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Research Article

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Inhibitory Effect of Hydroalcoholic Extracts of Barberry, Sour cherry and Cornelian Cherry on α-amylase and α-Glucosidase activities

Farideh Shishehbor[1], Mohamad Ebrahim Azemi[2*], Davood Zameni[3] and Azadeh Saki[4]

[1]*Nutrition and Metabolic Disease Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
[2]*Department of Pharmacognosy, Medicinal Plant and Natural Product Research Center, School of Pharmacy, Jundishapur University of Medical Sciences, Ahvaz, Iran*
[3]*Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran*
[4] *Department of Biostatistics and Epidemiology, School of health, Ahvaz Jundishapur University of Medical Science, Ahvaz, IR Iran*
**Email: M.E.Azemi@ajums.ac.ir*

ABSTRACT

Reducing postprandial hyperglycemia is an efficient medical therapy to improve the health of diabetic patients. One of the main strategies to reduce postprandial hyperglycemia is reducing or inhibiting the digestion and absorption of carbohydrates through inhibiting carbohydrate digestive enzyme. This study aimed to investigate the the inhibitory effects of hydroalcoholic extracts of barberry (Berberis vulgaris), sour cherry (Prunus cerasus) and cornelian cherry (Cornus mas L.) fruit extracts against pancreatic α-amylase and intestinal α-glucosidase. The findings showed that barberry extract was the most potent inhibitor against the α-amylase with the IC_{50} values of 2.38 mg/ml. Cornelian cherry was also the most effective inhibitor of α-glucosidase with the IC_{50} values of 6.87 mg/ml. The results of the present study suggest that the extracts of barberry, cornelian cherry and sour cherry fruits can be useful in controlling postprandial hyperglycemia. However, their effects should be further investigated in controlled in vivo studies.

Keywords: Postprandial hyperglycemia, α-glucosidase, α-amylase, *Prunus cerasus*, *Breberis vulgaris*, *Cornus mas*

INTRODUCTION

Diabetes mellitus (DM), characterized by increased blood glucose is caused by defects in insulin secretion, impaired insulin action, or both. The prevalence of this chronic disease is increasing all over the world and increases the risk of mortality and economic cost (1, 2). The common characteristic of diabetes is hyperglycemia which leads to many clinical complications such as hyperlipidemia, hyperinsulinemia, hypertension and atherosclerosis (2). Increasing evidence indicates that the acute increases of plasma glucose, which is known as postprandial hyperglycemia, plays a decisive role in the development of diabetes complications compared with fasting hyperglycemia (3, 4). Therefore reducing postprandial hyperglycemia is an efficient medical therapy to improve the health of diabetic patients (3). One of the main strategies that are used to reduce postprandial hyperglycemia, is reducing or inhibiting the digestion and absorption of carbohydrates by inhibiting carbohydrate digestive enzymes such as α-amylase and α-glucosidase (3, 5, 6). During recent years medicinal herbs have been dramatically developed in different medical, industrial, and environmental fields.

Acarbose, miglitol and voglibose that are synthetically produced as an inhibitor of gastrointestinal α-glucosidase enzymes lead to lower postprandial plasma glucose. However, because of their side effects such as flatulence,

diarrhea, abdominal pain, and bloating a great attention has been paid to the natural inhibitors of these enzymes(5, 7). In this regard, so far different extracts of several plants have been studied and positive effects have been observed (5, 8).

Fruits, in particular cherries and berries, contain numerous bioactive compounds such as phenolic compounds. The potential inhibitory effects of phenolic compounds against carbohydrate-digesting enzymes have been shown by *in vitro* and *in vivo* studies (9-11).

Fruits of barberry (*Berberis vulgaris*), sour cherry (*Prunus cerasus*) and cornelian cherry (*Cornus mas L.*) are rich in polyphenols (12-17). However, their effects on digestive enzymes have not been yet examined. Therefore, the aim of the present study was to investigate the inhibitory effects of hydro-alcoholic extracts of these fruits against α-amylase and α-glucosidase.

MATERIALS AND METHODS

Chemicals:
All the chemicals used including α-amylase (EC 3.2.1.1) and α-glucosidase (EC 3.2.1.20) were purchased from Sigma-Aldrich Chemie Gmbh (Germany) and Merck (Germany) companies. The chemicals were of analytical grade.

