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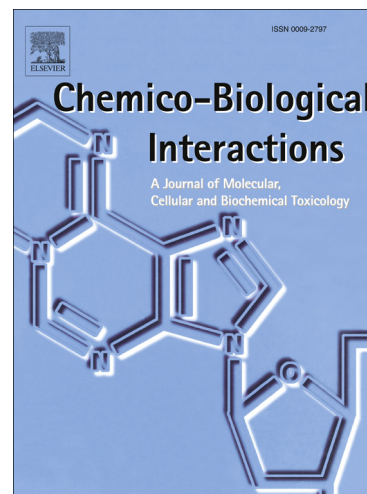
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***Smallanthus macroscyphus*: a new source of antidiabetic compounds.**

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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 g /kg 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, whose most relevant member is the species *S. sonchifolius* (Poepp. and Endl.) H. Robinson or “yacon” due to its wide range of medicinal and nutritional properties [1,2,3,4,5,6,7]. *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, 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 hypoglycemic 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 secretory 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 ^1H 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 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 *Smallanthus 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 *Smallanthus 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.

2.3.2. Thin-layer chromatography (TLC) analysis

TLC was developed to identify phenolic acids in decoction of *S. macroscyphus* by comparison with authentic samples using different solvents and detection systems as described previously [5]. Briefly, Merck aluminum sheets of Silica gel 60 F254 were used. For 3-caffeoylquinic acid (3-CQ) 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 (CAF) identification, plates were developed with n-hexane:ethyl acetate:acetic acid (4:6:0.15). Detection was performed by (i) fluorescence at 366nm (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 FeCl₃ in methanol.

2.3.3. HPLC analysis

The HPLC analysis of the aqueous extract of *S. macroscyphus* leaves was performed using a Gilson 322 HPLC (binary pump) with a Gilson UV/VIS-152 Detector, Rheodyne injector with 20 µL loop and Unipoint software. In order to characterize the phenolic acids, a GraceSmart RP₁₈ analytical column (5 µm; 4.6 mm x 250 mm) was employed using two different solvent programs, as previously described for characterize 3-CQ, CAF, 3,4-dicaffeoylquinic (3,4-DCQ) acid, 3,5-dicaffeoylquinic (3,5-DCQ) acid and 4,5 dicaffeoylquinic (4,5-DCQ) acid of *S. sonchifolius* leaves [25]. 3-CQ and CAF were obtained from Sigma Chemical Company. Reference samples of all the three DCQ acids were obtained from *Ilex paraguariensis* [26].

In order to characterize the presence of the sesquiterpene lactone polymatin A in aqueous extract of leaves, a third solvent program was used. The binary mobile phase consisted of solvent A (0.5% acetic acid water solution) and solvent B (0.5% acetic acid acetonitrile solution). Elution was achieved with the following linear gradient: 0 to 45% B in

30 min. Isocratic elution occurred with 45% B in 30 to 50 min, 45 to 100% B in 50 to 80 min. The flow rate was 1.3 mL min^{-1} . UV detection was carried out at 254 nm; 0.01 sensitivity. Injection volume: 20 μL . The presence of polymatin A in 10% decoctions was evaluated by comparison of retention times and UV spectra of purified polymatin A (see section 2.4).

2.3.4 Analysis by Gas Chromatography coupled to a Mass Detector (GC-MS)

Dried 10% decoction (50 mg) was suspended in water (20 mL) and then extracted with chloroform (15 mL). The organic phase was recovered and the solvent was evaporated to afford a dry residue. GC-MS analysis were recorded using a Hewlett-Packard 5973 selective mass detector coupled to a Hewlett-Packard 6890 Gas Chromatograph fitted with an Elite-5MS Perkin-Elmer column (5% phenylmethylsiloxane, 30 m x 0.25 mm i.d.; 0.25 μm film thickness); ionization energy 70 eV. Temperatures of injector, GC-MS interphase, ion source and selective mass detector were maintained at 220°C, 280°C, 230°C and 150°C, respectively; carrier gas was Helium with a flow rate of 1.1 mL min^{-1} . The oven temperature was programmed as follows: 140-300 °C at $2 \text{ }^\circ\text{C min}^{-1}$ and then held at 300°C for 5 min. The sample was injected (1.0 μL) as a 1% solution in methylene chloride. Polymatin A was characterized by: (a) comparison of their mass spectrum with a commercial GC-mass spectra library (National Institute of Standards and Technology, 1999, PC Version 1.7 of the NIST/EPA/NIH Mass Spectral Library. Perkin-Elmer Corp.: Norwalk, CT.) and with mass spectrum reported in the literature [19,20].

2.4. Isolation and Purification of polymatin A

Whole air-dried leaves (406 g) of *S. macroscyphus* were soaked one by one in chloroform (2.95 L) placed in a glass tank. In this procedure each leaf was soaked in the solvent for 20 seconds at room temperature with a continuous and gentle swinging motion using a steel forceps. The extraction solvent was filtered through filter paper to eliminate

small pieces of plant material and then was evaporated at reduced pressure to afford 7.06 g of residue which was dissolved with 120 mL of methanol at 40-45° C. Waxes were precipitated by adding dropwise water (50 mL) with magnetic stirring and the filtrate was evaporated to dryness to yield 5.33 g of a crude lactone mixture. The aim of this experimental procedure was to extract the contents of glandular trichomes of the leaf surface, which is rich in STLs.

To purify preparative amounts of polymatin A, a portion of crude lactone mixture (5.11 g) was processed by chromatography on Silica gel Merck (230-400 mesh, 160 g) using hexane-ethyl acetate mixtures of increasing polarity (9:1; 4:1; 7:3; 3:2 and 1:1); 52 fractions were collected and grouped according to their TLC profiles and monitored by Infrared (IR) spectroscopy. The fractions showing strong γ -lactone absorption ($1760-1780\text{ cm}^{-1}$) and containing fairly pure polymatin A (>92% by Gas chromatographic analysis) were reunited, the solvent evaporated and the gummy residue (3.32 g) was treated with ether (9 mL) and kept at 4 °C for one week. This treatment induced crystallization of polymatin A. Recrystallization from heptane-ethyl acetate afforded 2.89 g of crystalline polymatin A, melting point 121-122 °C (yield 0.71%, w/w of dry leaf weight). GC-MS analysis indicated a purity of 97.4% of polymatin A. NMR measurements were recorded on a Bruker 300 AVANCE.

2.5. Experimental animals

Adult male Wistar rats aged 8 to 12 weeks were selected for all the experiments. The animals were acclimated for 7 days, during which period each animal was examined to confirm suitability for the study. Suitability criteria were acceptable physical examination and body weight (b.w.), so that b.w. means for each group were comparable. At the beginning of the study, male rats weighed 200 ± 20 g. They were obtained from the colony bred at the Department of Developmental Biology, INSIBIO (CONICET-UNT), Tucumán, Argentina.

The rats were housed in individual cages. The photoperiod (light on from 07:00 to 19:00 h), air changes, room temperature ($22\pm 2^{\circ}\text{C}$) and humidity (60-70% relative) were controlled. Animals were given free access to a powdered certified rodent diet obtained from a commercial source (Standard Food -Asociación de Cooperativas Argentinas - SENASA N° 2706) and given tap water *ad libitum*. There were no known contaminants in food or water which were expected to interfere with the results of the study.

Throughout the experiments, all animals were maintained and handled according to international ethical guidelines for the care of laboratory animals (US Food and Drug Administration). The experimental protocol was approved by the Committee on Bioethics in Research of the National University of Tucumán and all experiments have complied with the current laws of Argentina (Ethical Framework of Reference for Biomedical Research in laboratory animals, Resol. D N° 1047 anexo II, 2005).

2.6. Induction of experimental diabetes

Stable diabetes was induced by a single intraperitoneal injection to rats of freshly prepared streptozotocin (STZ, Sigma Chemical Company, St. Louis, MO, USA) solution in 10mM sodium citrate buffer (pH 4.5), at a dose of 45 mg/kg body weight (b.w.). Control rats received only citrate buffer. Diabetes was achieved within 48 h in the majority of the animals, as determined by measuring of the fasting blood glucose levels and glucosuria. Only the animals with glycemia >350 mg/dL 2 days after STZ treatment were included in the study.

