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Inhibitory effect of dates-extract on α-Amylase and α-glucosidase enzymes relevant to non-insulin dependent diabetes mellitus

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Abstract

α-Amylase and α-glucosidase are key enzymes involved in carbohydrates breakdown and intestinal absorption, respectively. Inhibition of these enzymes hinders blood glucose level increase after a carbohydrate diet and can be an important strategy in the management of non-insulin-dependent diabetes mellitus (NIDDM). A main drawback of currently used inhibitors is their side effects such as abdominal distention, flatulence, meteorism and diarrhea, caused by excessive inhibition of pancreatic α-amylase resulting in abnormal bacterial fermentation of undigested carbohydrates in the colon. Natural inhibitors from plants have shown lower inhibitory effect against α-amylase activity and a stronger inhibitory activity against α-glucosidase and can be used as effective therapy for NIDDM with minimal side effects. In this work dates-extract (DE) inhibitory effect on α-amylase and α-glucosidase has been assessed. The inhibition percentages on α-amylase and α-glucosidase were in the range of 6-24% and 54%, respectively. The results clearly show the NIDDM treatment potential of DE.

Keywords: Diabetes, dates-extract, α-amylase, α-glucosidase, enzyme inhibition

Introduction

α-Amylase and α-glucosidase are enzymes involved in starch breakdown and intestinal absorption, respectively. The first enzyme is involved in the digestion of carbohydrates to produce simpler saccharides, whereas the second is involved in their absorption. It is believed that inhibition of the two enzymes would result in a lower blood glucose levels after a rich carbohydrate diet. The current available anti-diabetic drugs used to treat non-insulin dependent diabetes mellitus NIDDM, such as Acarbose, strongly inhibits both enzymes. However, patients using Acarbose usually suffer from abdominal distention, flatulence, meteorism and possibly diarrhea (Bischoff, 1994). These side effects are caused by the excessive inhibition of pancreatic α-amylase resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon (Bischoff, 1994; Horii, 1987). Therefore it is attractive to find a substance that has a strong inhibitory activity against α-glucosidase, but minor effect on α-amylase activity (Kwon et al., 2006). As dates represent large portion of sugar source in Middle Eastern diet, it would be advantages to find out if they have positive effect on NIDDM. The results of this work will open the door for further investigation into the active agents that cause the inhibitory effect, which can be extracted and used as a natural drug.

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Chemicals and Methods

Chemicals

Dates-syrup was purchased from local market and dates-pits granules were obtained from Al-Saad Dates Processing Factory, UAE. α-Amylase from *Aspergillus oryzae*, 1.5 units mg^{-1} and α-glucosidase from *Saccharomyces cerevisiae* Type I, lyophilized powder with ≥10 units mg^{-1} were purchased from Sigma-Aldrich, USA. All other chemicals were of analytical grade and purchased from Sigma-Aldrich, USA.

Dates- and dates-pits extracts preparation

Dates-extract (DE) was prepared by mixing 50 ml of dates-syrup thoroughly with to 50 ml of distilled water. The mixture was then centrifuged at 4000 rpm (ICE CL31 Multispeed Centrifuge, Thermoscientific, USA), and the supernatant was vacuum-filtered (Shel Lab, USA). Vacuum was generated using jet pump and the filter paper used was Whatman-Gade 1(11 μm).

Dates-pits extract (DPE) was prepared by initially washing the granules. Then they were dried and course grinded using Sanyo, Japan grinder and then fine grinded using Moulinex, France grinder. The particles were then screened in Pascal Eng. Co. Ltd., England using mesh 70-120, which gives particle sizes in the range of 125-212 μmand screened. 20 g of the collected granules were dispersed

in 50 ml distilled water in a screw capped bottle. The bottle was kept on a shaker (WSB-30, Korea) at 350 rpm for 24 hours to reach equilibrium. The mixture was then vacuum-filtered (Shel Lab, USA).

α-Amylase assay

The reactions took place in ten test tubes, each containing 1 ml distilled water (or DE solution), 1 ml of 100 g l^{-1} starch solution in 2 mM phosphate buffer solution (pH 6.9) and 1 ml of 0.05 g l^{-1} α-amylase in phosphate buffer solution. The test tubes were incubated at 37 ^{o}C (which represent normal body temperature). The enzymatic reaction was stopped by adding 50 µl of 1 M HCl to each test tube at different times in the range of 10 to 100 seconds, with 10 minutes intervals between the test tubes. The reducing sugar formed in the reaction was measured by 3,5-dinitrosalicylic acid (DNS) method using maltose as the standard (Miller, 1959) and was used to determine the activity of α-amylase. One unit of α-amylase was defined as the amount of enzyme required to produce 1 µmol of reducing equivalents per minute from soluble starch under the assay conditions. The experiment was then repeated at different initial amounts of substrate in the range of 33 g l^{-1} to 66 g l^{-1}.