Plant preparation:
The fruits of *Berberis vulgaris, Prunus cerasus* and *Cornus mas L.* were obtained from regions of Ghaenat County of Khorasan Province, Sardasht in Kurdistan Province and Alamut region of Qazvin province in Iran between July to October 2013. The plant material was identified at Department of Horticulture, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

The plant parts were dried in a drying room, with ventilation at ambient temperature. A voucher specimen for the plant was deposited at the Herbarium of the Department of Pharmacognosy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

The plant parts were dried in a dark room with ambient temperature. A voucher specimen for the plant was deposited at the Herbarium of the Department of Pharmacognosy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Extraction
All plants were extracted my maceration method, 100 g of each dried fruit was crushed as much as possible, and immersed in a sufficient amount of 80% ethanol solution for 72 hours. After filtration and evaporation of the solvent under reduced pressure using rotary evaporator, extracts were put in an oven with controlled temperature of 40° C as long as their weight was constant.

α-amylase inhibitory assay
In this assay we used Giancarlo et al.'s method with some modification (18). Different concentrations of the extracts were prepared by serial dilution and dimethyl sulfoxide (DMSO) and acarbose were used respectively as negative and positive controls. The hydrolysis of starch (as a substrate), by the enzyme, produces the reducing sugars and led to reduction of the color reagent (a mixture of 3, 5-Dinitrosalicylic acid [DNS] and potassium sodium tartrate). Generally, acarbose and possible extracts inhibit the enzyme and lead to a lesser reduction of the color reagent. Subsequently, the absorbance was read on the wavelength of 540 nm. To eliminate the effect of absorbance caused by other factors, in addition to the test tube, a tube blank was prepared for each concentration of the extract. The absorbance for each concentration was obtained from the difference between test and blank. The α-amylase inhibitory activity of the extracts was calculated using the following formula:

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

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

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

α- glucosidase inhibitory assay

The alpha-glucosidase inhibitory test was performed according to the Kapustka method with modification (19). Briefly, 1 ml of maltose solution and 200 microliter of α- glucosidase solution was added to 100 ml of different concentrations of extract and incubated at 37 ° C for 30 minutes. The reaction was terminated by the addition of 200 µl of perchloric acid. Thereafter, 80 µl of DIAN solution and 1.2 ml of PGO were added to 40 µl of the previous stage solution. The reaction was maintained at 37 ° C for 30 minutes. Then, the absorbance of the samples was recorded at a wavelength of 500 nm. In this assay, a blank was also prepared for each concentration of the extract. Inhibitory activity was calculated using the formula described in the α-amylase inhibitory assay.

Statistical analysis

The experiments were performed in triplicate and data were expressed as means ± standard error (S.E) for n=3. The IC50 was calculated using simple linear regression between log of the concentration and inhibition rate of the extract. Data were analyzed using one-way analysis of variance (ANOVA) with Tukey's post-test. For comparing the average of inhibition rate in the various groups, the analysis of covariance (ANCOVA) was used. Statistical analysis was performed using IBM SPSS Statistics (23). Differences were considered significant at $p < 0.05$.

RESULTS

The results of drying and extraction

Hydroalcoholic extracts of plants were prepared by maceration method. The extraction yield of each plant is presented in table 1.

B: Results of enzyme inhibition assay

The percentage of inhibition of the α-amylase and α-glucosidase enzymes were calculated by respectively different concentrations of fruit extracts, and according to the formula mentioned. The $I_{\alpha\text{-amylase}}$ % and $I_{\alpha\text{- glucosidase}}$ % 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 the sample necessary to decrease the absorbance of α-amylase or α- glucosidase by 50%. The results are presented in figures 1 and 2 and table 2.

IC50 was calculated using a linear chart of the inhibition of enzyme versus the logarithm of the concentration of each sample (table 2). IC50 showed a concentration of the sample that causes to inhibit 50 % of the enzyme activity and whatever is less indicative of a more inhibitory effect of extracts on the desired enzyme.