2.7. Biochemical determinations

Blood glucose was measured with an Accu-chek® Active (Roche Diagnostics GnbHD-68298 Mannheim, Germany), based on glucose dye oxido-reductase mediator reaction. Urine glucose was determined by a reagent-based glucose oxidase-peroxidase enzymatic method (Bayer S.A. Diagnostic). Plasma insulin was determined by an Enzyme-

linked immunosorbent assay (rat/mouse Insulin ELISA kit, Linco Research, Inc.). Hemoglobin A1c (HbA1c) was separated by a chromatographic method using a cationic exchange resin (Hemoglobin A1c Kit, BioSystem S.A, Barcelona-Spain) and quantified by spectrophotometric reading at 415nm.

2.8. Experimental design

2.8.1. Dose optimization. Oral glucose tolerance test in normal healthy rats

In order to choose the minimum effective hypoglycemic dose of the 10% decoction of *S. macroscyphus* leaves a range of variable doses (70, 140 and 280 mg dried extract/kg b.w.) was evaluated in normal healthy rats with the oral glucose tolerance test (OGTT), whereas the hypoglycemic action of polymatin A isolated from leaves was assessed with doses of 7, 14 and 28 mg dried powder/kg b.w.

OGTT was performed as described: the overnight fasted normal rats were divided into groups of six animals each. Basal (pre-treatment) blood glucose levels of all animals were recorded. Then, each group received orally, different doses of 10% decoction or polymatin A as mentioned above. The control group received vehicle (distilled water), whereas the standard drug glimepiride (5 mg/kg b.w.) was orally administered to rats in the positive control group. Thirty minutes later, the blood glucose level of each group was evaluated, and considered as 0 h value. Then, all the animals received orally a glucose solution (2 g/kg b.w.) and blood glucose was recorded at 15, 30, 45, 60 and 120 min after glucose loading.

2.8.2. Assessment of hypoglycemic effect in normoglycemic rats

2.8.2.1. Single-dose effect on fasting blood glucose.

Normal healthy male rats were fasted overnight (for 12 h) and pre-treatment fasting blood glucose levels were determined. The animals were divided into four groups of six rats each, and were given orally vehicle (distilled water, 1mL), 10% decoction (140 mg dried

extract/kg b.w), polymatin A (14 mg/kg b.w.) or glimepiride (5 mg/kg b.w.). Blood samples were collected from the tail vein 1, 2 and 4 h after extract administration.

2.8.2.2. Single-dose effect on post-prandial blood glucose.

Normal healthy male rats fasted overnight (for 12 h) were divided into four groups (n = 6 rats) and were given orally vehicle (distilled water, 1 mL), 10% decoction (140 mg dried extract/kg b.w), or polymatin A (14 mg/kg b.w.). Blood glucose levels were determined and referred to as 0 h value. Then, all the animals received sucrose (2g/kg b.w.) at 6:00 pm, and post-prandial blood glucose levels were monitored after 30, 60 and 120 min.

2.8.3. Assessment of hypoglycemic activity in diabetic rats

STZ-diabetic rats were randomly divided into four groups (n=10 rats), and each group was treated with vehicle (distilled water, 1 mL), 10% decoction (140 mg dried extract/kg b.w), polymatin A (14 mg/kg b.w.) or glimepiride (5 mg/kg b.w.), once a day for 4 weeks. All dry extracts were dissolved in distilled water (1 mL) and administered at 6:00pm before eating using an intragastric tube. Fasting blood glucose level was determined in the blood sample from the tail vein every 7 days during the experimental period. Body weight, urine glucose level and plasma insulin levels were recorded weekly. Food intake was recorded daily.

2.9. Acute toxicity studies

Acute oral toxicity of 10% decoction and pure polymatin A was studied by a single-dose experiment. Healthy Wistar rats were randomly divided into groups of six animals. Each group included three males and three females with a weight of 200.0 ± 20.0 g that were orally given a single dose of 10% decoction or polymatin A. The doses tested were 3.5, 7.0 and 14.0 g dry extract/kg b.w. for 10% decoction, representing 25, 50 and 100 times the effective hypoglycemic dose, and 0.7, 1.4 and 2.8 g dried powder/kg b.w. for pure crystalline polymatin A, representing 50, 100 and 200 times the effective hypoglycemic dose. The

control group received only the vehicle (distilled water). Rats were observed continuously for 1 h after treatment to evaluate their behaviour (reduction in locomotion, aggressiveness, reaction to stimuli as tail pinch, noise, social interactions), neurologic (posture, exploratory movements, stereotypes, presence of clonic or tonic movements) and autonomic activity (piloerection, lacrimation, pupil size, respiratory pattern) and toxic effects. Then, the controls were carried out at 3.0 and 6.0 h post-dose on day 0 and twice daily (morning and afternoon) thereafter for 14 days. Food consumption, feces and urine (using reactive strips, Bayer S.A. Argentina) were also examined at the intervals specified above.

2.10. Statistical analysis

The results from three independent experiments were presented as mean \pm SD. The significance of differences was evaluated using the paired Student's *t*-test. When more than one group was compared with one control, significance was evaluated by one-way analysis of variance (ANOVA). When correspond, comparisons of blood glucose levels in different times and treatments were performed by two-way ANOVA followed by Bonferroni *post-hoc* test. A *p* value <0.05 was considered statistically significant

3. Results

3.1. Phytochemical analysis of the aqueous extract (10% decoction) of *S.*

macroscyphus leaves

In order to identify new active principles, we analyzed the constituents of a 10% decoction of *S. macroscyphus* leaves. First, IR spectroscopy was performed. Absorption corresponding to γ -lactone carbonyl ($1755\text{--}1780\text{cm}^{-1}$) was observed in the IR spectrum, indicating that SLTs are present in this extract. The IR spectrum (KBr) of the 10% decoction displayed strong absorptions at 3300cm^{-1} (phenol O-H stretch) and at 1600cm^{-1} (alkenyl C=C stretch), indicating that phenolic compounds are present in this extract.

TLC screening using authentic samples as reference and three different detection systems showed the presence of 3-CQ, CAF and caffeic acid derivatives, probably dicaffeoylquinic acids, in *S. macroscyphus* decoction. HPLC chromatograms of this extract using two different solvent programs (see Section 2.3.3) are shown in Fig. 2. Fig. 2A shows HPLC chromatogram using a Mobile phase I (detection at 326 nm) with peaks corresponding to 3-CQ and CAF. In Fig. 2B, the chromatogram using a Mobile phase II shows peaks of 3,4-DCQ, 3,5-DCQ and 4,5-DCQ acids (detection UV at 326 nm). The chromatogram registered at 254 nm using a third solvent program shows that the 10% decoction of leaves has polar compounds and many peaks with low retention times (Fig. 3A) It also shows a single peak with intermediate retention times (1) and therefore of intermediate polarity, likely an STL. The presence of polymatin A in 10% decoction was evaluated by comparison of this retention times with an authentic sample (Fig. 3B). The Fig. 3C shows an overlay of the spectra of polymatin A and decoction, indicating that peak (1) is likely polymatin A. A rapid and sensitive GC-MS method allowed the identification of the lactone as a minor component in the decoction (Fig. 4)

3.2. Chemical characterization of polymatin A isolated from *S. macroscyphus* leaves.

Crystalline polymatin A, was obtained as described in section 2.4. ^1H NMR (300 MHz, CDCl_3) δ in ppm (J in Hz; assignment): 6.85 dd (9.9 and 7.3 Hz; H-1), 6.33 dd (8.4 and 1.8; H-8), 6.24 d (3.2; H-13a); 6.11 qq (7.1 and 1.4; H-3'); 5.67 d (3.4; H-13b); 5.08 dd (10.5 and 9.5; H-6); 4.94 broad d (10.5; H-5); 4.00 dd (10 and 8.4; H-9), 3.80 s (3H; $-\text{CO}_2\text{Me}$); 2.82 d (10; $-\text{OH}$), 2.65 dddd (9.5, 3.4, 3.2 and 1.8; H-7), 2.48 m (H-2a), 2.33 m (H-3b), 2.24 m (H-2b), 2.09 m (H-3a), 1.98 dq (3H; 7.3 and 1.4; H-4'), 1.88 broad s (6H; H-15 and H-5'). The ^1H NMR spectra matched exactly with the data reported in the literature [19, 20]. The previously unreported ^{13}C NMR data follow: ^{13}C NMR (75 MHz, CDCl_3): 169.21 (C-12), 167.42 (C-14), 166.84 (C-1'), 144.19 (C-1), 139.46 (C-3'), 137.58 (C-4), 134.77 (C-11), 133.71 (C-10), 126.89 (C-2'), 126.40 (C-5), 121.09 (C-13), 75.56 (C-6), 71.65 (C-8), 71.08 (C-9), 52.11 (OMe), 51.42 (C-7), 36.65 (C-3), 25.71 (C-2), 20.40 (C-5'), 16.81 (C-15), 15.75 (C-4'). MS (EI, 70 eV, GC-MS): m/z (%) M^+ 390 (0.06), 359 (0.1), 307 (1), 290 (1), 272 (1), 258 (3), 240 (2), 212 (3), 193 (2), 128 (3), 105 (4), 83 (100), 44 (43). 0.06] (38), 211 (43), 167 (8), 151 (11), 149 (4), 125 (28), 109 (100), 108 (9), 83 (26), 82 (15), 81 (13), 79 (11), 67. HRMS: M^+ 390.1684 (calcd for $\text{C}_{21}\text{H}_{26}\text{O}_7$ 390.1678).

