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Safety assessment of aqueous extract from leaf *Smallanthus sonchifolius* and its main active lactone, *enhydrin*

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ABSTRACT

Ethnopharmacological Relevance: Leaves of *Smallanthus sonchifolius* (Poepp. & Endl.) H. Robinson (yacon) have been used since pre-Columbian times in the Andean region to prepare medicinal herbal tea with beneficial health properties. However, there are still disagreements about the safe use. This work was carried out to evaluate the toxicity profile of both, 10% decoction of yacon leaves and their major active lactone, enhydrin.

Materials and methods: In vitro cytotoxicity assays were performed with Hep-G2, COS1, CHO-K1 and Vero cell lines using a test of metabolic competence based upon assessment of mitochondrial performance. *In vivo* toxicity study was performed in adult Wistar rats. In the acute oral toxicity each group of rats was orally given a single dose of 10% decoction or enhydrin. General condition, behavior and mortality were recorded for up to 14 days post treatment. In subchronic toxicity studies, both products were given orally for 90 days to rats. Body weight and food intakes were observed weekly. Hematological, clinical chemistry parameters and organ weight were determined in all animals at the end of the experimental period.

Results: Cell viability decreased in a concentration dependent fashion when cells were incubated with 2–200 μg of 10% decoction and 0.015–7.5 μg of enhydrin. In acute study in rats, there were no deaths or signs of toxicity observed after oral administration of single doses of 10% decoction or enhydrin at any dose level up to the highest dose tested (14.0 g/kg and 0.32 g/kg, respectively). In subchronic studies in rats, both products administered orally for 90 days at daily doses of 0.07, 0.14 and 0.28 g 10% decoction/kg and 0.4, 0.8 and 8.0 mg enhydrin/kg, did not caused haematological, biochemical and histological alterations.

Conclusions: The results presented in this paper lead us to the conclusion that the use of 10% decoction and enhydrin is safe in rat at doses in which it is demonstrated the hypoglycaemic effect.

1. Introduction

Yacon (*Smallanthus sonchifolius* (Poepp. & Endl.) H. Robinson) is a perennial herb native to South America that belongs to the family Compositae or Asteraceae. Yacon roots have a long history of use as part of the diet of the inhabitants of the Andes since pre-Columbian times (Grau and Rea, 1997) and yacon cultivation and consumption have expanded in recent decades to several Asian and European countries. With regard to the medicinal uses of the plant, some beneficial properties have been attributed to yacon leaves, which are used to prepare a medicinal tea (Aybar et al., 2001; Valentová and Ulrichová, 2003). In the past decade numerous studies have shown that the extracts of dried yacon leaves have a variety of pharmacological activities, including antimicrobial (Joung et al., 2010; Lin et al., 2003), anti-inflammatory (Hong et al., 2008), antioxidant and free radical scavenging properties (Valentová et al., 2003, 2005).

In a previous study, we demonstrated the hypoglycaemic effect of the water extract of *S. sonchifolius* leaves in normal and diabetic rats (Aybar et al., 2001). Interestingly, treatment of diabetic rats with aqueous extract of yacon leaves for 30 days improved the general condition, body weight and renal function of the animals. More recently, we found that the butanolic extract of yacon leaves showed a high effective hypoglycaemic activity in normoglycaemic, transiently hyperglycaemic and diabetic rats in a dose-dependent manner (Genta et al., 2010). On the basis of the

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high polarity of this extract and the presence of caffeic and chlorogenic acid and their dicaffeoil quinic derivates, we may assume that these major constituents are active principles related to the hypoglycaemic effect of yacon leaves.

Further phytochemical investigations allowed the isolation and identification of a variety of compounds including melampolide-type sesquiterpene lactones (STLs) such as fluctuanin, uvedalin and enhydrin (Hong et al., 2008; Schorr and Da Costa, 2005). Enhydrin is the main lactone isolated from the leaves of yacon and, in recent years, studies have suggested that it has anti-diabetic properties, so much so that it was included in a patented anti-diabetic pharmaceutical formulation (Kawashima et al., 2001). In order to identify the metabolites with hypoglycaemic activity, in a previous work we isolated crystalline enhydrin from leaf extracts of *S. sonchifolius*. We found it to be an active compound that helped in the decrease of post-prandial blood glucose levels and that was useful in the treatment of diabetic Wistar rats (Genta et al., 2010).

The beneficial effects associated with the consumption of organic or aqueous extracts of yacon leaves for long periods might suggest that they have a high safety margin. Although some acute toxicity tests have been evaluated (Genta et al., 2010), studies on the toxicity of these extracts after prolonged periods of consumption need to be extensively reviewed.

