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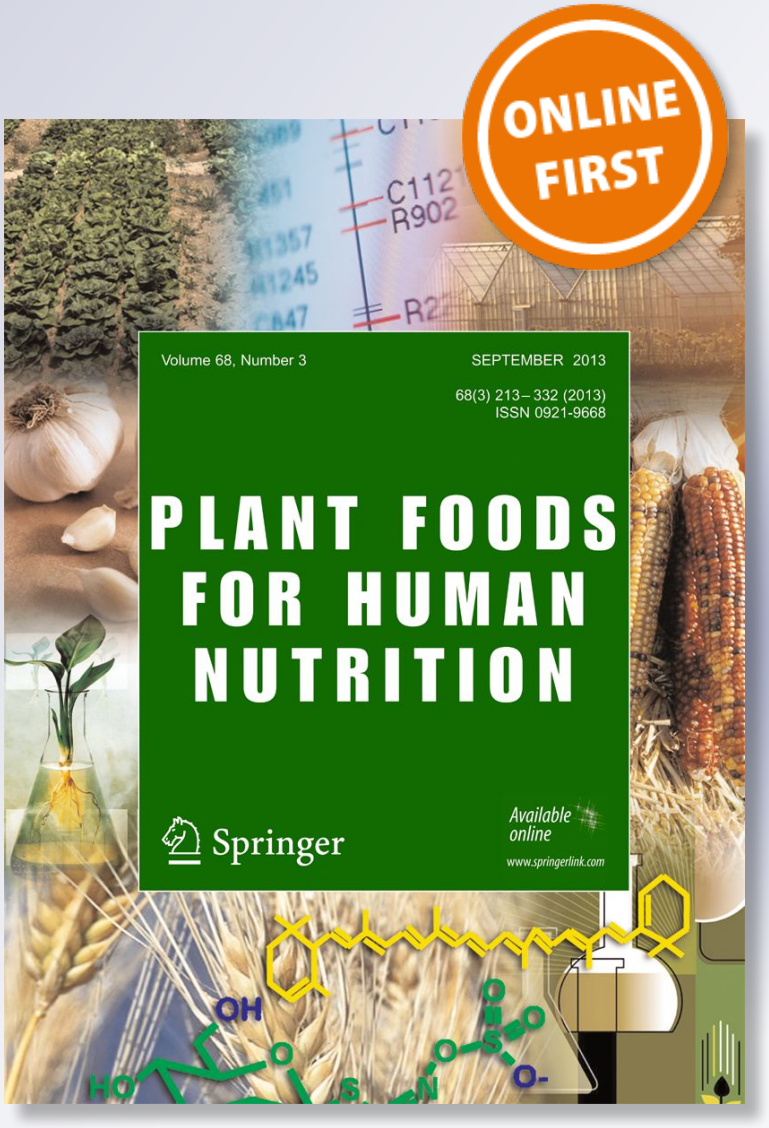

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ORIGINAL PAPER

Enhydrin Regulates Postprandial Hyperglycemia in Diabetic Rats by Inhibition of α-Glucosidase Activity

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Abstract During the last few years, numerous attempts were made to identify effective α-glucosidase inhibitors from natural sources in order to develop new alternatives for diabetes management. *Smallanthus sonchifolius* (yacon) leaves were found to be effective in controlling postprandial hyperglycemia. Enhydrin, a constituent of yacon leaves, was noted for its significant hypoglycemic properties in diabetic rats. These properties were also demonstrated for yacon leaves decoction, which is rich in phenolic compounds such as chlorogenic acid and its derivatives. The purpose of the present study was to evaluate the potential of yacon leaves decoction and the isolated compound enhydrin to inhibit α-glucosidase enzyme, a possible mechanism of the above antihyperglycemic effect. *In vitro* assays showed that both 10% decoction and enhydrin significantly inhibited the activity of the yeast α-glucosidase enzyme in a dose-dependent manner, IC_{50} values being 50.40 and 134.17 μg/ml, respectively. *In vivo* experiments showed a rapid decrease in the hyperglycemic peak after sucrose load (2 g/kg body weight) in normal rats treated with the 10% decoction (140 mg/kg) and enhydrin (0.8 mg/kg). Both treatments caused a significant decrease in blood glucose levels in diabetic rats after sucrose load compared to diabetic control. These results suggest that both products assayed could be effective in the management of postprandial hyperglycemia through inhibition of α-glucosidase in the small intestine.