α-Glucosidase assay

A modified method to the one used by (Kwon et al., 2008) has been developed to follow the production of *p*-nitrophenol continuously with time. α-Glucosidase activity was assayed using 1 ml of 0.35 mM *p*-nitrophenyl-α-D-glucopyranoside solution in 100 µmol of acetate buffer (pH 4.5) mixed with 1 ml of distilled water (or DE or DPE solutions), 0.5 ml of 200 mM Na_2CO_3 and 0.1 ml of 1 mg ml^{-1} α-glucosidase solution. The reaction took place in a special vial placed in the spectrophotometer, and the absorbance at 405 nm was recorded as a function of time. The absorbance was compared to that of standard solutions of *p*-nitrophenol and the results were used to determine the activity of α- glucosidase. One unit of α-glucosidase was defined as the amount of enzyme required to produce 1 µmol of *p*-nitrophenol per minute under the assay conditions. The experiment was then repeated at different initial amounts of substrate in the range of 0.07 mM to 0.42 mM. The experiment was also run at substrate concentration of 0.35 mM in absence of α-glucosidase as a reference.

Results and Discussion

α-Amylase inhibition

The rate of reduced sugar production was determined using different initial starch concentrations, with and without the presence of DE. To eliminate the effect of reduced sugar initially found in the date-extract, the results were presented in terms of deviation maltose concentration, **P**, defined as difference between the concentration of reduced sugar at anytime and that at time zero. An example of the deviation maltose production with time, with and in absence of DE, at initial starch concentration of 33 g l^{-1} is shown in Fig. 1. All experiments were done in duplicate, and the reproducibility of the results is confirmed from the small error bars shown in the figure. Although slight inhibition effect was observed, the results show that the presence of DE has insignificant effect on the activity of α-amylase. Similar results were also observed at other initial starch concentration in the range of 33 to 66 g l^{-1}.

α-Amylase activity was determined at different initial substrate concentrations and the results are shown in Table 1, with and without the presence of DE. It can be seen that the presence of DE slightly inhibited α-amylase with less than 10% inhibition at concentrations 33 and 50 g l^{-1}. However, the inhibition effect increased at higher substrate concentration reaching 24% at initial substrate concentration of 66 g l^{-1}.

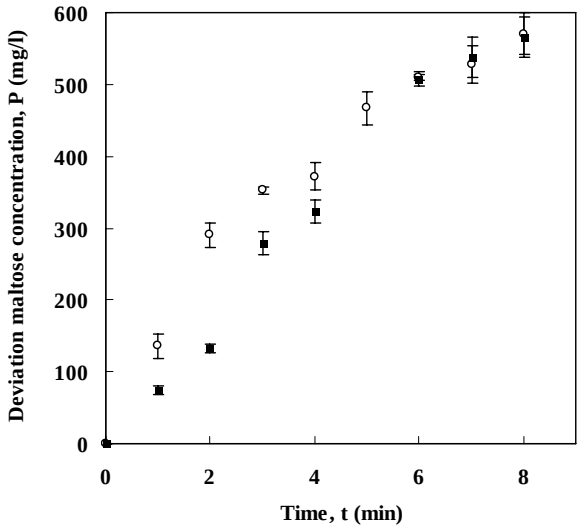

Figure 1: Effect of DE on enzymatic production of deviation maltose at starch concentration of 33 g l^{-1} (o without DE and ■ with DE)

α-Glucosidase inhibition

The rate of *p*-phenol production was determined using different initial *p*-nitrophenyl-α-D-glucopyranoside solution, with the presence of DE and DPE and in absence of any inhibition. For reference, the experiment was also repeated in absence of α-glucosidase. The results at initial substrate concentration of 0.35 mM are shown in Fig. 2. All experiments were done in duplicate, and the reproducibility of the results is confirmed from the small error bars shown in the figure. The results show that the presence of DE has significant effect on the activity of α-glucosidase. On the other hand, only slight inhibition effect was observed in the presence of DPE. Table 2 shows α-glucosidase inhibition of DE and DPE. It can be seen that the presence of DE significantly inhibited α-glucosidase, and reduced its activity by 54%, which is almost equal to the absence of enzyme. This indicated that the DE almost completely inhibited the enzyme. On the other hand, date-pits extract, inhibited the enzyme much less and percentage reduction of only 30% was observed. Similar results were observed at other initial starch concentration in the range of 0.07 - 0.42 mM, and the results are shown in Fig. 3.