3. 3. Dose optimization. Effect on OGTT of normal healthy rats

In a preliminary test we have demonstrated that the aqueous extracts of dried *S. macroscyphus* leaves exert a hypoglycemic effect in rats [13]. In order to determine the minimum effective dose of both 10% decoction and polymatin A isolated from leaves of this plant, we have performed an OGTT.

As shown in Figure 5, a 10 % decoction of *S. macroscyphus* leaves has effective hypoglycemic activity. This effect was observed as early as 15 min after the glucose load, with all doses assayed (70, 140 and 280 mg/kg b.w.). Interestingly, 140 mg dry extract/kg b.w. was the only dose able to inhibit the hyperglycemic peak with an important reduction of

blood glucose level at 30 min after glucose pulse, so it was selected for further experiments as the most effective dose. In addition, when the glycemic response was expressed as the increase of total area under the curve (IAUC), the values of IAUC of this treated group (S.m 140 mg/kg b.w.) were significantly lower than the ones found in the others groups (Figure 5). Compared to control (H₂O), the whole glycemic response was reduced by 62.25%, 75.40%, 38.85% and 36.20% in animals treated with 70, 140 and 280 mg/kg b.w. of dry extract and 5 mg/kg b.w. of glimepiride, respectively.

Figure 6 shows the result of OGTT for polymatin A at the doses assayed (7, 14 and 28 mg/kg b.w.). From this test, we concluded that the lactone obtained from *S. macroscyphus* leaves has an important hypoglycemic effect, the minimum effective dose being 14 mg/kg b.w. This dose was able to inhibit the hyperglycemic peak at 15 min after glucose load and kept blood glucose levels stable up to 30 min. The glycemic response expressed as IAUC values showed a significant decrease in the group treated with a dose of 14 mg/kg with respect to the control group (H₂O), it being significantly lower than that in the other groups (Figure 6). Indeed, the whole glycemic response was reduced by 74.59% in animals treated with 14 mg/kg b.w. of crystalline lactone compared with control group; therefore, this dose was selected for the further experiments.

3.4. Effect on blood glucose levels of normoglycemic rats

3.4.1. Single-dose effect on fasting blood glucose

This experimental protocol was carried out with a single dose of 10% decoction of *S. macroscyphus* leaves (140 mg dry extract/kg b.w.) or polymatin A (14 mg /kg b.w.) to evaluate their effects on the fasting blood glucose of normoglycemic rats. The extract of leaves caused a moderate hypoglycemic effect 2 h after oral administration. As shown in Table 1, this effect was significant but not as strong as that of glimepiride. Conversely,

treatment of fasting normoglycemic rats with polymatin A (14 mg/kg) had no significant glucose-lowering effect.

3.4.2. Single-dose effect on post-prandial blood glucose

The ability of 10% decoction of *S. macroscyphus* and polymatin A to inhibit postprandial hyperglycemia (anti-hyperglycemic effect) was evaluated using rats in the postprandial state, after sucrose overload. In rats treated with pure polymatin A (14 mg/kg), the blood glucose level reached a peak at 30 min after sucrose administration and returned to the initial level at 60 min (Table 2). This response was significantly different in control rats receiving H₂O, in which blood glucose levels reached a peak at 30 min but failed to return to the initial level in the course of 120 min. Moreover, a single-dose of 10% decoction (140 mg/kg) significantly prevented the hyperglycemic peak at 30 min after sucrose ingestion and was able to maintain blood glucose levels throughout the experiment (Table 2). Additionally, the treatment of normal rats with glimepiride, a positive control drug, produced a marked reduction in blood glucose that was maintained until the end of the experiment, as shown in table 2.

3.5. Antidiabetic effect in STZ-diabetic rats

This study was carried out in order to evaluate the effect of a leaf extract (10% decoction) of *S. macroscyphus* and pure polymatin A on blood glucose levels in STZ-diabetic rats. This is the first report addressing the potential therapeutic effect of *S. macroscyphus* leaves on diabetes mellitus. The treatment for 4 weeks of diabetic animals with a 10% decoction at a dose of 140 mg/kg/day was able to produce a significant decrease in blood glucose levels compared with untreated diabetic animals (Figure 7). At the end of the experimental period, the blood glucose level was significantly lower with respect to the pre-

treatment values ($p < 0.05$) and also compared to the glucose level of untreated diabetic group ($p < 0.001$). At this time, glycemia was approximately 32% lower than before treatment.

When diabetic rats received polymatin A (14 mg/kg) daily for 4 weeks a significant reduction in fasting blood glucose was detected (Figure 7). At the end of the experimental period, the blood glucose levels was significantly lower with respect to the untreated diabetic group ($p < 0.001$) and statistically different to pre-treatment values ($p < 0.05$). Interestingly, at this point, the statistical analysis showed that polymatin A (14 mg/kg) was as effective as glimepiride and was more effective than *S. macroscyphus* decoction.

Body weight of treated and control animals after 28 days of treatment are shown in Table 3. There were no significant differences in the initial weight of treated animals compared to controls (data not shown, see section 2.5 Criteria of suitability). At the end of the experimental period (4 weeks), a marked decrease in body weight was observed in diabetic rats compared to normal healthy animals as a consequence of disease progression. Interestingly, daily administration of a 10% decoction or polymatin A resulted in a significant increase in body weight compared to untreated diabetic rats which was similar to the one caused by treatment with glimepiride (Table 3).

The diabetic animals treated with 10% decoction, polymatin A or glimepiride showed a decrease in daily standard rodent diet consumption compared with the untreated diabetic group but these intakes were significantly higher than those in the normal group (Table 3).

It was demonstrated that after a 4-week treatment of diabetic rats with a 10% decoction or polymatin A, urinary glucose excretion was significantly decreased compared with the untreated diabetic group, although these values were higher than in the normal group (Table 3). These results are in agreement with the improvement in blood glucose levels of treated diabetic rats.

As shown in Table 3, the relative concentration of Hb A1c in normal rats at the end of the experimental period was $6.03 \pm 1.82\%$. These levels increased in untreated STZ-diabetic

animals as a result of poor glycemic control in the previous 4 weeks (equivalent to the lifetime of the erythrocytes). HbA1c levels declined significantly 4 weeks following the treatment with the 10% decoction or polymatin A, indicating that both treatments led to acceptable glycemic control in diabetic animals.

Figure 8 shows the effects of different treatments on blood glucose and plasma insulin levels of diabetic rats at the end of the experimental period (day 28). At this time, both the 10% decoction and polymatin A produced a significant decrease in blood glucose levels, although there was no significant increase in plasma insulin levels. In this experimental model, similar results were observed in rats treated with glimepiride, a reference hypoglycemic drug (Fig. 8). However, all treatments significantly improved the fasting blood glucose/insulin ratio compared to the diabetic control group by 37.6% with the 10% decoction treatment, 61.9% with polymatin A treatment and 67.7% with glimepiride treatment.

3.6. Acute toxicity studies

Acute oral toxicity of the 10% decoction and pure polymatin A was evaluated in normal healthy rats. The doses assayed were 25, 50 and 100 times greater than the effective hypoglycemic dose of the 10% decoction and 50, 100 and 200 times than the effective hypoglycaemic dose of polymatin A. No deaths or acute toxic effect (changes in behavior or posture, presence of convulsions or occurrence of secretions) were reported within 14 days. All treatments were well tolerated and did not produce adverse nutritional effect. No gastrointestinal symptom such as diarrhea or constipation were observed at the doses assayed. Volume, pH and urine specific gravity were within normal ranges. No nitrites, protein or blood were detected in the urine samples of the animal groups treated with different doses of the 10% decoction or polymatin A throughout 14 days of control. In this experiment no evidence of acute toxicity was found at any dose level up to the highest dose tested of the 10% decoction (14.0 g dry extract/kg b.w., 100 times the effective hypoglycemic

dose) or of polymatin A (2.8 g dried powder /kg b.w., 200 times the effective hypoglycemic dose). These doses represent the no-observed-adverse-effect level (NOAEL), indicating that the safety margin of the extract and polymatin A is high.