Keywords *Smallanthus sonchifolius* · Enhydrin · Antihyperglycemic effect · α-glucosidase inhibition

Introduction

Many natural products and health foods have been recognized for their properties to improve overall well-being as well as for their beneficial effects in the prevention and/or treatment of several diseases. Although several plants are neither food *per se* nor dietary supplements, they are an important source of many active compounds (usually secondary metabolites) with relevant medicinal properties [1].

Diabetes mellitus (DM) is a common endocrine disease in which chronic hyperglycemia is the main factor in the development and progression of micro and macrovascular complications [2]. This is why a conventional treatment is now widely used to reduce postprandial hyperglycemia primarily by interfering with the carbohydrate- digesting enzymes and delaying glucose absorption [3].

Digestion of complex starches, oligosaccharides and disaccharides is facilitated by enteric enzymes, including α-glucosidases attached to the brush border of intestinal cells. Inhibition of these enzymes leads to a reduction in disaccharide hydrolysis which in turn has beneficial effects on glycemic control in diabetic patients. Acarbose and miglitol, which are competitive inhibitors of intestinal α-glucosidase, play an important therapeutic role in type 2 DM [4]. However, these therapeutic agents are usually accompanied by numerous side effects such as abdominal distention, flatulence and diarrhea

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[5]. Hence, there is permanent interest in new compounds from plant species with inhibitory activity against α-glucosidase in order to develop alternative antidiabetic drugs with clinically relevant efficacy and scarce adverse effects [6]. Several works have reported that numerous phytoconstituents such as flavonoids, alkaloids, terpenoids, anthocyanins, glycosides and phenolic compounds could interact with proteins and hence inhibit enzymatic activity [7].

Yacon (*Smallanthus sonchifolius* [Poepp. & Endl.] H. Robinson, Asteraceae) is a native Andean herb used for centuries by the inhabitants of many South American regions in traditional folk medicine due to its beneficial health properties [8]. In a previous study, we demonstrated the hypoglycemic effect of water extract from yacon leaves in normal and diabetic rats [9]. More recently, we reported an interesting protective action of this extract against early diabetic nephropathy [10]. Phytochemical studies of yacon leaves showed the presence of high polarity compounds such as caffeic, chlorogenic and three dicaffeoilquinic acids. These major compounds have an effective hypoglycemic effect on transiently hyperglycemic and diabetic rats in a dose-dependent manner [11]. Moreover, we also found that the sesquiterpene lactone enhydrin isolated from yacon leaves caused a significant decrease in postprandial blood glucose levels in diabetic rats. These findings would strongly support the use of yacon leaves decoction or some of its constituents to achieve satisfactory glycemic control. However, the precise mechanism of this effect remains unknown. Therefore, in the present study we tried to determine the *in vivo* and *in vitro* inhibitory activity of both yacon leaves decoction and crystalline enhydrin on α-glucosidase.

Materials and Methods

Plant Materials

Yacon leaves (Clone LIEY97–1) used in this study were collected in Horco Molle, Province of Tucumán, between March and April. Voucher specimens are deposited in the herbarium of the "Fundación Miguel Lillo", Province of Tucumán, Argentina (No. 607173LIL). The 10% decoction was obtained as follows: 10 g dried leaves were steeped in 100 ml of boiling water for 10 min, allowed to cool at room temperature, filtered, lyophilized (yield: 1.73 g dry residue) and stored at −20 °C until use. In this work we selected a dose of 140 mg dry extract/kg body weight (b.w.) of 10% *S. sonchifolius* leaves decoction based on its previously assayed hypoglycemic efficacy [12].

The isolation and purification of enhydrin from the glandular trichomes of the yacon leaf surface were previously described by Genta et al. [11]. The effective hypoglycemic dose (0.8 mg enhydrin/kg b.w.) selected was previously determined in our laboratory [11, 12].

In Vitro Enzyme Assay of α-Glucosidase Activity

In order to evaluate the *in vitro* effect of 10% *S. sonchifolius* leaves decoction and pure enhydrin on α-glucosidase activity, a protocol described by Li et al. [13] was used. α-glucosidase type I from baker's yeast (EC 3.2.1.20) was obtained from Sigma Chemical Co. (St. Louis, MO). The test sample (dry extract of 10% decoction or enhydrin) and acarbose (positive control) were assayed at varying concentrations (1.5–195.0 μg/ml). The reaction mixture without extract or enhydrin was used as a control and the mixture without enzyme served as a blank to correct background absorbance.