Table 1: Substrate concentration effect on α-amylase activity

[S] (g l^{-1})	α-amylase activity(U)		percentage reduction
	Without DE	With DE	
33	195	183	6.3 %
50	240	221	7.9 %
66	470	356	24.2 %

The use of DE in the treatment of NIDDM

Results indicated that DPE slightly inhibits the activity of α-amylase. The inhibition percentage was in the range of 6-24%, depending on the initial substrate concentration. The inhibition effect of DE was less than that of egg-plant extract used by Kwon et al. (2008), which was in the range of 14-38%. This is a favourable

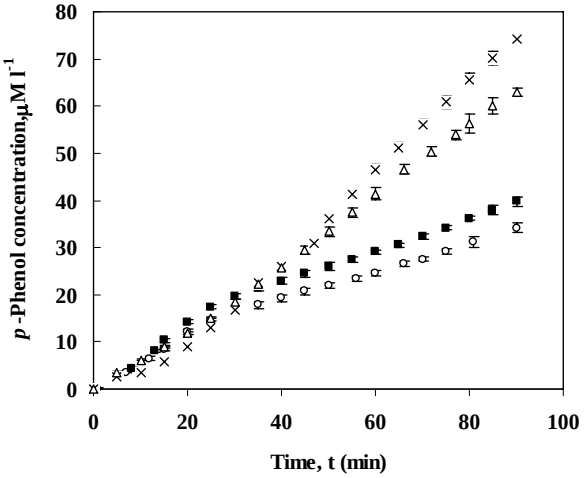

Figure 2: DE and DPE effect on *p*-phenol production, at initial substrate concentration of 0.35 mM (o without enzyme, ■ with DE, Δ with DPE and x without inhibition)

result, since as explained earlier, the excessive inhibition of α-amylase results in abnormal bacterial fermentation of undigested carbohydrates in the colon, which intern results in abdominal distention, flatulence, meteorism and possibly diarrhea (Bischoff, 1994). On the other hand, it was found that DPE strongly inhibits

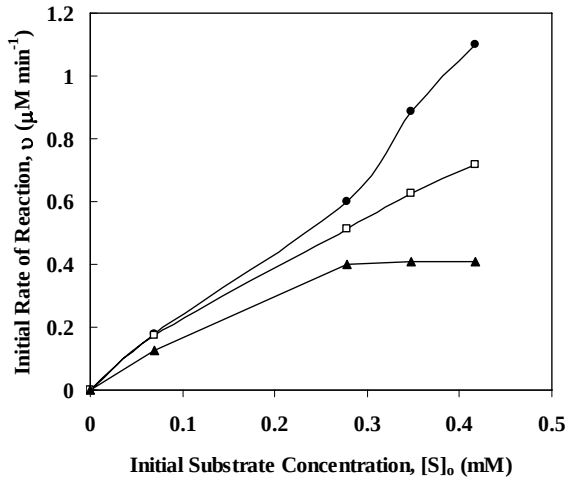

Figure 3: Substrate concentration effect on α-glucosidase activity (● without inhibitors, □ with DPE, ▲ with DE)

the activity of α- glucosidase and renders it almost inactive. The inhibition percentage using DE is found to be 54%, which is higher than the egg plant extract inhibition which was in the range of 5-46% (Kwon et al., 2008).

Table 2: Effect of inhibitors on α-glucosidase activity

Case	α-glucosidase activity(U)	Percentage reduction
No enzyme	370	58.4 %
No inhibition	890	0.0 %
With DPE	620	30.3 %
With DE	410	54.0 %

Conclusion

The inhibition effect of DE on the activity of α-amylase and α-glucosidase has been experimentally assessed. The results show that DE inhibits α-glucosidase more than it does on α-amylase, which is a positive result. The findings of this work clearly show the DE treatment potential on NIDDM.

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References

Bischoff H (1994) Pharmacology of glucosidase inhibitor. Eur J Clin Invest 24:3–10

Horii S (1987) Synthesis and α-D-glucosidase inhibitory activity of Nsubstituted valiolamine derivatives as potent oral antidiabetic agents. J Med Chem 29:1038–1046

Kwon YI, Apostolidis E, Shetty K (2008) In vitro studies of eggplant (Solanum melongena) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. Biores Technol 99:2981-2988

Kwon YI, Vattem DV, Shetty K (2006) Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension. Asia Pac J Clin Nutr 15:107–118

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428

Rosetti LL, Giacarri A, Defronzo RA (1990) Glucose toxicity. Diabetes Care 13:610–630

Shetty K, Curtis OF, Levin RE, Wikowsky R, Ang W (1995) Prevention of verification associated with in vitro shoot culture of oregano (Origanum vulgare) by Pseudomonas spp. J Plant Physiol 147:447–451