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4. Discussion

In recent years, the prevention and control of complications associated with diabetes have become one of the key issues in medical research, and the search for new pharmacologically active agents obtained by screening natural sources such as plants has become particularly relevant [27,28].

In view of the above aspects the aim of the present study was to investigate the glucose-lowering activity of the leaf extract of *S. macroscyphus* and of polymatin A, the most abundant sesquiterpene lactone in its aerial parts.

S. macroscyphus is a species closely related to *S. sonchifolius* and possibly with similar biological activities [1]. An intensive search of the existing literature demonstrated that this is the first comprehensive report that analyzes its hypoglycemic effect. Since there is no precedent for the use of this plant in traditional medicine or of its biological activities, we selected a 10% decoction of *S. macroscyphus* leaves to test its glucose-lowering effects. This concentration was chosen taking into account that in a previous work we demonstrated the safety and hypoglycemic efficacy of a 10% decoction of *S. sonchifolius* leaves, a species used in folk medicine and closely related to *S. macroscyphus*. [25].

S. macroscyphus represents a slight departure from previously studied *Smallanthus* species that contains a relatively large proportion of a class of 8-OH-9 acylmelampolides but otherwise fits the overall chemical profile of the genus [22]. The phytochemical analysis of the 10% leaf decoction showed that its major constituents were phenolic compounds. CAF and 3-CQ were readily detected and three dicaffeoil quinic derivatives (3,4-DCQ, 3,5-DCQ and 4,5-DCQ) were found also found to be constituents of the decoction. Moreover, a rapid and sensitive GC-MS method allowed the identification of polymatin A as a minor component in the decoction.

A number of studies have shown that the caffeic acid and chlorogenic acid present in several plants exhibited hypoglycemic activity and antioxidant properties in experimental animals [29,30,31,32]. The presence of these compounds together with dicaffeoyl quinic derivatives led us to think that they could be hypoglycemic principles of the decoction. Indeed, in the current study, we noticed for the first time an important hypoglycemic activity in the 10% decoction of *S. macroscyphus* leaves in a dose-dependent manner, as shown by an OGTT in normal rats. While all doses tested were effective in the prevention of a hyperglycemic peak within 15 minutes of the glucose load, 140 mg dry extract/kg b.w. was the only dose able to significantly reduce the blood glucose level at 30 min and maintain basal levels until the end of the test. The glycemic response, expressed as IAUC, was significantly lower with this dose than with the others ones assayed, so this was selected as the more effective hypoglycemic dose.

OGTT is an easy and widely used procedure that was originally developed to classify carbohydrate tolerance [33], and in the present study it was used to determine the ability of plant extracts to maintain the homeostasis of blood glucose after a glucose load in normal rats and to select an effective dose. The efficacy of the 10% decoction at a dose of 140 mg dry extract/kg b.w. was compared to the standard anti-diabetic drug glimepiride (5 mg/kg b.w.). In view of our results, it is possible that the glucose-lowering effect of the decoction could be mediated by an improvement in the glycemic control mechanisms, including insulin secretion and/or extrapancreatic pathways, as was suggested for sulfonylurea drugs [34].

Oral administration of a single-dose of the 10% decoction (140 mg/kg) to fasted normal rats caused a slight lowering in plasma glucose without any serious hypoglycemic effect during the 4 h of the test. On the other hand, this extract effectively inhibited the post-prandial hyperglycemic peak at 30 min after sucrose ingestion and maintained near-normal blood glucose levels throughout the experiment. The latter finding suggest that this treatment would prevent carbohydrate absorption after food intake via an inhibition of the enteric

enzymes including α -glucosidase and α -amylase present in the intestinal brush borders [35], with a consequent potential to control the post-prandial hyperglycemia.

In the present study, the oral administration of glimepiride (5 mg/kg b.w.) as a positive control produced a significant hypoglycemic effect in either fasting or post-prandial conditions. It is known that glimepiride, a third-generation sulfonylurea, stimulates insulin secretion, but also reduces blood glucose levels acting in the liver and other extra-pancreatic tissues improving the insulin action or by an insulin-like effect [36]. In keeping with our findings, it is possible that the 10% decoction or some of its constituents could act by a similar mechanism, contributing to the observed hypoglycemic effect without hypoglycemia risk. Indeed, it has been shown that some bioactive plant compounds with an aromatic hydroxyl group may potentiate insulin action which, together with their function as antioxidants, can exert a beneficial effect on the control of glucose in the diabetes [37].

In order to identify new metabolites with hypoglycemic activity, we isolated crystalline polymatin A from *S. macrocyphus* leaves. Interestingly, we determined by OGTT in normal rats that this sesquiterpene lactone was an active hypoglycemic compound, the minimum effective dose being 14 mg/kg b.w. This dose was effective in the prevention of the hyperglycemic peak within 15 minutes of the glucose load, with a quick return to basal blood glucose values. So, this was selected for further experiment.

In a previous study we found that enhydrin, the most abundant sesquiterpene lactone in yacon leaves, was also effective in reducing post-prandial glucose and useful in the treatment of diabetic animals [5]. Sesquiterpene lactones are secondary plant metabolites widely distributed within the Asteraceae family that in recent years have acquired therapeutic relevance as single components for the local treatment of inflammation and for cancer therapy [38]. The present work represents the first report that analyzes the hypoglycemic effect of polymatin A isolated from *S. macrocyphus* leaves.

Interestingly, polymatin A at a dose of 14 mg/kg b.w. had no significant glucose-lowering effects in fasting normoglycemic rats. However, it effectively normalized blood glucose level at 60 min of sucrose ingestion and the glycaemic response expressed as IAUC was significantly lower than in the untreated control group (data not shown). This finding suggests that polymatin A at the assayed dose would exert its pharmacological properties by stimulation of insulin release or by an insulin-like effect, without reaching the magnitude of the effect of glimepiride. It should be noted that polymatin A improved the glycaemic control without risk of hypoglycemia, which is one of the most undesirable side effects of diabetes treatments.

Control of blood glucose levels is critical in the early treatment of diabetes mellitus to prevent complications of the disease [39]. Consequently, investigation of the glucose lowering effects of the 10% decoction of *S. macroscyphus* leaves and Polymatin A on STZ-induced diabetic rats was a matter of interest. Daily treatment with a 10% decoction or polymatin A significantly decreased the blood glucose levels and maintained the effect throughout the experimental period (4 week). This hypoglycaemic effect was particularly relevant at the end of the experimental period, in which the blood glucose levels were close to normal. These findings lead us to believe that perhaps a longer treatment is required to achieve tight glycaemic control.

The HbA1c concentration in blood measures chronic glycaemia and is widely used to judge the adequacy of diabetes treatment and adjust therapy [40,41]. In keeping with our findings, treatments of diabetic animals for 4 weeks with a 10% decoction of *S. macroscyphus* leaves or polymatin A significantly decreased HbA1c levels, indicating an acceptable glycaemic control in treated rats.

In pre-clinical studies, the anti-diabetic effect of certain plant extracts is generally dependent on the dose of the diabetogenic agent and therefore on the degree of β -cell destruction [42]. Under our experimental conditions, STZ induced rapid and incomplete β -cell

destruction, evidenced by lower plasma insulin levels and hyperglucemia. While treatments of STZ-diabetic rats with a leaf decoction or with polymatin A significantly lowered fasting blood glucose, they produced no marked increase in plasma insulin level. Interestingly, both treatments significantly improved the fasting blood glucose/insulin ratio. Under our experimental conditions, this ratio indicates that the same level of insulinemia is able to appropriately compensate the fasting hyperglycemia in treated animals compared with diabetic controls and therefore, there is a possibility that decoction and polymatin A could act as insulin-mimetic agents and/or improved the insulin action at the cellular level. Further studies are in progress to determine the precise mechanism involved.

Decreased body weight, polyphagia and increased urine glucose excretion are well-known manifestations of diabetic state. In our studies, treatment of STZ-diabetic rats with a 10% decoction or polymatin A for 4 weeks caused both a significant gain in body weight and a decrease in daily food intake compared with untreated STZ-diabetic rats. Moreover, the treated animals reduced urinary glucose excretion. These results would be related to the high effectiveness of the extract and of polymatin A in decreasing the fasting blood glucose levels at 4 weeks of treatment. This is an additional advantage, confirming thereby the antidiabetic activity of the bioactive compounds.

