Chemico-Biological Interactions 209 (2014) 35–47

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Smallanthus macroscyphus: A new source of antidiabetic compounds

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ARTICLE INFO

Article history:
Received 15 July 2013
Received in revised form 28 October 2013
Accepted 24 November 2013
Available online 2 December 2013

Keywords:
Smallanthus macroscyphus leaves
Polymatin A
Hypoglycemic activity

ABSTRACT

The aim of the present study was to analyze the *in vivo* hypoglycaemic effects of both decoction of *Smallanthus macroscyphus* leaves and pure crystalline polymatin A isolated from its leaves. Phytochemical analysis of the leaf decoction showed that its major constituents were caffeic, chlorogenic and three dicaffeoilquinic acids, together with the sesquiterpene lactone polymatin A. Oral glucose tolerance test in normal rats was performed to evaluate the hypoglycemic activity and to choose the minimum effective dose of the decoction and polymatin A. They have effective hypoglycemic activity at the minimum dose of 140 mg dry extract and 14 mg crystalline powder/kg body weight, respectively, and were selected for the following experiments. Oral administration of a single-dose of decoction produced a moderate lowering effect in fasting glycemia of normal rats, whereas polymatin A had no significant effect. We also assessed the effect of a single-dose on post-prandial blood glucose, resulting in an inhibition of the hyperglycemic peak after sucrose overload. Daily administration of decoction or polymatin A for 4 weeks produced an effective glycemic control in diabetic animals, with a decrease in urinary glucose excretion and a significant reduction in the HbA1c levels. Although there were no significant increases in plasma insulin levels, both treatments improved the fasting blood glucose/insulin ratio. *In vivo* acute toxicity studies were performed in adult Wistar rats. There were no deaths or signs of toxicity observed after oral administration of decoction or polymatin A at any dose level up to the highest dose tested (14.0 and 2.8 g/kg, respectively).

The results presented here strongly support the notion that *S. macroscyphus* represents a new source of antidiabetic compounds that could help to manage diabetes more efficiently and safely.

1. Introduction

Smallanthus macroscyphus (Baker ex Martius) A. Grau (Heliantheae, Asteraceae), a perennial herb commonly known as "wild yacon", is indigenous to South America from southern Bolivia to northwestern Argentina [1]. This specie is an invader of abandoned sugar cane fields as well as of the free space between the plots of land. It belongs to the *Smallanthus* genus, whosemost relevant member is the species *Smallanthus sonchifolius* (Poepp. and Endl.) H. Robinson or "yacon" due to it wide range of medicinal and nutritional properties [1–7]. *S. sonchifolius* is probably an allotetraploid species, with "wild yacon" (*S. macroscyphus*) as one of the putative parents [1]. Yacon is a pre-columbian culture whose consumption has gained in importance during the last decades by consumption from its roots as a functional food or nutraceutical and its leaves as a hypoglycemic infusion. This allows us to hypothesize that the leaves of *S. macroscyphus*, species belonging to the same genus could also contain active principles with hypoglycemic activity.

Unlike yacon, there is no available oral or written information concerning the use of the wild species of the genus *Smallanthus* in folk medicine and even less of their biological activities. There is only one study carried out in farming communities in the Upper Bermejo Basin, province of Salta, Argentina, documenting the use of *S. macroscyphus* as a plant in the manufacture of "yista", which is used during the insalivation of "coca" (*Erythroxylum coca* Lam.-var. coca) leaves [8]. Considering geographical distribution, growth habitat and morphology of the aerial parts, *S. macroscyphus* appears as specie closely related to *S. sonchifolius* and perhaps with a similar chemical composition and biological activities [1].

Diabetes mellitus is a metabolic disorder which characterizes by hyperglycemia resulting from defects in insulin secretion, insulin action or both. It has been demonstrated that tight control of blood glucose is effective in reducing clinical complications and improves the quality of life of diabetic patients [9,10]. However,

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http://dx.doi.org/10.1016/j.cbi.2013.11.015

treatments with modern drugs are also associated with side effects and fail to significantly alter the course of diabetic complications, suggesting that alternative treatment strategies are required. In this regard, the World Health Organization has recommended the evaluation of medicinal plants or their extracts that can help to reverse disease progression and plans to incorporate "traditional medicine" into the next revision of its International Classification of Diseases [11]. Numerous studies conducted over the last few years have been focused on the discovery, development and evaluation of plants and their byproducts for therapeutic management of diabetes because of their ready availability, affordability and anti-diabetic effectiveness [12].

In a preliminary experimental test to evaluate new medicinal plants with anti-diabetic activity, we demonstrated that the aqueous extracts of dried *S. macroscyphus* leaves exert a hypoglycemiant effect on rats [13], greater than the one demonstrated by yacon leaves [5]. These findings represented a first step toward the assessment of *S. macroscyphus* leaves as a product with beneficial action on diabetes and have led us to continue the study of the medicinal properties of this species.

