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Artigo

Traditionally used plants in diabetes therapy - phytotherapeutics as inhibitors of α-amylase activity

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RESUMO: "Plantas tradicionalmente utilizadas na terapia da diabetes – fitomedicamentos como inibidores da atividade α-amilase". Diabetes mellitus é uma desordem metabólica caracterizada pela hiperglicemia crônica. Existem diversas estratégias terapêuticas no tratamento da diabetes Tipo 2. A inibição da atividade da α-amilase é apenas uma possibilidade de reduzir os níveis de glicose posprandiais. Nos nossos estudos *in vitro* pudemos demonstrar que diferentes plantas, especialmente as tradicionalmente usadas em terapia comum de diabetes na África ou Europa, são capazes de inibir a α-amilase, a qual é responsável pela quebra dos oligossacarídeos em monossacarídeos, os quais são absorvidos. Uma inibição da atividade da α-amilase da ordem de 90% foi observada com o extrato das folhas de *Tamarindus indica*. Para quantificar os graus de inibição, acarbose foi usada (IC_{50}: 23,2 μM). O maior grau de inibição de acarbose no nosso modelo de teste foi de cerca de 85%. Adicionalmente testes com compostos polifenólicos puros poderão explicar a atividade biológica das plantas selecionadas.

Unitermos: Diabetes mellitus Tipo 2, inibidores da atividade α-amilase, plantas utilizadas tradicionalmente no tratamento da diabetes, substâncias polifenólicas.

ABSTRACT: Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycaemia. There are many and diverse therapeutic strategies in the management of Type 2 diabetes. The inhibition of α-amylase activity is only one possibility to lower postprandial blood glucose levels. In our in-vitro studies we could demonstrate that different plants, mostly traditionally used in common diabetic therapy in Africa or Europe, are able to inhibit α-amylase, which is responsible for the breakdown of oligosaccharides into monosaccharides which are absorbed. An inhibition of α-amylase activity of 90% was seen with the extract of the leaves of *Tamarindus indica.* To quantify inhibtion rates, acarbose was used (IC_{50}: 23.2 μM). Highest inhibition level of acarbose in our testmodel was about 85%. Additionally tests with pure polyphenolic compounds might explain the biological activity of the selected plants.

Keywords: Type 2 Diabetes mellitus, inhibitors of α-amylase activity, plants traditionally used in diabetes treatment, polyphenolic compounds.

INTRODUCTION

Diabetes mellitus is the most common endocrine disease worldwide. About 173 million people suffer from Diabetes mellitus. The number of people with diabetes mellitus will more than double over the next 25 years to reach a total of 366 million by 2030. This multiple disorder is characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from absolute or relative lack of insulin secretion and insufficient cellular effect of insulin respectively. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs (WHO, 1999, 2003).

There are two forms of diabetes. The WHO recommends that the terms Type 1 and Type 2 should be reintroduced, because they classify the patients on the basis of the pathogenesis and not on the basis of treatment. The Type 1 diabetes includes the cases which can be attributed to an autoimmune process and/or those with β-cell destruction for which unknown pathogenesis. The Type 2 includes the common major form of diabetes which results from defects in insulin secretion or rather insulin resistance (WHO, 1999).

The only therapy of Type 1 diabetes is the substitution of insulin. Many and diverse therapeutic strategies for the treatment of Type 2 diabetes are known. Conventional treatments include the reduction of the demand for insulin, stimulation of endogenous insulin secretion, enhancement of the action of insulin at the target tissues and the inhibition of degradation of oligo- and disaccharides (Groop et al. 1997, Perfetti et al. 1998). One group of drugs introduced in the management of Type 2 diabetes is represented by the inhibitors of α-glucosidase. The enzymes summarized as α-glucosidase are responsible for the breakdown of

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oligo- and/or disaccharides to monosaccharides. The inhibition of these enzymes leads to a decrease of blood glucose level, because the monosaccharides are the form of carbohydrates which is absorbed through the mucosal border in the small intestine. There are two α-glucosidase inhibitors used in antidiabetic therapy in Germany: Acarbose and Miglitol.

