Alpha amylase inhibitory activity of some plant extracts with hypoglycemic activity

Rawand S. Abu Soud, Imad I. Hamdan, Fatma U. Afifi*

Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of
Jordan, Queen Rania Street, Amman, Jordan

Abstract

Thirteen plant species which are claimed to have anti-diabetic activity (based on folk medicine and/or scientific reports) were tested for alpha amylase inhibitory activity. Two of the screened plants exhibited significant (more than 80%) alpha amylase inhibitory activity. IC_{50} of these plants was estimated based on the dried crude extract and found to be 0.08, and 0.2 mg/ml for *Aloe vera* and *Paronychia argentea* respectively. In *A. vera* the activity was most likely due to cinnamic acid derivatives. In *P. argentea* the activity was attributed to flavonoid components. These findings support the hypoglycemic activity of these species and give insight about the potential mechanism of their hypoglycemic activity.

Keywords: amylase inhibitors, anti-diabetics, *Aloe vera*, *Paronychia argentea*.

Introduction

Several plant species have been advocated in the Jordanian folk medicine for their hypoglycemic effects [1]. The hypoglycemic effect of some of these plants have also been supported by scientific studies on experimental animals [2-7]. However in many cases, neither the active principle nor the mechanism of action have been identified. One possible mechanism for hypoglycemic effect could be through decreasing the absorption of ingested sugars. This could be achieved if the enzyme responsible for the degradation of complex carbohydrates (i.e. alpha amylase) was inhibited. Alpha amylase has been looked at as a potential target to control diabetes since more than 30 years [8]. However it was not before the early nineties when the

first alpha glucosidase (alpha amylase as well) inhibitor came into clinical use [8]. This inhibitor, known as acarbose, was the result of long screening program for glucosidase inhibitors and has been reported to be quite efficient in controlling blood glucose levels [9]. A major side effect associated with acarbose is flatulence [9]. It has been suggested that alpha amylase inhibitors would result in a better side effect profile (compared to alpha glucosidase inhibitors) as they do not lead to the accumulation of maltose and oligosacharides in the gut [10]. Therefore the aim of this study was to screen some plants commonly used in the folk medicine in Jordan in a search for new alpha amylase inhibitors and to give insight concerning the mechanism of their potential hypoglycemic activity.

Experimental

Plants and Materials

All of the examined plants (Table 1) were obtained from the local market (herbalists shops) in a dried form and authenticated by comparison with the herbarium specimens in the Faculty of Science-University of Jordan. A. vera was further characterized according to the identification test described in British Pharmacopoeia, 1993. Emodine, aloin, aloe emodine, barbaloin, hydroquinone and cinnamic acid were obtained from Sigma (USA). Anthracene was from Hnawa Chemicals (China). Purified porcine pancreatic alpha amylase was obtained from ICN Biochemicals (Ohio, USA). All other reagents and solvents were obtained from Merck (Germany). Distilled water was used for the preparation of the different solutions.

Preparation of the extracts

All plants were extracted with 50% methanol in water according to the method described by Hansawasdi [11] with some modifications. The dried plant was ground to pass through a 40-mesh sieve. Of the sieved material 1 g was extracted with 10 ml of 50% methanol water by incubating in a water bath at 50 °C for 30 minutes. The obtained extract was centrifuged and filtered before being subjected to alpha amylase inhibitory test.

For the purpose of estimating the IC_{50}, the concentration of the whole extract was estimated by evaporating the solvent to dryness (using rotary evaporator) and weighing the dried residue. The percentage yields (based on the dried residue) together with the result of the phytochemical screening, according to Wagner [12] for all of the examined plant extracts are presented in Table 1.

Alpha amylase inhibitory test

Iodine solution was prepared by dissolving 0.254 g I_2 and 4.0 g KI in 1L of distilled water. Starch solution was prepared by dissolving 1g of starch in 10 ml of distilled water, gently boiling, cooling and completing to 100 ml with distilled water. Amylase solution was prepared by transferring 6 μl of the standard porcine pancreatic amylase suspension (40 mg/ml) to 8 ml of phosphate buffer (pH 6.9).