Table1. The yield of drying and extraction of *Berberis vulgaris, Prunus cerasus* and *Cornus mas* L. fruits [*]

Fruit	**Drying results** (%)	**Extraction efficiency** (%)
Berberis vulgaris	38.3	14
Prunus cerasus	24	19
Cornus mas L.	28.7	17

[*] *The results are from fresh fruit with seed and normal wastes.*

Table2. IC_{50} values obtained for *Berberis vulgaris, Prunus cerasus* and *Cornus mas* L. fruit and acarbose against the α-amylase and α-glucosidase enzymes

sample	**(α-amylase) IC50 (mg/ml)***	**(α-glucosidase) IC50 (mg/ml) ***
Berberis vulgaris	2.379	34.002
Prunus cerasus	11.715	9.361
Cornus mas L.	6.047	6.868
Acarbose	0.023	0.043

** Values have been expressed in mg/ml of the extract.*

DISCUSSION

Hyperglycemia is the common characteristic of diabetes which leads to many clinical complications (3, 4). Compared with high fasting blood glucose, postprandial hyperglycemia plays a decisive role in the development of diabetes complications (4, 6). Therefore, reduction of postprandial hyperglycemia is one the main approaches of diabetes management (6). Synthetic inhibiters such as acarbose, are commonly used as an inhibitor of gastrointestinal α-glucosidase enzymes to reduce post-meal hyperglycemia. However, because of their side effects, a great attention has been paid to the natural inhibitors of these enzymes by many researchers(5, 7).

Recent studies indicate that extracts from a number of fruits possess inhibitory activity against α-amylaseand α-glucosidase (10, 20, 21).

In addition to the traditional medicinal use of barberry, sour cherry and cornelian cherry, the anti-diabetic effects of these fruits have been observed in some studies (15-17, 22, 23).

According to the results obtained in our study, the IC_{50} for hydro-alcoholic extract of barberry against the α-amylase was 2.379. The IC_{50} of barberry extract indicated a stronger inhibition capacity in comparison to sour cherry and cornelian cherry extracts.

In this work, the inhibition activities of the extracts obtained from *Berberis vulgaris*, *Prunus cerasus* and *Cornus mas* L. were investigated on the α –amylase enzyme and IC_{50} values were calculated. All the plants studied, demonstrated inhibitory concentration dependent effects on the α –amylase and α-glucosidase activity. The strongest activity on the α –amylase (IC_{50}=2.379) was shown by *Berberis vulgaris* and The strongest activity on the α-glucosidase (IC_{50}=6.868) was shown by *Cornus mas* L.

The IC_{50} obtained for the extracts showed that cornelian cherry extract was more effective than sour cherry extract in inhibiting α-amylase.
Regarding the inhibition of α-glucosidase, the IC_{50} obtained for cornelian cherry (6.868) is a negligible amount less than the IC_{50} obtained for sour cherry and is nearly 5 times less than barberry (34.002). Therefore, cornelian cherry extract has a stronger inhibitory effect of α-glucosidase compared with barberry and sour cherry.

As shown in the diagrams, a direct and linear relationship is seen between the percentage of inhibition of both enzymes and the logarithm of the extract concentration of each fruit and also acarbose. Furthermore, different concentrations of the fruit extracts inhibit the investigated enzymes in a wide range (3.27 – 93.28 %) that represents the effect of the concentration of the extract on the inhibition of these enzymes.

To date, no study has examined the inhibition of carbohydrate-digesting enzyme by barberry and cornelian cherry, or their extracts. However, in a study conducted by Podsędek (2014), the effect of crude extracts of 30 commonly consumed fruits such as berries as well as sour cherry on the inhibition of α-glucosidase and α-amylase enzymes was examined (24). In the present study, the IC_{50} obtained against α-amylase and α-glucosidase (respectively 257 and 205.25 mg per ml of fresh fruit) is consistent with IC_{50} obtained against these enzymes in Podsędek's study (> 200 and 258 mg/ml, respectively). Similar to the Podsędek's study, our results also showed that the IC_{50} difference against both enzymes by sour cherry is scarce. The IC_{50} for sour cherry against both the α-amylase and α-glucosidase enzymes is respectively, about 2 and 1.5 times more than the IC_{50} obtained for cornelian cherry, showing that the inhibitory effect is weaker in both cases. However, this difference was not significant. A study conducted by Capanoglu et al. shows that the content of proanthocyanidins is much higher in sour cherry than cornelian cherry (25). Thus, the difference in the inhibition activity of these fruits against α-amylase and α-glucosidase observed in the present study may be referred to their different phenolic and anthocyanins contents.