The aim of our study was the screening for α-amylase inhibition of plants traditionally used in anti-diabetic treatment and pure natural products. The anti-diabetic potency was defined by the inhibition of α-amylase activity. In previous studies we established a kinetic assay for the screening of test compounds (Funke; Melzig, 2005). Therefore, we adapted the determination of α-amylase using maltooligosaccharides of defined chain length with a 4-nitrophenyl-group as chromogenic substrate, which was originally used for diagnostic purpose of pancreatic diseases. This substrate is cleaved by α-amylase, the released nitrophenol can be continuously monitored at 405 nm.

MATERIAL AND METHODS

Plant material

The tested drugs were obtained from CAELO Germany (cinnamon, melissa, sage, beans, bilberry, fenugreek, dandelion, rosemary), goat´s rue was obtained from Heinrich Klenk GmbH Germany. The African drugs (*Balanites aegyptiaca* (L.) Del., *Khaya senegalensis* (Desr.) A. Juss., *Holarrhena floribunda* (Don) Durand & Schinz, *Mitragyna inermis* (Willd.) O. Ktze., *Tamarindus indica* L., *Securidaca longepedunculata* (Fresen) were supplied by Dr. Bizimana (Berlin).

Preparation of plant material (Table 1)

Table 1. Preparation of plant material

number	preparation
1	dissolving in buffer
2	extraction with buffer, roomtemperature (0,5 g plant material + 2500 µl buffer, 20 min)
3	extraction with buffer, boiling (0,5 g plant material + 2500 µl buffer, 20 min)
4	extraction with water, boiling (0,5 g plant material + 2500 µl water, 20 min)

Test compounds

Tested substances were obtained from Sigma-Aldrich Chemie GmbH [Steinheim, Germany] (ferulic acid, rosmarinic acid), Carl Roth GmbH & Co. [Karlsruhe, Germany] (luteolin, luteolin-7-glucoside, apigenin-7-glucoside, isochlorogenic acid, esculin), Merck KgaA [Darmstadt, Germany] (chlorogenic acid) and Fluka [Buchs SG, Switzerland] (fisetin).

Kinetic assay

p-Nitrophenyl-α-D-maltopentaoside (PNPG5) was obtained from Megazyme [Bray, Co.Wicklow, Ireland]. HEPES was purchased from Lancaster [Mühlheim, Germany]. α-amylase (EC 3.2.1.1) from porcine pancreas was obtained from Sigma-Aldrich Chemie GmbH [Steinheim, Germany]. DMSO was obtained from Merck KgaA [Darmstadt, Germany]. Acarbose was a gift from the Bayer AG [Wuppertal-Aprath, Germany].

The α-amylase assay was performed in 96-well-plates (Greiner, Germany). All reagents were dissolved in buffer (HEPES 50 mM, pH 7.1), DMSO or 5%-(v/v) DMSO-buffer-solutions. The highest concentration of DMSO was 5%. The preparation of extracts is shown in table 1. Controls were prepared by using the identical solvent to consider the influence of DMSO on reaction. 50 µl of substrate solution (PNPG5, 25 mM, dissolved in buffer) and solutions of investigated substances and extracts (10-100 µl) were pipetted into the wells. Buffer (HEPES, 50 mM, pH 7.1) was added up to a volume of 150 µl. Reaction was started by rapid addition of the enzyme solution (50 µl porcine pancreatic α-amylase in buffer, 100 U/ml).

Absorption (405 nm) was measured at 3-min intervals for a total period of 90 min at 37°C using a Tecan Spectra Fluor. The increase of the absorbtion was monitored as a function of time to provide a progress curve for the reactions. The slope of each reaction was analysed by linear regression and used for calculation of the inhibition rates, expressed in percent to controls without inhibitors. Blind samples without enzyme and substrate were measured in each experiment. All assays were performed at least two times with duplicate samples. IC_{50} values were determined from dose-effect-curves by linear regression using Microsoft Excel. The data were expressed as mean ± SD. As positive control and well established inhibitor of α-amylase activity we used acarbose.