Alpha amylase inhibitory activity was based on the starch-iodine method described by Hansawasdi [11] with some modifications. Briefly, control and test solutions were prepared as follows: 0.3 ml of amylase solution were transferred to a sample tube containing 0.3 ml of the extract to be tested (substituted by the solvent of extraction in the case of control) and 0.6 ml phosphate buffer (pH 6.9). The mixture was incubated at 37 °C for 15 minutes. 0.4 ml aliquots of that incubate were transferred to sample tubes containing 3 ml starch (1g%) and 2 ml of phosphate buffer (pH 6.5) and the mixture was re-incubated for 45 minutes. At zero time and at the end of the incubation period 0.1 ml of the reaction mixture was withdrawn from each tube after mixing and discharged into 10 ml of iodine solution. Solutions were thoroughly mixed and the absorbance measured immediately at 565 nm. Percentage inhibition was calculated according to the formula:

$$[(A_0-A_t)_{control} -(A_0-A_t)_{sample}/(A_0-A_t)_{control}] \times 100\%$$

where A_0 and A_t are the absorbance values at zero time and at the end of the incubation, respectively. Each experiment was repeated three to four times and the average value was used for obtaining the relevant plots.

Results and discussion

Thirteen plant extracts were tested for alpha amylase inhibitory activity using a literature method that was optimized and validated in the author's labs [13].   Results were calculated as percentage inhibition (under experimental conditions)   and summarized in Table 1. Of the thirteen plant species tested only two extracts exhibited significant (more than 80%) alpha amylase inhibitory activity; namely *A. vera*, and *P. argentea*. For these plants the inhibitory activity was also shown to be concentration dependent. Dose response curves were obtained for the two most active plant extracts and compared to that of acarbose as a standard alpha glucosidase and amylase inhibitor (Fig. 1). The IC_{50} of each plant extract was determined (from Fig. 1) as the inhibitor concentration (estimated on basis of the dried extract) required to inhibit 50% of alpha amylase activity under the described experimental conditions. Acarbose appeared to be about 20 times more active (IC_{50} = 0.004 +/- 0.0003 mg/ml) than *A. vera* (IC_{50}= 0.081 +/- 0.003mg/ml) and 50 times more active than *P. argentea* (IC_{50} = 0.20 +/- 0.02 mg/ml).   However, the two plant extracts are considered to have a significant inhibitory activity that might contribute to their claimed anti-diabetic activities. Particularly because the IC_{50} was estimated based on the weight of the crude dried extract in this experiment, and thus has to be higher than the IC_{50} of the respective pure active component responsible for the effect.

Although both of these plants have been advocated in folk medicine for anti-diabetic activity [1]   only *A. vera* has been shown by scientific studies to have such activity [14]. Therefore alpha amylase inhibition is responsible (at least in part) to the hypoglycemic activity of *A. vera*, and *P. argentea*. As regard to other plants which did not show significant alpha amylase inhibitory activity they may still be active as hypoglycemic plants but through a different mechanism of action; particularly those which have been supported by scientific studies on animals.

Table 1: Percentage inhibition of alpha amylase activity.

Plant (family)	percentage yield	Constituents (phytochemical screening)	Part used	Percentage inhibition
Nigella sativa Linn. (Raunuclaceae)	12.5	e.o	Seeds	NA
Teucrium polium L. (Palmaceae)	9.5	e.o, flav	Leaves, stem	5 % +/- 1.2
Varthemia iphionoides L. (Labiatae)	5.5	flav, e.o	Leaves, stem	NA
Trigonella foenum-graecum L. (Leguminoseae)	4.3	alk, e.o	Seeds	10% +/- 1.6
Aloe vera L. (Liliaceae)	7.2	anth, coum, flav	Leaves (juice)	99% +/- 2.5
Artemisia herba alba asso. (Labiatae)	3.5	coum, flav, e.o	Leaves, seeds	NA
Olea europea L. (Oleaceae)	7.5	coum, flav, e.o	Leaves	NA
Origanum syriacum L. (Labiatae)	4.2	coum, flav, e.o	Leaves, stem	NA
Matricaria aurea (Labiatae)	7.8	coum, flav, e.o	Stem, Leaves, flowers	NA
Paronychia argentea Lam. (Caryophyllaceae)	2.5	coum, flav	Stem, Leaves,	85% +/- 4.3
Lupinus albus L. (Leguminoseae)	6.5	alk, coum, flav	flowers	3% +/- 0.6
Elatteria cardamomum L. (Zingiberaceae)	3.5	e.o	Seeds	NA
Petroselinum sativum Hoffm. (Umbelliferae)	7.5	coum, flav, e.o	Stems, leaves	NA