In addition, some studies have observed a strong positive correlation between phenolic content, antioxidant capacity and anthocyanin content (21, 26, 27), whereas other studies have proven otherwise (10, 24). Therefore, the difference between the inhibitory effects of the extracts of fruits on digestive enzymes can be induced from the difference of phenolic profile and the compounds forming these fruits rather than phenolic content or anthocyanin content. Therefore, it seems that the determination of the constituents of the studied fruits is necessary.

Differences between results achieved in this study and other studies may be due to the different in procedures applied, as the variety of extraction methods had very significant effects on the results of studies in order to evaluate the inhibition of carbohydrate digestive enzymes (27-30).

CONCLUSION

Taken together, the results of the present study showed that barberry, cornelian cherry and sour cherry fruits can be useful as functional foods in controlling postprandial hyperglycemia. However, their effects should be investigated in further *in vivo* studies.

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Declaration of Interest
The authors report no conflicts of interest.

REFERENCES

[1] Alberti KGMM, Zimmet Pf. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabetic medicine*. **1998**;15(7):539-53.
[2] Association AD. Diagnosis and classification of diabetes mellitus. *Diabetes care.* **2010**;33(Supplement 1):S62-S9.
[3] Rizza RA. Pathogenesis of fasting and postprandial hyperglycemia in type 2 diabetes: implications for therapy. *Diabetes*. **2010**;59(11):2697-707.
[4] Standl E, Schnell O, Ceriello A. Postprandial Hyperglycemia and Glycemic Variability Should we care? *Diabetes Care.* **2011**;34(Supplement 2):S120-S7.
[5] Gerich JE. Clinical significance, pathogenesis, and management of postprandial hyperglycemia. *Archives of internal medicine.* **2003**;163(11):1306-16.
[6] Ceriello A. Postprandial hyperglycemia and diabetes complications is it time to treat? Diabetes. 2005;54(1):1-7.
[7] Krentz AJ, Bailey CJ. Oral antidiabetic agents. *Drugs*. **2005**;65(3):385-411.
[8] Hanhineva K, Törrönen R, Bondia-Pons I, Pekkinen J, Kolehmainen M, Mykkänen H, et al. Impact of dietary polyphenols on carbohydrate metabolism. *International Journal of Molecular Sciences*. **2010**;11(4):1365-402.
[9] McDougall GJ, Stewart D. The inhibitory effects of berry polyphenols on digestive enzymes. *Biofactors.* **2005**;23(4):189-95.
[10] Zhang L, Li J, Hogan S, Chung H, Welbaum GE, Zhou K. Inhibitory effect of raspberries on starch digestive enzyme and their antioxidant properties and phenolic composition. *Food chemistry.* **2010**;119(2):592-9.
[11] Törrönen R, Sarkkinen E, Tapola N, Hautaniemi E, Kilpi K, Niskanen L. Berries modify the postprandial plasma glucose response to sucrose in healthy subjects. *British Journal of Nutrition.* **2010**;103(8):1094.
[12] Imanshahidi M, Hosseinzadeh H. Pharmacological and therapeutic effects of Berberis vulgaris and its active constituent, berberine. *Phytotherapy Research*. **2008**;22(8):999-1012.
[13] Šarić A, Sobočanec S, Balog T, Kušić B, Šverko V, Dragović-Uzelac V, et al. Improved antioxidant and anti-inflammatory potential in mice consuming sour cherry juice (Prunus Cerasus cv. Maraska). *Plant foods for human nutrition*. **2009**;64(4):231-7.
[14] Tural S, Koca I. Physico-chemical and antioxidant properties of cornelian cherry fruits (< i> Cornus mas</i> L.) grown in Turkey. *Scientia Horticulturae.* **2008**;116(4):362-6.