Based on their results, Ogose et al. (2009) judged that the administration of yacon extracts to rats for two generations had no effects on either the reproductive functions or the development of the liveborn pups. In a recent work, de Oliveira et al. (2011) showed that prolonged oral administration (90 days) of extracts of *S. sonchifolius* was associated with kidney damage and attributed it to the presence of sesquiterpene lactones in the extract. However, the analysis of the results presented by these authors shows that the aqueous extract of yacon leaves triggers biochemical and histopathological changes when administered at high doses (100 mg/kg). This represents a dose six times higher than that used in folk medicine and is a question that requires further studies.

The toxic and medicinal properties of many plants have been shown to correlate with the biological activity of sesquiterpene lactones isolated from their extracts (Scmidt, 1999). In this regard, enhydrin is the main sesquiterpene lactone isolated from the leaves of yacon (Dou et al., 2010) and the determination of its potential toxic effects is an important issue to establish potential risks associated with its intake.

The degree of side effects or toxicity presented by extracts or compounds of medicinal plants depends on many complex factors. The effects of a single large dose of a toxic substance may not necessarily reflect the risks associated with the long-term low-level consumption commonly used in folk medicine. In addition, long-term studies are essential to determine a range of bioactivities to a no-observed-adverse-effect level (NOAEL) (Alexeeff et al., 2002).

Thus, this work was undertaken to evaluate the *in vitro* and *in vivo* toxicity of both the aqueous extract of *S. sonchifolius* leaves and enhydrin isolated from yacon leaves. It is an important issue in order to continue assessing their potential antidiabetic use.

2. Materials and methods

2.1. Plant material

Leaves of *S. sonchifolius* (Poepp & Endl.) H. Robinson (clone LIEY 97-1) used in this study were collected on February 2010 from plants cultivated in an experimental field belonging to the Regional Ecology Institute (IER), National University of Tucumán, located at Horco Molle, Yerba Buena, province of Tucumán, Argentina. Voucher specimens (LIL607173) are deposited in the herbarium of "Fundación Miguel Lillo", San Miguel de Tucumán, Argentina.

2.2. Preparation of the aqueous extract of S. sonchifolius

The plant material was carefully dried under air flow in an oven between 40 and 45 °C.

Aqueous extract of the plant was prepared by boiling 10 g dried leaves in 100 mL distilled water under reflux for 10 min. The decoction obtained (10%) was filtered, frozen at −20 °C and then lyophilized. The extract yielded 1.7 g of dry residue, 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, we selected a 10% decoction based on its efficacy hypoglucemiant previously assayed in our laboratory (Aybar et al., 2001) and on the approximate dose used in traditional medicine (Grau and Rea, 1997). In addition, we tested different doses of 10% decoction in Wistar rats, being 0.14 g dry residue/kg a dose hypoglucemiant more effective.

2.3. Isolation and purification of enhydrin

To isolate preparative amounts of enhydrin, a procedure described by Genta et al. (2010) was used. The aim of this experimental procedure was to extract the contents of glandular trichomes (rich in STLs) of the leaf surface and from there to purify enhydrin.

2.4. Phytochemical analysis of the aqueous extract of S. sonchifolius

2.4.1. Infrared (IR) spectroscopy

The analysis of the 10% decoction was performed by IR spectroscopy. The samples were prepared in KBr tablets and the IR spectra were performed in a Perkin-Elmer 1600 FT-IR spectrophotometer.

2.4.2. Thin-layer chromatography (TLC) analysis

TLC analysis enabled identification of caffeic acid and chlorogenic acid (3-caffeoylquinic acid) by comparison with authentic samples using different solvents and detection systems. Merck aluminum sheets of Silica gel 60 F254 were used. For chlorogenic acid identification, plates were developed with *n*-butanol:acetic acid:water (10:1.75:8) or ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:27). For caffeic acid identification, plates were developed with *n*-hexane:ethyl acetate:acetic acid (4:6:0.15). Detection was performed by (i) fluorescence at 366 nm (Mineral Light Lamp, Model UV GL, multiband UV 254/366, UVP San Gabriel, USA), (ii) spraying with a 1% solution of 2-aminoethyldiphenylborinate in methanol and then with a 5% solution of polyethylene glycol in ethanol and (iii) spraying with a 1% solution of $FeCl_3$ in methanol.

2.4.3. HPLC analysis

For the HPLC analysis, a Gilson 322 HPLC (binary pump) with a Gilson UV/VIS-152 Detector, Rheodyne injector with 20 μL loop and Unipoint software was used. The column employed was a Grace Smart RP_{18} analytical column (5 μm; 4.6 mm × 250 mm) using two different solvent programs. Mobile phase I: solvent A (5% acetic acid–water solution); solvent B (methanol). Elution was achieved with the following linear gradient: 20 to 33.5% B in 60 min. Flow rate: 0.7 mL/min. UV detection was carried out at 326 nm; 0.01 sensitivity. Injection volume: 20 μL. Mobile phase II: solvent A (2% acetic acid–water solution); solvent B (2% acetic