Listed values of percentage inhibition are average of three complete determinations(+/- RSD). NA=no activity. Alk= alkaloid, anth = anthracene derivative, coum= coumarins, flav= flavonoids, e.o= essential oil.
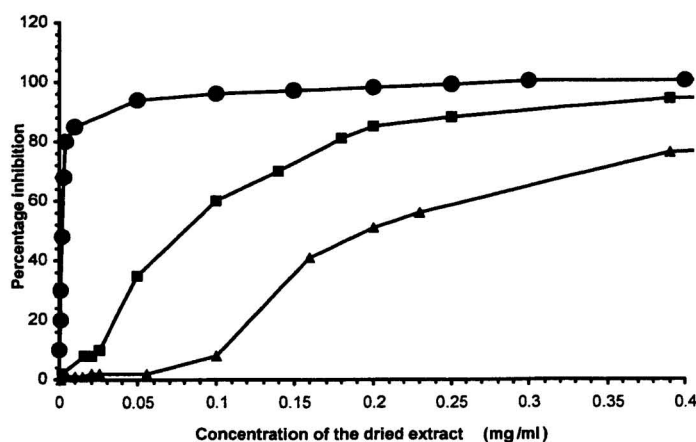
Fig. 1: Dose response curves for acarbose (•) and the crude extracts of *A. vera*
(■) and *P. argentea* (▲). At each concentration point the average of three
independent measurements was used for constructing the plot. R.S.D. was
less than 5 in all cases.

Since *A. vera* is one of the well studied plants with known-major constituents,
attempts to identify the biologically active principles were made by testing the
commercially available purified constituents. Major constituents of *A. vera* include the
anthraquinone derivatives: emodine, aloin and aloe emodine. When tested for alpha
amylase inhibitory activity emodine and aloe-emodine exhibited maximum of 1.5 and
2.5 % inhibition at saturated concentrations respectively, but aloin showed no activity
up to concentrations of 20 mg/ml. It was thought that the observed low activity was
attributed to the low solubility of these compounds in the test solvent (methanol:water).

It might be possible that other constituents of the whole extract would facilitate
the solubility of anthraquinones leading to higher activity. Addition of surfactant (tween
80) to the test solvent (and the control) was found to improve the solubility of both of
emodine and aloe-emodine. The observed increase in solubility was accompanied by
increase in activity: 9% and 16% for emodine and aloemodine, respectively. Thus the

activity of the purified emodine and aloemodine was still far below that observed for the whole extract (100%) which suggested the presence of other active constituents. Anthracene, anthracene-9-carboxylic acid, anthraquinone, 1,2,5,8 tetrahydroxyanthraquinone, and hydroquinone are other related anthraquinone derivatives that were available in pure form. When these were tested for alpha amylase inhibitory activity no inhibition was observed even at saturation concentrations. Therefore, the high alpha amylase inhibitory activity could not be attributed to the anthraquinone derivatives.

Other reported constituents of *A. vera* include ester derivatives of parahydroxy cinnamic acid [12]. As these were not available, pure cinnamic acid and cinnamaldehyde were tested using the same procedure. At saturation concentrations a maximum of 30% inhibition was observed for the pure cinnamic acid and zero inhibition for cinnamaldehyde. In order to ensure that the effect of cinnamic acid was a specific one rather than a non specific deactivation by decreasing the pH of the medium, the pH was measured and found to be within the expected range (6.6). These findings tend to suggest that the principal activity resides in one or more of the hydroxy cinnamic acid derivatives. For further evidence, the extract was run on preparative TLC plates using a standard mobile phase that is recommended for the separation of the constituents of *A. vera* [12]. Aloin and aloe-emodine were used as standards on a separate lane on the TLC plate. After development and visualization under UV light spots were removed from the plate and subjected to alpha amylase inhibition test.

The obtained TLC-chromatograms were comparable to the reference one and showed 14 distinguishable spots. According to Wagner et al. [12] the spots above aloin and below aloe-emodine (obtained R_f 0.5-.99) were attributed to various cinnamic acid derivatives e.g. p-coumaric acid methyl ester which exhibit blue fluorescence under the UV light 365nm. Of the fourteen fractions examined only two exhibited significant activity; those were with R_f values of 0.91 and 0.61 and produced inhibitory activity of 37 +/- 4% and 30 +/- 2% respectively. When examined under UV light at 365nm, these fractions exhibited blue fluorescence. Thus combining these data with those obtained from experiments on purified constituents it would be evident that the alpha amylase

inhibitory activity of A. vera resides in some of the cinnamic acid derivatives rather than the anthraquinone derivatives.

It would be expected that amylase inhibitors should possess a sugar-like structure e.g. acarbose.   However alpha amylase inhibitory activity has been demonstrated for other non-sugar naturally occurring products such as hibiscus acid [11] and flavonoids including luteolin. As the carboxyl group is the common structural feature between cinnamic acid and hibiscus acid it might be possible that carboxylic acid group together with other structural features (e.g. size) is important for alpha amylase inhibitory activity. However, further studies are needed and currently in progress to isolate and identify the constituents responsible for alpha amylase inhibitory activity in the active plant species A. vera, and P. argentea.

Acknowledgement

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