RESULTS AND DISCUSSION

Effects of plants on α-amylase activity are shown in Table 2. Our data demonstrate that several plants are able to influence α-amylase activity. The inhibition rates of the tested plants are different. For our tests we selected plants which are traditionally used in folk medicine for the treatment of diabetes in Europe or Africa. We

Table 2: Influence of plants on α-amylase activity

test material	preparation of plant material (table 1)	Inhibition
Acarbose	1	+++
Balanites aegyptiaca (L.) Del.: bark	2	++
Camellia sinensis L.: dried extract of the leaves (Chinese tea)	1	++
Cinnamomum verum J.S.Presl: bark (Cinnamon)	2	-
Galega officinalis L.: herb (Goat´s rue)	2	-
Galega officinalis L.: herb (Goat´s rue)	3	+
Holarrhena floribunda (Don) Durand & Schinz: leaves	2	+
Khaya senegalensis (Desr.) A. Juss.: bark	2	++
Melissae officinalis L.: leaves (Melissa)	2	++
Mitragyna inermis (Willd.) O. Ktze.: leaves	2	++
Phaseolus vulgaris L.: pericarp (Bean)	4	++
Rosmarinus officinalis L.: leaves (Rosemary)	2	++
Salvia officinalis L.: leaves (Sage)	2	-
Securidaca longepedunculata (Fresen): root	2	+
Tamarindus indica L.: leaves (Tamarind)	2	+++
Taraxacum officinale Web. ex Wigg.: herb (Dandelion)	2	+
Trigonella foenum-graecum L.: seeds (Fenugreek)	4	-
Vaccinium myrtillus L.: leaves (Bilberry)	2	+++

- inhibition lower than 20%
+ inhibition between 20-45%
++ inhibition between 45-75%
+++ inhibition higher than 75%

made a selection between leaves, barks, and roots. Main compounds in those plants are - as far as known - polyphenolic compounds. An overview of the effects of inhibitory potency of some pure substances is shown in Figure 1. It shows the IC_{50} values of some phenolic compounds. It is possible to divert the wide ranged effect from that figure.

The tested extracts of plants from Mali influenced α-amylase activity partly strong. Traditional use of plants is common in the treatment of diabetes. A blood sugar lowering effect of some of the tested plants has been shown in animal tests (Neuwinger, 2004). Plants are usually prepared as decoction. Extraction of plant material with boiling water or buffer should mimic that kind of preparation. *Balanites aegyptiaca* and *Holarrhena floribunda* are described as high potential antidiabetic plants. In both plants we were able to detect a moderate inhibitory effect on α-amylase activity. The extract of the leaves of *Mitragyna inermis* induced an inhibition of 75%. The extract of the leaves of *Tamarindus indica* led to an α-amylase inhibition of 90%.

Traditional use of plants is widespread in Europe, too. Labiates are main compounds in antidiabetic tea species. Effects of sage, rosemary, and melissa were quite different. The inhibition range of sage was at the level of 15%. Melissa led to a 50% inhibition of α-amylase activity, and rosemary caused an inhibition of 60%. The content of hydroxyl cinnamic acid derivatives is 8% in rosemary and 3-5% in sage and melissa, respectively (Brand, 1994, Stahl-Biskup 1993, 1994). Figure 1 shows the different inhibitory potencies of selected hydroxy cinnamic acid derivatives. Probably, the composition of those derivatives varies in the single plants considerably. In former studies we already showed the ability of these compounds and some flavonoids to inhibit the α-amylase activity (Funke; Melzig, 2005). By comparison