In order to evaluate acute toxic effect in healthy adult rats, a wide range of doses of a 10% decoction of *S. macroscyphus leaves* and polymatin A were assayed. The lack of toxic effects at all doses up to the highest dose tested, suggested that the lethal dose 50 (LD50) of the 10% decoction and of polymatin A would be above 14.0 and 2.8 g/kg b.w., respectively, which are significantly higher than the effective hypoglycaemic doses. These doses represent the no-observed-adverse-effect level (NOAEL), indicating that the safety margin of the extract and the polymatin A is high.

Conclusion

The present study reveals that the 10% decoction of *S. macroscyphus* leaves and polymatin A, the main sesquiterpene lactone of the aerial part, have significant hypoglycemic and antidiabetic potential. This report represents a first step toward the assessment of a new medicinal plant and of new chemical entities isolated from it, which could help to manage diabetes more efficiently and safely.

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Conflict of interest statement

The authors declare that there was no conflict of interest.

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Figure captions

Figure 1. Chemical structure of polymatin A

Figure 2. HPLC chromatogram of 10% decoction from *S. macroscyphus* leaves. Grace Smart RP18 analytical column (5 μm ; 4.6 mm x 250 mm). **A)** Mobile phase I: solvent (a), 5% acetic acid water solution; solvent (b), methanol. Linear gradient: 20 to 33.5% (b) in 60 min. Flow rate: 0.7 mL min^{-1} . **B)** Mobile phase II: solvent (a), 2% acetic acid water solution; solvent (b), 2% acetic acid–methanol solution. Gradient: 15-40% (b) in 30 min; 40-75% (b) in 10 min and 75-85% (b) in 5 min. Flow rate of 0.7 mL min^{-1} . UV detection: 326 nm. CAF: caffeic acid, 3-CQ: 3-caffeoylquinic acid (chlorogenic acid), DCQ: dicaffeoylquinic acid.

Figure 3. HPLC chromatogram of 10% decoction from *S. macroscyphus* leaves. Grace Smart RP18 analytical column (5 μm ; 4.6 mm x 250 mm). **A)** Chromatogram of 10% decoction: Mobile phase: solvent (a) 0.5% acetic acid water solution; solvent (b) 0.5% acetic acid acetonitrile solution. Gradient: 0-45% (b) in 30 min, isocratic elution with 45% (b) in 30-45 min, 45-100% (b) in 50-80 min. Flow rate: 1.3 mL min^{-1} . UV detection: 254 nm. **B)** Chromatogram of purified polymatin A obtained from *S. macroscyphus* leaves. Column and conditions were identical as **A**. **C)** Overlaying chromatograms A and B.

Figure 4. GC-MS analysis of 10% decoction from *S. macroscyphus* leaves extracted with chloroform (see Material and Methods section). Polymatin A was characterized by comparison of its mass spectrum with commercial GC-mass spectra libraries and with those reported in the literature.

Figure 5. Dose Optimization of 10% decoction of *S. macroscyphus* leaves (S.m.) through oral glucose tolerance test (OGTT). Normal rats administered S.m.: (-◆-) 70 mg/kg b.w.; (-■-) 140 mg/kg b.w.; (-▲-) 280 mg/kg b.w.; (...×...) normal rat administered glimepiride 5 mg/kg b.w.; (---●---) normal rats administered water, control group. Data are expressed as mean \pm SD. n= 6 rats / group. **A)** The blood glucose concentration was measured at the indicated times. **B)** Glycemic response is expressed as the increase of total area under curve (IAUC) in %.

* $p < 0.05$ is considered to be statistically significant when compared with normal rats administered water. ** $p < 0.05$ is considered to be statistically significant when compared with normal rat administered glimepiride.

Figure 6. Dose Optimization of polymatin A (PA) through oral glucose tolerance test (OGTT). Normal rats administered PA: (-◆-) 7 mg/kg b.w.; (-■-) 14 mg/kg b.w.; (-▲-) 28 mg/kg b.w.; (...×...) normal rat administered glimepiride 5 mg/kg b.w.; (---●---) normal rats administered water, control group. Data are expressed as mean \pm SD. n= 6 rats / group. **A)** The blood glucose concentration was measured at the indicated times. **B)** Glycemic response is expressed as the increase of total area under curve (IAUC) in %.

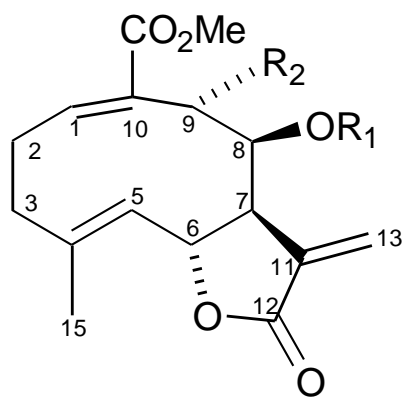
* The mean is considered to be statistically significant when compared with normal rats administered with water ($p < 0.05$). ** The mean is considered to be statistically significant when compared with normal rat administered with glimepiride ($p < 0.05$).

Figure 7. Effect of a 4 weeks oral treatment with 10% decoction (S.m.) and polymatin A (PA) of *S. macroscyphus* leaves on blood glucose levels of diabetic rats. (-◆-) S.m.140 mg/kg b.w./day.; (-■-) PA 14 mg/kg b.w./day; (-▲-) glimepiride 5 mg/kg b.w./day; (...×...) water (diabetic control rats). Data are expressed as mean \pm SD. Data was analyzed by two-way

ANOVA followed by Bonferroni post-hoc test. The mean is considered to be statistically significant when compared to pre-treatment values of respective treatment group ($^{\#}p<0.05$). The mean is considered to be statistically significant when compared with blood glucose levels of diabetic control rats ($* p<0.05$, $**p<0.01$, $***p<0.001$). $n=10$ rats / group.

Figure 8. Effect of an oral treatment with 10% decoction of *S. macroscyphus* leaves (S.m.) and polymatin A (PA) on **A)** fasting blood glucose levels and **B)** plasma insulin levels of diabetic rats at the end of the experimental period (day 28). Data are expressed as mean \pm SD. $n=10$ rats / group. * The mean is considered to be statistically significant when compared with diabetic control rats ($p<0.05$). ** The mean is considered to be statistically significant when compared with normal rats ($p<0.05$).

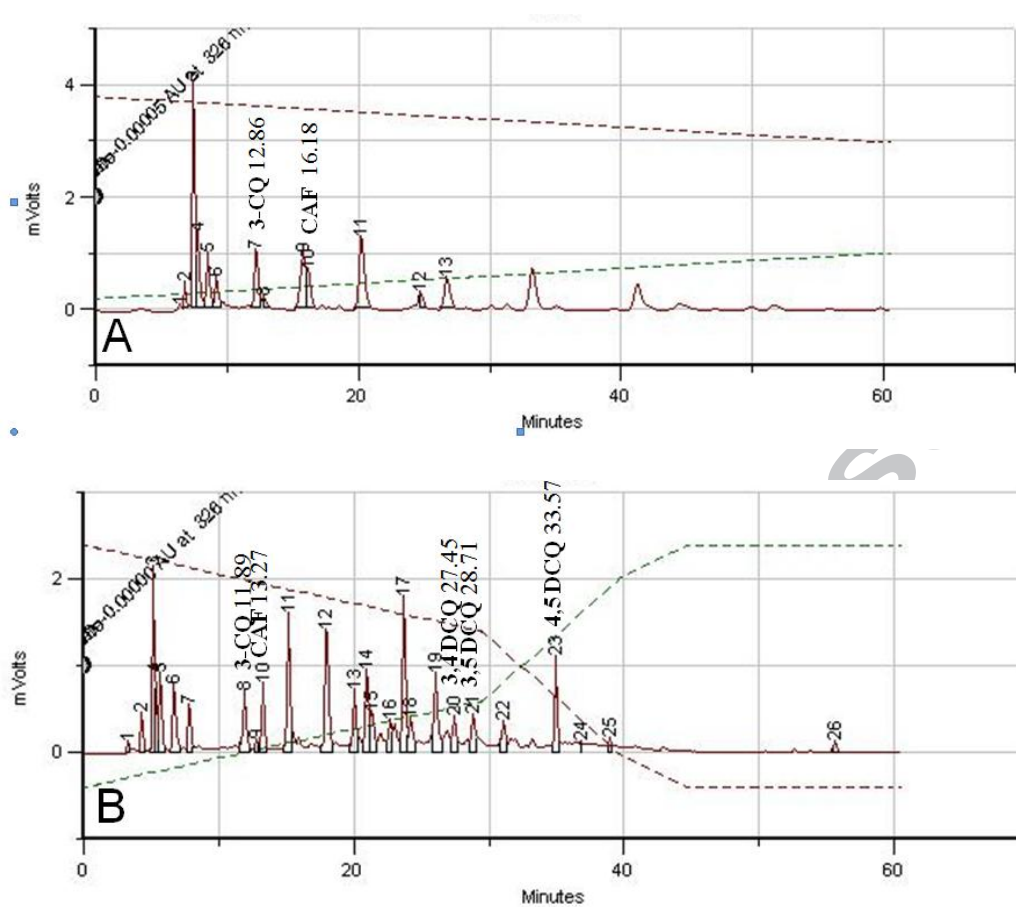
Figure 1



R₁: Ang; R₂: OH

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Figure 2.



