of each the extracts and/or fractions and 1 ml of the enzyme solution were mixed in a test tube and incubated at 25°C for 30 min. To 1 ml of this mixture was added 1 ml of the starch solution and the tube was further incubated at 25°C for 3 min. Then, 1 ml of the color reagent was added and the stoppered tube was placed into an 85°C water bath. After 15 min, the reaction mixture was removed from the water bath and cooled thereafter, diluted with 9 ml distilled water and the absorbance value determined at 540 nm using 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 before the addition of starch solution and then, the tube was placed into the water bath. Then, the method was followed as described above. Controls were conducted in an identical manner, replacing extracts and/or fractions with 1 ml DMSO. Acarbose solution 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 sample concentration and a logarithmic regression curve was obtained in order to calculate the IC_{50} value which is concentration of sample (mg/ml) necessary to decrease the absorbance of α-amylase solution by 50%.

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 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 presence of significant differences ($P<0.001$) between the extracts (or fractions). All of the statistical analyses were accomplished using the computer software GraphPad Prism 3.02 for Windows (GraphPad Software, USA).

Results and Discussion

Carbohydrate hydrolyzing enzymes play main roles in the carbohydrate digestion and absorption. The enzymes degrade poly- and oligosaccharides to monosaccharides before they can be absorbed. The inhibition of these enzymes would delay the degradation of the complex sugars such as starch and prolong overall carbohydrate digestion time which would in turn cause a reduction in the rate of glucose absorption and consequently the decrease of postprandial glucose concentration rise (9). The stable blood glucose level is important for diabetic patients, because it prevents the hyperglycemia and the complications associated with diabetes. Therefore, carbohydrate hydrolyzing enzyme inhibitors are one of the essential drugs for managing type II diabetes (10).

In this work, the inhibition activities of the crude extracts obtained from *M. alba*, *M. alba* var. *nigra*, *M. nigra* were investigated on the α-amylase enzyme and IC_{50} values were calculated (Figure 1 and Table 1). Among the plants studied, two species, *M. alba*, and *M. alba* var. *nigra*, demonstrated inhibitory concentration-dependent effects on the α-amylase activity. The strongest activity was shown by the extract of *M. alba* var. *nigra* [IC_{50}=13.26 (12.86-13.66) mg/ml]. *M. alba* extract revealed a weaker activity

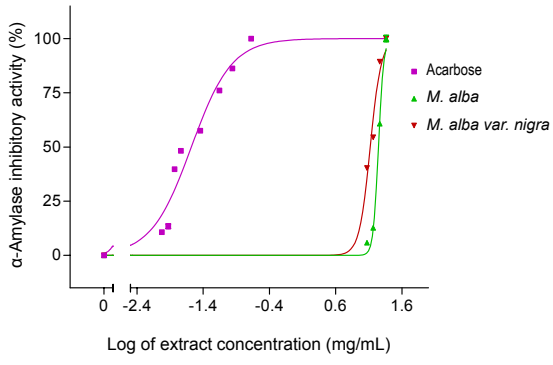

Figure 1. α-Amylase inhibitory activities of the studied *Morus* spp. extracts. Each point represents the mean of five experiments and the vertical bars represent the SEM.

[IC_{50}=17.60 (17.39-17.80) mg/ml] ($P < 0.001$). Although, enzyme inhibition activity was observed by the extract of *M. nigra* , but it was not concentration-dependent inhibition (Table 1). The phenomena probably due to the fact that at high concentrations of the extract, there is a conformational change derived from binding of compounds to the enzyme (1, 17, 18). The results of this study indicate that the administration of some of *Morus* species can probably manage the postprandial blood glucose levels and confirm the usage of these plants prescribed as a treatment of diabetes in Traditional Medicine in Iran and other countries.

Since *M. alba* var. *nigra* extract displayed a favorable inhibitory activity on α-amylase, we focused on the fractions of the extract. The extract was partitioned by the stepwise solvent-solvent extraction process and fractions were collected and checked for their inhibitory activities on the enzyme. The incubation of graded concentrations of the fractions with α-amylase and starch resulted in a noticeable and concentration-dependent reduction in the enzyme activity and starch breakdown. The IC_{50} values for *n*-hexane, chloroform and ethyl acetate fractions were 0.43 (0.41-0.44) mg/ml, 0.42 (0.39 - 0.46) mg/ml, 0.41 (0.39- 0.44) mg/ml, respectively, and no significant differences were observed on IC_{50} values of the fractions ($P > 0.05$). However, aqueous fraction had a fewer effect against α-amylase [IC_{50}= 0.58 (0.53 - 0.62) mg/ml] than the organic fractions ($P < 0.001$). These findings reveal that active compounds in *M. alba* var. *nigra* are of various molecules which belong to the different classes of natural products.