IC_{50} values, defined as the concentration of the product that causes a 50% inhibition of the enzyme activity were calculated by applying logarithmic regression analysis (XLStat 2012.4.02) from the mean inhibitory values. All assays were performed in triplicate.

In Vivo Experimental Design

Animals

Adult male Wistar rats, aged 8 to 12 weeks (weight 200 ± 20 g), were used in the experiments. They were raised in the animal facility of the Department of Developmental Biology-INSIBIO (CONICET-UNT), Tucumán, Argentina. The animals were housed in individual cages. The photoperiod (07:00 to 19:00 h), air exchange, room temperature (22 ± 2 °C) and relative humidity (60–70%) were controlled. Experimental rats were given free access to a powdered certified rodent diet obtained from a commercial source (Standard Food-Association of Argentinean Coop.-S.E.N.A.S.A. No. 2706) and tap water. All animals were maintained and handled according to International Ethical Guidelines for the Care of Laboratory Animals (U.S. Food and Drug Administration).

Induction of Experimental Diabetes

Stable diabetes was induced in normal male rats by a single intraperitoneal injection of freshly prepared streptozotocin (STZ, Sigma Chemical Company, St. Louis, MO, USA) solution in 10 mM sodium citrate buffer (pH 4.5) at a dose of 45 mg/kg b.w. Control rats received only citrate buffer. Diabetes was achieved within 48 h in the majority of the animals, as determined by measuring fasting blood glucose levels (>350 mg/dL) and glucosuria.

Single-Dose Effect on Blood Glucose after Sucrose Administration

This experimental procedure was carried out in normal and diabetic rats fasted overnight. The animals were divided into four groups (n = 6 rats each) of normal rats and four groups of

diabetics rats (n = 6 rats each). Normal and diabetic control groups received only vehicle (distilled water), while positive control groups were given acarbose (250 mg/kg b.w.)

Treated groups received 10% decoction (140 mg dried extract/kg b.w.) or enhydrin (0.8 mg/kg b.w.). All administrations were performed by the oral route. Thirty minutes later, the blood glucose level of each group was evaluated and considered as 0 h value. Then, all animals received an oral solution of sucrose (2 g/kg b.w.) and postprandial blood glucose levels were monitored after 30, 60, 90 and 120 min.

Biochemical Determinations

Blood glucose was measured with an Accu-chek® Active (Roche Diagnostics, Mannhein, Germany), based on glucose dye oxido reductase mediator reaction. Urine glucose was determined by a glucose oxidase-peroxidase enzymatic method (Bayer Diagnostics, Buenos Aires, Argentina).

Statistical Analysis

All data were expressed as mean ± standard deviation (SD) from three independent experiments. The significance of differences was evaluated using a paired Student's t-test. When more than one group was compared with the control, significance was evaluated by one way analysis of variance (ANOVA). A p value <0.05 was considered statistically significant.

Results and Discussion

Yacon leaves decoction is a traditional derivative used because of its beneficial health properties such as a decrease in postprandial blood glucose levels [11, 12]. In order to explore these functionalities, we focused on the potential α-glucosidase inhibitory effect of 10% decoction of yacon leaves and enhydrin.

In vitro tests are useful to assess antihyperglycemic activity in a preliminary way before *in vivo* preclinical antihyperglycemic test. The *in vitro* α-glucosidase inhibitory activities of 10% decoction and pure enhydrin were evaluated using yeast α-glucosidase. The highest concentration tested (195.0 μg/ml) showed a maximum inhibition of nearly 66.30 and 67.12% for decoction and enhydrin, respectively. However, as shown in Table 1, IC_{50} values indicate a lower inhibitory ability for enhydrin compared to decoction or acarbose. It is noteworthy that many natural compounds are more effective inhibitors of yeast α-glucosidase activity than mammalian enzyme [14].