About 1000 plants worldwide are used ethnopharmacologically to treat symptoms of diabetes mellitus or experimentally to confirm their efficacy, mechanism of action and safety, the *Fabaceae* and *Asteraceae* families being most frequently cited. At the present, there are more than 200 pure compounds from plant sources reported to show blood glucose lowering activity [14,15] and the wide variety of classes of chemical compounds indicates that different mechanisms must be involved in this effect.

Naturally occurring chemicals produced by plants are stored in various organs including leaves, stems and roots. In particular, the glandular trichomes on the leaf surfaces are specialized secretors structures that might be the sites of production or accumulation of various bioactive secondary metabolites [16]. These chemical compounds may have diverse functions, including the plant defense [17], and could be of interest as pesticides or pharmaceutical products. Melampolide-type sesquiterpene lactones (STLs) are characteristic secondary metabolites of the genus *Smallanthus* produced in glandular trichomes of the foliar surface [18]. Polymatin A is a STLs isolated as a gum from *Smallanthus siegesbeckia* [19] and *Smallanthus maculatus* var. maculatus [20]. So far, nineteen melampolide-type STLs have been isolated and identified in *S. sonchifolius* leaves [21], enhydrin being the more abundant. With regard to the chemical constituents of *S. macroscyphus* leaves, de Pedro et al. [22] have determined the presence of small amounts of a mixture of STLs among which polymatin A was identified by ^{1}H NMR spectrometry and MS analysis as the predominant STL in this species. Fig. 1 shows the chemical structure of the polymatin A.

In a previous work we isolated enhydrin, the main lactone from leaf extracts of *S. sonchifolius*, and we found it to be an active

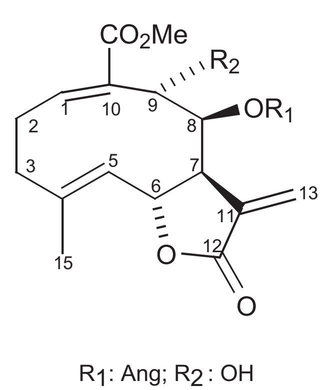

Fig. 1. Chemical structure of polymatin A.

compound useful in the decrease post-prandial blood glucose levels and in the treatment of diabetic Wistar rats [5]. These results strongly suggest that the STLs would be very important active anti-diabetic principles from the leaves of the *Smallanthus* genus. Since *S. macroscyphus* is a slight departure from the previously studied specie *S. sonchifolius*, we think that polimatin A may represent another interesting chemical entity with anti-diabetic properties.

Despite their natural origin, the use of medicinal plants extracts containing a very complex mix of chemical compounds may cause adverse effects and medicine interactions [23]. In addition, STLs also may cause acute or chronic toxicity [24]. In a recent study we provided evidence that the use of a 10% decoction of yacon leaves or pure crystalline enhydrin isolated from yacon leaves was safe in rats at doses in which the hypoglycemic effect was demonstrated [25]. Further studies are required to assess the efficacy/safety ratio of *S. macroscyphus* leaves as a product with beneficial action on diabetes.

In view of the close relationship between yacon and the wild species *S. macroscyphus*, it is important to characterize the biological activity as well as the potential toxic effects of the latter species. Therefore, the aim of the present study was to analyze the hypoglycemic effects in normal and diabetic rats of both the aqueous extract of *S. macroscyphus* leaves and the pure crystalline polymatin A isolated from them. This study represents a first step toward the assessment of new medicinal plants and new chemical entities isolated from them, which could help to manage diabetes with greater efficiency and safety.

2. Materials and methods

2.1. Plant material

Leaves of *S. macroscyphus* (Baker ex Martius) Grau were collected in February 2010 from an experimental field (Regional Ecology Institute (IER), National University of Tucumán, located at Horco Molle, Yerba Buena, province of Tucumán, Argentina, 26°47′ S, 65°19′ W, 547 m.a.s.l. The plant material at the experimental field was grown from rhizomes possessing young buds of wild plants collected at Rearte, Trancas Department, province of Tucumán, Argentina, 26°20′ S, 65°32′ W, 1450 m.a.s.l. A voucher specimen (LIL607375) is on deposit in the herbarium of "Fundación Miguel Lillo".

2.2. Preparation of the aqueous extract

Fresh plant material was carefully dried under air flow in an oven between 40 and 45 °C and ground to a powder. Aqueous extract of the leaves (decoction) was prepared boiling 10 g dried powder in 100 mL distilled water under reflux for 10 min. The decoction obtained (10%) was filtered, frozen at −20 °C and then lyophilized. The yield in dry residue was 1.8 g (18%, w/w), which was stored at −20 °C until used. The appropriate amount of dry residue was dissolved in distilled water immediately before each experiment. In the present work, a 10% decoction was the dose level selected based on the hypoglycemic efficacy previously assayed with *S. sonchifolius* leaves [25].

2.3. Phytochemical analysis of the aqueous extract

2.3.1. Infrared (IR) spectroscopy

The analysis of the dry lyophilized residue from 10% decoction was performed by IR spectroscopy using a Perkin-Elmer 1600 FT-IR spectrophotometer.