In general, our *in vitro* studies indicated that *Morus* species, especially *M. alba* var. *nigra*, can serve as natural α-amylase inhibitors and might possess therapeutic antidiabetic effects in the type II diabetes mellitus.

References

(1) 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

(2) 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

(3) Li Y, Wen S, Kota BP, Peng G, Li GQ, Yamahara J and Roufogalis BD. *Punica granatum* flower extract, a potent alpha-glucosidase inhibitor, improves postprandial hyperglycemia in Zucker diabetic fatty rats. *J. Ethnopharmacol.* (2005) 99: 239-244

(4) Funke I and Melzig MF. Traditionally used plants in diabetes therapy-phytotherapeutics as inhibitors of α-amylase activity. *Braz. J. Pharmacogn.* (2006) 16: 1-5

(5) Ye F, Shen Z and Xie M. alpha-Glucosidase inhibition from a Chinese medical herb (*Ramulus mori*) in normal and diabetic rats and mice. *Phytomedicine* (2002) 9: 161-166

(6) Bhat M, Zinjarde SS, Bhargava SY, Kumar AR and Joshi BN. Antidiabetic Indian plants: a good source of potent amylase inhibitors. *Evid. Based Complement. Alternat. Med.* doi:10.1093/ecam/nen040

(7) Zhong L, Furne JK and Levitt MD. An extract of black, green, and mulberry teas causes malabsorption of carbohydrate but not of triacylglycerol in healthy volunteers. *Am. J. Clin. Nutr.* (2006) 84: 551-555

(8) Singab AN, El-Beshbishy HA, Yonekawa M, Nomura T and Fukai T. Hypoglycemic effect of Egyptian *Morus alba* root bark extract: effect on diabetes and lipid peroxidation of streptozotocin-induced diabetic rats. *J. Ethnopharmacol.* (2005) 100: 333-338

(9) Bhandari MR, Jong-Anurakkun N, Hong G and Kawabata J. α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb, Pakhanbhed (*Bergenia ciliate* Haw.). *Food Chem.* (2008) 106: 247-252

(10) Büyükbalci A and Sedef Nehir El. Determination of *in vitro* antidiabetic effects, antioxidant activities and phenol contents of some herbal teas. *Plant Foods Hum. Nutr.* (2008) 63: 27-33

(11) Amin G. *Popular Medicinal Plants of Iran.* Vice-Chancellorship of Research, Tehran University of

Medical Sciences, Tehran (2005)
(12) Asano N, Yamashita T, Yasuda K, Ikeda K, Kizu H, Kameda Y, Kato A, Nash RJ, Lee HS and Ryu KS. Polyhydroxylated alkaloids isolated from mulberry trees (*Morus alba* L.) and silkworms (*Bombyx mori* L.). *J. Agric. Food Chem.* (2001) 49: 4208-4213
(13) Asano N, Oseki K, Tomioka E, Kizu H and Matsui K. N-Containing sugars from *Morus alba* and their glycosidase inhibitory activities. *Carbohydr. Res.* (1994) 259: 243-255
(14) Yamada H, Oya I, Nagai T, Matsumoto T, Kiyohara H and Omura S. Screening of alpha-glucosidase II inhibitor from Chinese herbs and its application on the quality control of mulberry bark. *Shoyakugaku Zasshi* (1993) 47: 47-55
(15) Hansawasdi C and Kawabata J. alpha-Glucosidase inhibitory effect of mulberry (*Morus alba*) leaves on Caco-2. *Fitoterapia* (2006) 77: 568-573
(16) Nickavar B, Abolhasani L and Izadpanah H. alpha-Amylase inhibitory activities of six *Salvia* species. *Iran. J. Pharm. Res.* (2008) 7: 297-303
(17) Kim JS, Kwon CS and Son KH. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. *Biosci. Biotechnol. Biochem.* (2000) 64: 2458-2461
(18) Gowri PM, Tiwari AK, Ali AZ and Rao JM. Inhibition of alpha-glucosidase and amylase by bartogenic acid isolated from *Barringtonia racemosa* Roxb. seeds. *Phytother. Res.* (2007) 21: 796-799

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