Table 1 *In vitro* inhibitory activity of 10% decoction of *S. sonchifolius* leaves and enhydrin against α-glucosidase type I from yeast (EC3.2.1.20)

	IC_{50} (μg/ml)
Dry extract of 10% leaves decoction	50.40 ± 12.50
Enhydrin	134.17 ± 42.00*
Acarbose	46.25 ± 11.00

The *in vivo* experimental design was conducted with 140 mg dry extract/kg b.w. of 10% leaves decoction and 0.8 mg enhydrin/kg b.w./day. As shown in Fig. 1a, in normal animals both treatments significantly ($p < 0.01$) reduced blood glucose levels (22.21 ± 0.82 and 31.15 ± 1.02 mg/dl, respectively) at 30 min after sucrose ingestion compared with the untreated controls (44.45 ± 1.34 mg/dl), glycemia returning to near baseline values at 120 min. Treatment with the positive control drug acarbose reduced blood glucose levels to 9.04 ± 0.98 mg/dl. Figure 1b shows the whole glycemic response (0–120 min) after sucrose loading in animals treated with decoction, enhydrin and acarbose. The results, expressed as the total increase in the area under the curve (IAUC), show a significant reduction by 46.2, 51.5 and 89.3%, respectively, compared with the untreated group.

In order to determine the effective antihyperglycemic effect of decoction and enhydrin under pathological conditions, the

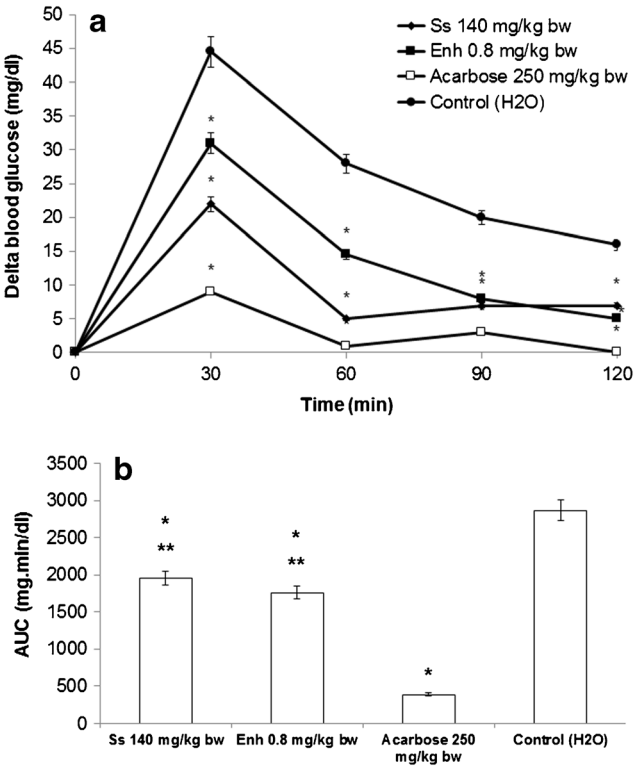

Fig. 1 Effect of 10% *S. sonchifolius* leaves decoction (Ss) and enhydrin (Enh) on blood glucose levels of normal rats after oral administration of sucrose. (**a**) Glycemic response curve in normal rats after sucrose loading (2 g/kg b.w.) expressed as change from baseline plasma glucose. (**b**) Incremental AUC0–120 min in normal rats after sucrose administration. Data are expressed as the mean ± SD, n = 6 rats/group. *The mean is considered to be statistically significant when compared with untreated normal rats ($p < 0.05$). **The mean is considered to be statistically significant ($p < 0.05$) when compared with normal rats administered acarbose (250 mg/kg b.w.)

glycemic response after sucrose loading was evaluated in treated STZ-diabetic rats. Ten percent decoction and enhydrin at the doses assayed caused a strong and significant reduction ($p < 0.01$) in blood glucose levels (19.41 ± 0.65 and 38.32 ± 1.64 mg/dl, respectively) in comparison with the diabetic control group (173.52 ± 4.39 mg/dl) 30 min after sucrose loading (Fig. 2a). A low glycemic variation was observed along the experimental period (120 min). As shown in Fig. 2b, the effects on the whole glycemic response (IAUC) indicated good postprandial glycemic control in treated diabetic animals. The IAUC was significantly ($p < 0.05$) reduced by 77.4 and 62.4% in animals treated with decoction and enhydrin, respectively, compared with the untreated diabetic group.

In a previous work, we found that phenolic compounds, mainly caffeic and chlorogenic acids together with the three isomeric dicaffeoylquinic acids, are major constituents of yacon leaves decoction [12]. We think that these compounds could contribute to effective α-glucosidase inhibition as demonstrated for other plants [15, 16].