[15] Shamsi F AS, Rafieian M, Kazemi S AA. Effects of Cornus mas L. on blood glucose, insulin and histopathology of pancreas in alloxan-induced diabetic rats. *Journal of Isfahan Medical School* (IUMS). 1390;29(147):929-38.
[16] Meliani N, Dib MEA, Allali H, Tabti B. Hypoglycaemic effect of Berberis vulgaris L. in normal and streptozotocin-induced diabetic rats. *Asian Pac J Trop Biomed*. **2011**;1(6):468-71.
[17] Shidfar F, Ebrahimi SS, Hosseini S, Heydari I, Shidfar S, Hajhassani G. The Effects of Berberis vulgaris Fruit Extract on Serum Lipoproteins, apoB, apoA-I, Homocysteine, Glycemic Control and Total Antioxidant Capacity in Type 2 Diabetic Patients. Iranian journal of pharmaceutical research: **IJPR**. *2012*;11(2):643.
[18] Nickavar B, Yousefian N. Evaluation of α-amylase inhibitory activities of selected antidiabetic medicinal plants. *Journal für Verbraucherschutz und Lebensmittelsicherheit.* **2011**;6(2):191-5.
[19] Menichini F, Tundis R, Bonesi M, Loizzo MR, Conforti F, Statti G, et al. The influence of fruit ripening on the phytochemical content and biological activity of< i> Capsicum chinense</i> Jacq. cv Habanero. *Food Chemistry.* **2009**;114(2):553-60.
[20] Li Z-Q, Zuo D-Y, Qie X-D, Qi H, Zhao M-Q, Wu Y-L. Berberine acutely inhibits the digestion of maltose in the intestine. *Journal of ethnopharmacology.* **2012**;142(2):474-80.
[21] McDougall GJ, Shpiro F, Dobson P, Smith P, Blake A, Stewart D. Different polyphenolic components of soft fruits inhibit alpha-amylase and alpha-glucosidase. *Journal of agricultural and food chemistry.* **2005**;53(7):2760-6.
[22] Jayaprakasam B, Olson LK, Schutzki RE, Tai MH, Nair MG. Amelioration of obesity and glucose intolerance in high-fat-fed C57BL/6 mice by anthocyanins and ursolic acid in Cornelian cherry (Cornus mas). *Journal of agricultural and food chemistry.* **2006**;54(1):243-8.
[23] Ataie-Jafari A, Hosseini S, Karimi F, Pajouhi M. Effects of sour cherry juice on blood glucose and some cardiovascular risk factors improvements in diabetic women: A pilot study. *Nutrition & Food Science.* **2008**;38(4):355-60.
[24] Podsedek A, Majewska I, Redzynia M, Sosnowska D, Koziołkiewicz M. In Vitro Inhibitory Effect on Digestive Enzymes and Antioxidant Potential of Commonly Consumed Fruits. *Journal of agricultural and food chemistry.* 2014.
[25] Capanoglu E, Boyacioglu D, de Vos RC, Hall RD, Beekwilder J. Procyanidins in fruit from Sour cherry (Prunus cerasus) differ strongly in chainlength from those in Laurel cherry (Prunus lauracerasus) and Cornelian cherry (Cornus mas). *Journal of Berry Research*. **2011**;1(3):137-46.

[26] Johnson MH, Lucius A, Meyer T, Gonzalez de Mejia E. Cultivar evaluation and effect of fermentation on antioxidant capacity and in vitro inhibition of α-amylase and α-glucosidase by highbush blueberry (Vaccinium corombosum). *Journal of agricultural and food chemistry.* **2011**;59(16):8923-30.
[27] Mai TT, Thu NN, Tien PG, Van Chuyen N. Alpha-glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. *Journal of nutritional science and vitaminology.* **2007**;53(3):267-76.
[28] Wangensteen H, Bräunlich M, Nikolic V, Malterud KE, Slimestad R, Barsett H. Anthocyanins, proanthocyanidins and total phenolics in four cultivars of aronia: Antioxidant and enzyme inhibitory effects. *Journal of Functional Foods.* **2014**;7:746-52.
[29] Wang Y, Xiang L, Wang C, Tang C, He X. Antidiabetic and antioxidant effects and phytochemicals of mulberry fruit (Morus alba L.) polyphenol enhanced extract. *PloS one.* **2013**;8(7):e71144.
[30] Lachin T. Effect of Antioxidant Extract from Cherries on Diabetes. *Recent patents on endocrine, metabolic & immune drug discovery.* **2014**;8(1):67-74.