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Figure 3.

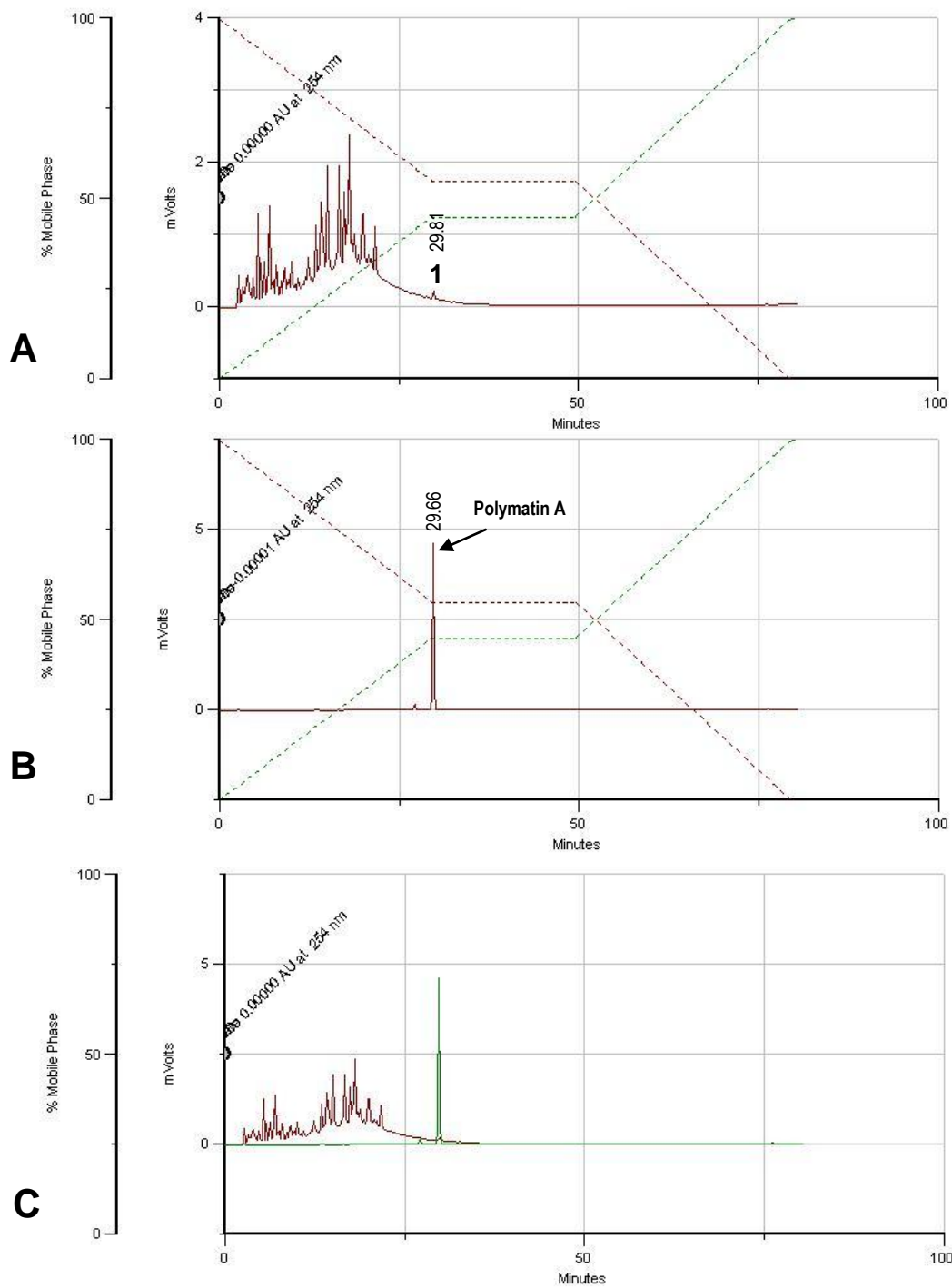


Figure 4.

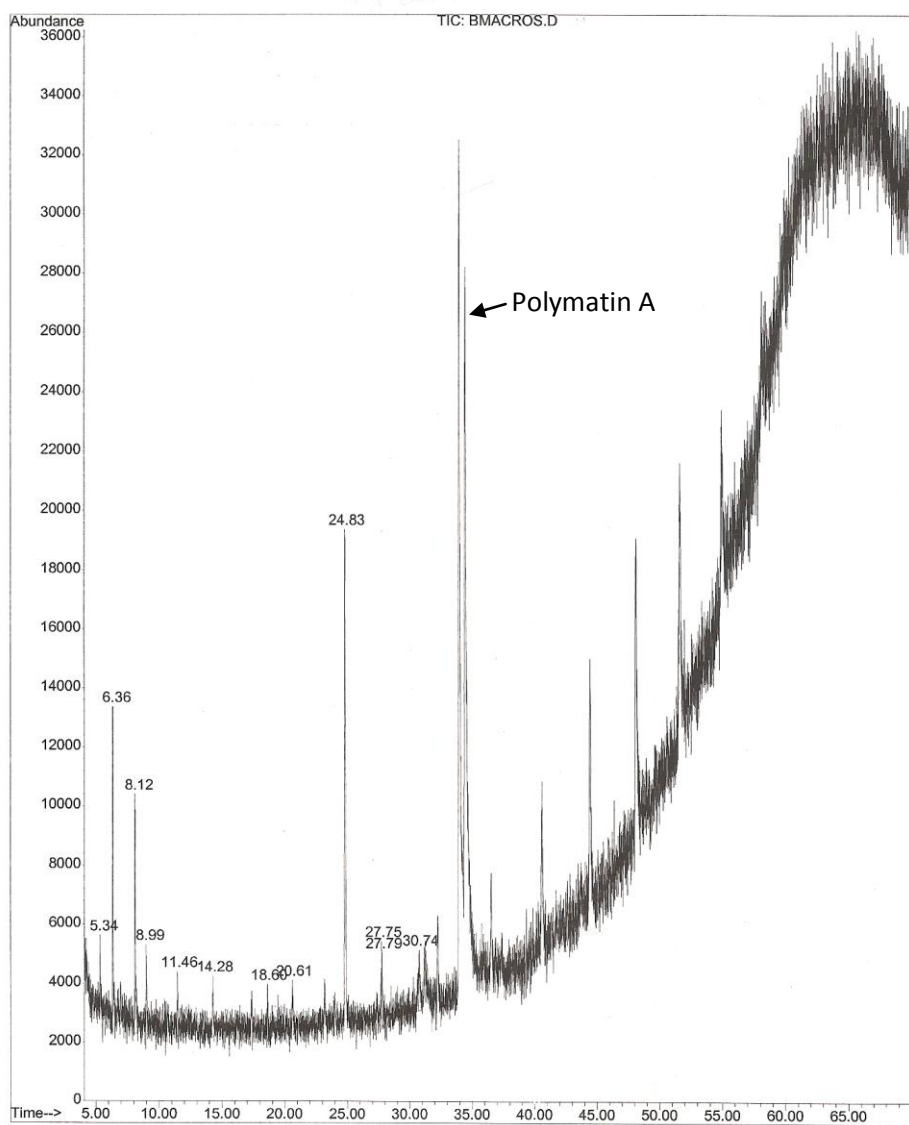


Figure 5.