Taken together, *in vitro* and *in vivo* results led us to establish that both yacon leaves decoction and enhydrin delay the quick digestion of sucrose, thus lengthening the time of glucose absorption.

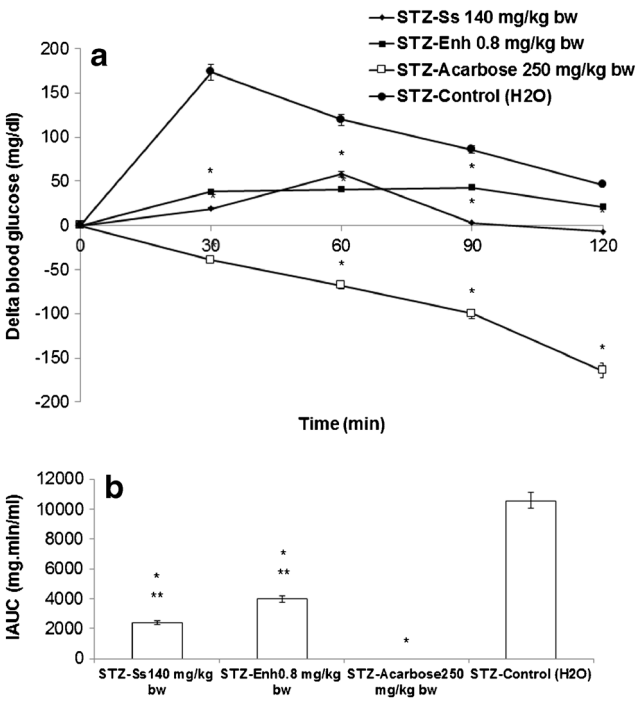

Fig. 2 Effect of 10% *S. sonchifolius* leaves decoction (Ss) and enhydrin (Enh) on blood glucose levels of diabetic rats (STZ) after oral administration of sucrose. **(a)** Glycemic response curve in STZ-diabetic rats after sucrose loading (2 g/kg b.w.) expressed as change from baseline plasma glucose. **(b)** Incremental AUC0–120 min in diabetic rats after sucrose administration. Data are expressed as the mean ± SD, $n = 6$ rats/group. *The mean is considered to be statistically significant ($p < 0.05$) when compared with untreated diabetic rats. **The mean is considered to be statistically significant ($p < 0.05$) when compared with diabetic rats administered acarbose (250 mg/kg b.w)

It is well known that inhibition of α-glucosidase by chlorogenic acid derivatives appears to be non-competitive, suggesting binding to or interaction with the enzyme at a site other than the active site [17]. Since caffeic, chlorogenic and three dicaffeoilquinic acids are the main chemical compounds present in yacon leaves decoction [12], they could be responsible for the *in vivo* suppression of the postprandial rise in blood glucose.

To the best of our knowledge, there is no information concerning the *in vivo* inhibition of the α-glucosidase enzyme by enhydrin. It is widely accepted that some bioactivities of sesquiterpene lactones are mediated by alkylation of nucleophiles, especially the cysteine sulfhydryl residues in proteins, through their α-methylene-γ-lactone moiety [18]. This structure was found in the enhydrin molecule [11, 19] and therefore could react with the sulfhydryl group(s) of the α-glucosidase enzyme, thus causing its inhibition.

The therapeutic use of sesquiterpene lactones as pure chemicals should necessarily consider the safety-efficacy ratio. Interestingly, in a previous work we demonstrated the safety and absence of gastrointestinal adverse effects of a 0.8 mg enhydrin/kg b.w. dose in rats [12].

The leaves decoction (rich in phenolic compounds) was significantly ($p < 0.05$) more active than enhydrin as evidenced by greater glycemic control after sucrose loading in diabetic rats (Fig. 2). Such differences could be associated with intrinsic factors of the active molecules. In fact, enhydrin is a lipophilic molecule while phenolic compounds such as chlorogenic acid have polar structures, which would give them a higher capacity for direct interaction with the enzyme.

In summary, our research leads us to conclude that one of the mechanisms of the antidiabetic action of yacon leaves occurs through the inhibition of the α-glucosidase enzyme from the small intestine. Thus, the decoction of *S. sonchifolius* leaves and enhydrin could be an alternative for the treatment of DM.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that there is no conflict of interest.

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