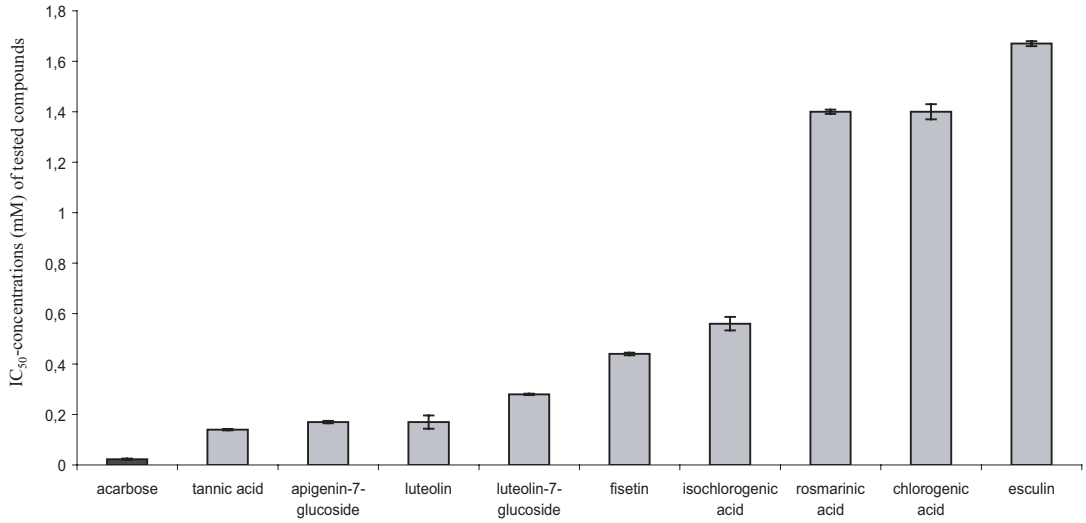

Figure 1. IC_{50}-values of tested substances

of IC_{50}-values of chlorogenic acid (1.4 ± 0.03 mM) and isochlorogenic acid (0.56 ± 0.027 mM), we could give evidence that the steric position of the hydroxyl groups in the molecule is important for the inhibition rate.

The method of extraction had a crucial influence on the results. Diverse prepared extracts of *Galega officinalis* (Goat´s rue) showed different effects. The extract prepared at room temperature had nearly no effect, the plant extracted with boiling buffer led to a 35% inhibition of the enzyme. The hypoglycaemic effect of Goat´s rue was attributed to galegin, which is a metformin-like isoamylene guanidine (Witters, 2001).

Some drugs had no effect on the enzyme activity. Antidiabetic activities of fenugreek and cinnamon were described before (Shane-Mc-Worther, 2001, Yeh et al., 2003, Khan et al., 2003); inhibitory effects on α-amylase activity were not detectable.

CONCLUSION

In 2002 around 173 million people suffered from diabetes mellitus. Around two thirds of these people lived in developing countries. Diabetes epidemic spreads in the developing world; the number of people with diabetes will increase by 150% in the next 25 years, with an increasing proportion of affected people in younger age groups (WHO, 2003). Costs of diabetes are estimated to be 5-10% of the national health budgets. Additional costs result by cardiovascular disease, blindness, amputation, and kidney failure which may be caused by diabetes. Cardiovascular disease is causing about 50-80% of deaths in people with diabetes. In developing countries people in the middle, the productive years of their lives who are particularly affected by diabetes. In these countries three-quarters of all patients with diabetes are under 65 years old, 25% of all adults with diabetes are younger than 44. In developed countries more than half of all people with diabetes are older than 65 and only 8% of adults with diabetes are younger than 44 (WHO, 2004).

These data show that a reliable, cost saving therapy with traditionally used plants could be a possibility to lower the problems of untreated diabetes because of a lack of synthetic drugs. On the other side medicinal plants contain an enormous potential for the development of new drugs and the efficient treatment of diabetes. But it is necessary that their effectiveness is proofed. In our study we could show that plants are able to inhibit the α-amylase activity. This mechanism belongs to first line therapies in diabetes treatment. The demonstrated results might be a base for further studies with plants also from South, Central, and North America which are traditionally used in diabetes therapy (Barbosa-Filho et al., 2005).

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