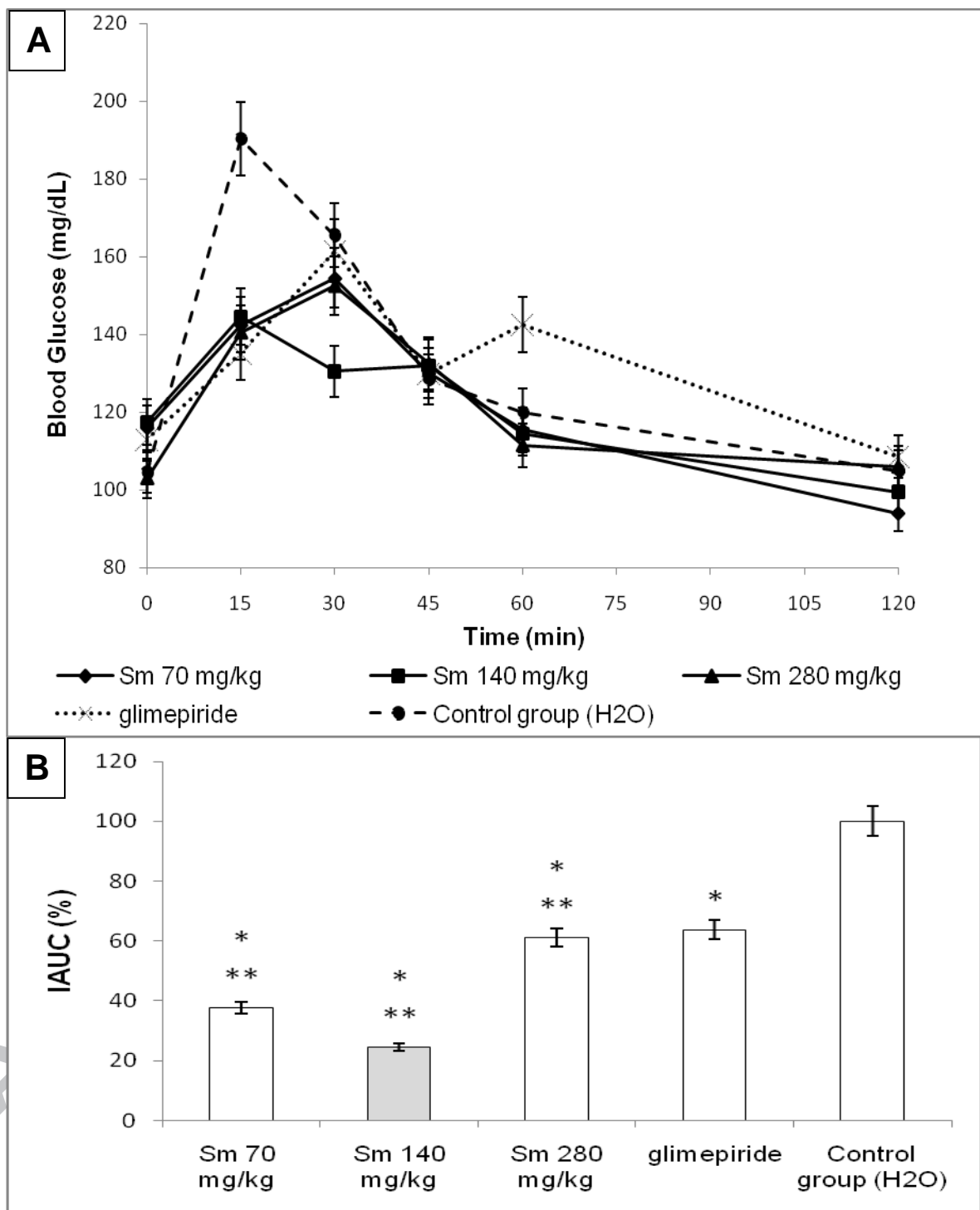


Figure 6.

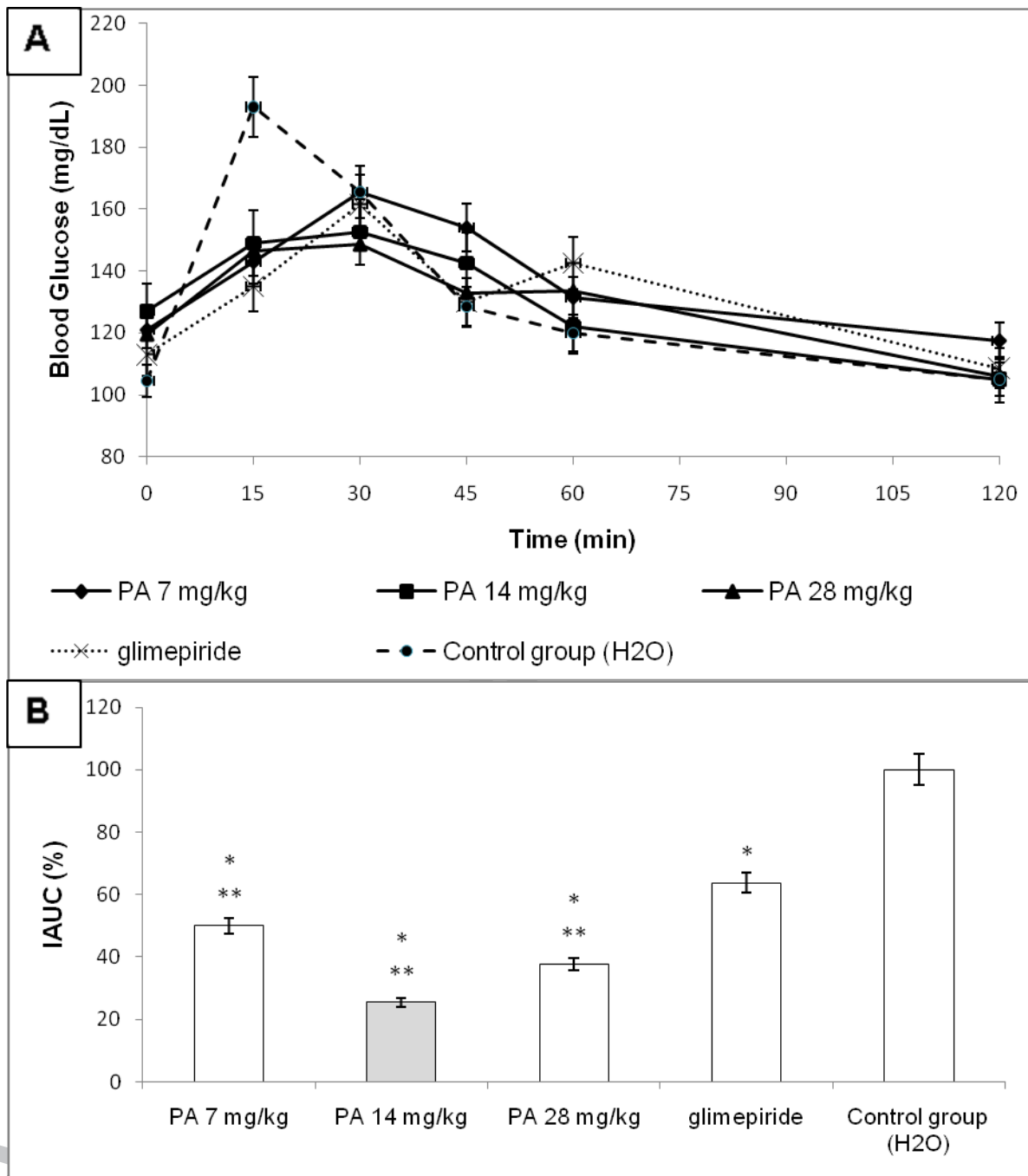
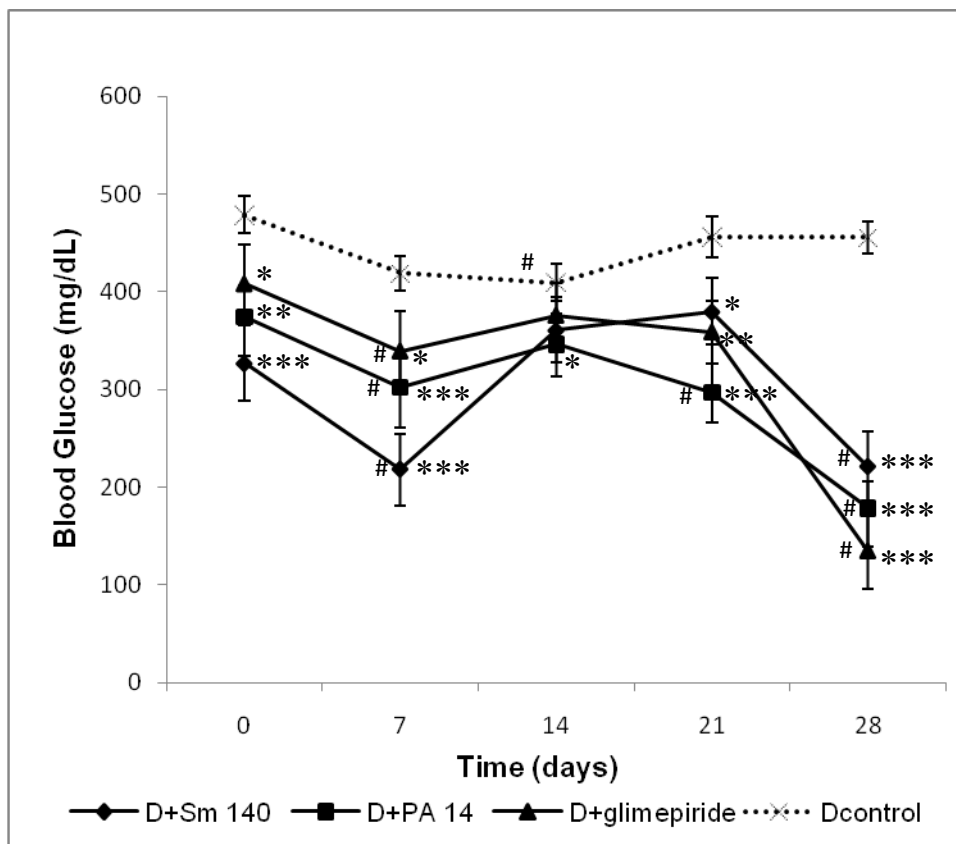
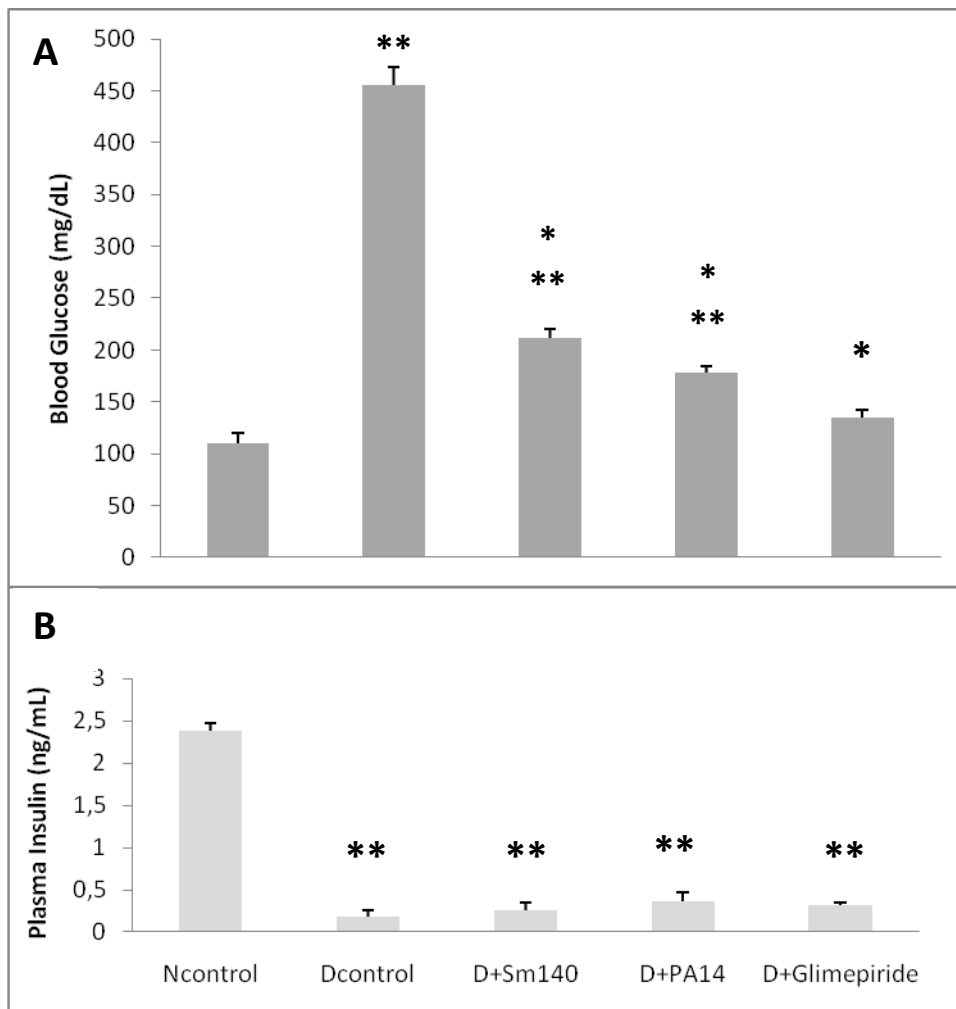


Figure 7.



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Figure 8



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Table 1. Effect of 10% decoction of *S. macroscyphus* leaves and polymatin A on fasting blood glucose level in normal rats.

Animal Groups	Glucose (mg/dl) Post-treatment			
	Pre-treatment Glucose (mg/dl)	1 h	2 h	4 h
Normal +distilled water (control)	90.1±3.3	91.6±27	89.8±3.2	79.8±5.1 ²
Normal+10%decoction (140mg/kg)	90.3±4.1	86.3±2.7	64.2±3.2 ^{1,2}	78.3±4.1 ²
Normal+polymatin A (14 mg/kg)	88.6±3,7	86.9±3.8	85.0±2.2	80.7±2.7 ²
Normal+glimepiride (5mg/kg)	89.2±3.3	79.2±3.1	43.4±3.7 ^{1,2}	48.3±4.0 ^{1,2}

Data are mean±SD. N=6 rats per group.

¹ Mean is considered to be statistically significant when compared with Normal+distilled water (control) group ($p<0.05$).

² Mean is considered to be statistically significant when compared with pre-treatment value ($p<0.05$).

Table 2 . Effect of 10% decoction of *S. macroscyphus* leaves and polymatin A on post-prandial blood glucose level in normal rats

Animal Groups	Post-prandial (sucrose overload) blood glucose level (mg/dl)			
	0 min	30 min	60 min	120 min
Normal +distilled water (control)	105.7±3.5	140.3±2.5	136.3±2.5	147.7±2.5
Normal+10%decoction (140mg/kg)	113.2±4,5	126.0±4,0 ¹	131.8±4.0	125.4±13.0 ¹
Normal+polymatin A (14 mg/kg)	112.0±5.5	142.7±5.0	108.3±3.0 ¹	110.0±7.5 ¹
Normal + glimepiride (5mg/kg)	108.7±2.5	97.7±3.4 ¹	66.4±3.5 ¹	50.1±4.0 ¹

Data are mean±SD. N=6 rats per group.

¹ Mean is considered to be statistically significant when compared with Normal+distilled water (control) group ($p<0.05$).

Table 3. Effect of 10% decoction of *S. macrocyphus* leaves and polymatin A on body weight, food intake, urine glucose and HbA1c level in STZ-diabetic rats (after 28 days of treatment)

Animal Groups	Body weight (g)	Food intake (g/kg b.w./day)	Urine glucose ()	Hb A1c (%)
Normal (control)	294.7±7.3	56.1±0.1	ND	6.03±1.82
Diabetic control	166.20± 9.0	60.3±0.3	1,000±10	14.99±0.17 ²
Diabetic+10%decoction (140mg/kg/day)	241.73±13.66 ¹	59.7±0.2 ^{1,2}	750±10 ^{1,2}	9.73±2.52 ¹
Diabetic+polymatin A (14 mg/kg/day)	227.53±14.53 ¹	58.4±0.2 ^{1,2}	750±10 ^{1,2}	8.89±0.75 ^{1,2}
Diabetic+glimepiride (5mg/kg/day)	214.70±14.14 ¹	58.2±0.5 ^{1,2}	500±10 ^{1,2}	6.94±1.24 ¹

Data are mean±SD. N=10 rats per group.

¹ Mean is considered to be statistically significant when compared with Diabetic control group ($p<0.05$).

² Mean is considered to be statistically significant when compared with Normal (control) group ($p<0.05$).

Highlights

- This is the first report on *Smallanthus macroscyphus* as a new source of antidiabetic compounds.
- A 10% decoction of *S. macroscyphus* leaves has a significant hypoglycemic effect.
- Polymatin A, a sesquiterpene lactone of the leaf, has a significant hypoglycemic activity.
- Acute toxicity studies in rat demonstrated that the use of decoction and polymatin A is safe.