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Research Article

Yacon leaf aqueous extract: Effects on diabetic oxidative stress and its therapeutic implications

Carolina Serra-Barcellona[1], Natalia Cecilia Habib[1,3], María Inés Mercado[2], Sara Serafina Sánchez[1, 3*] and Susana Beatriz Genta[1,3*]

1. Instituto de Biología "Dr. Francisco D. Barbieri", Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Chacabuco 461, San Miguel de Tucumán (T4000INI), Argentina.
2. Instituto de Morfología Vegetal, Área Botánica, Fundación Miguel Lillo, Miguel Lillo 251, San Miguel de Tucumán (T4000JFE), Argentina.
3. Instituto Superior de Investigaciones Biológicas (INSIBIO- CONICET). Chacabuco 461, (T4000INI) San Miguel de Tucumán, Tucumán, Argentina.

Abstract

Oxidative stress, resulting from an imbalance between free radical production and antioxidant defenses, plays a key role in the pathogenesis of various diseases, including diabetes mellitus. In recent times, the search for safer natural antioxidants has intensified. *Smallanthus sonchifolius* [Poepp. & Endl.] H. Robinson (yacon) is a traditional Andean crop recognized for its pharmacological properties. We have previously demonstrated the efficacy and safety of aqueous extract of yacon leaves for treating hyperglycemia in rodents. This study aimed to analyze the major chemical components of a 10% yacon leaf decoction and evaluate its antioxidant capacity in a streptozotocin-induced diabetes model in Wistar rats. The decoction was found to contain 77.47 ± 1.23 mg GAE/g of total phenolic compounds, with caffeic and chlorogenic acids identified as constituents by TLC and HPLC. Antioxidant activity was initially assessed *in vitro* using DPPH and β-carotene/linoleic acid assays, which revealed moderate radical scavenging activity. Streptozotocin-induced diabetic Wistar rats treated with the decoction (140 mg /kg/day) for 30 days exhibited a significant reduction in malondialdehyde levels and decreased activities of superoxide dismutase and catalase in liver and kidney homogenates, compared with untreated diabetic animals. In contrast, glutathione peroxidase activity and reduced glutathione levels were significantly increased. These findings show that yacon leaf decoction could be helpful in reducing problems related to oxidative stress in diabetes.

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Corresponding Authors
Prof. Dr. Susana Beatriz Genta
E-mail: susana.genta@fbqf.unt.edu.ar
Tel: +54 381 4247752, Ext. 7214
Prof. Dr. Sara Serafina Sánchez
E-mail: sara.sanchez@fbqf.unt.edu.ar
Tel: +54 381 4247752, Ext. 7214

Keywords
Experimental diabetes, natural antioxidants, oxidative stress, phenolic compounds, *Smallanthus sonchifolius*, Yacon leaves extract.

Introduction

Free radical production is a normal biological process strictly controlled to maintain cellular homeostasis. Under physiological conditions, cells possess potent antioxidative defense systems to maintain oxidative balance, thereby preventing damage to biological targets such as cellular membranes, subcellular organelles, and biomolecules (DNA, proteins, and lipids) [1]. Oxidative stress is defined as an imbalance between free radical production and antioxidant defenses, and is linked to the pathogenesis of many

diseases, including diabetes mellitus [2].

Experimental evidence suggests that free radical generation under chronic hyperglycemia occurs through several molecular mechanisms, including increased electron transfer to the mitochondrial electron transport chain, resulting in enhanced superoxide anion production [3]. Mitochondrial oxidative stress may contribute to increased lipid peroxidation and damage cell membranes and DNA, activating a cascade of signaling events that worsen the disease. Additionally, the oxidation of carbohydrates and phospholipids, generation of toxic by-products, and non-enzymatic glycosylation of proteins and lipids are key mechanisms in free radical generation associated with diabetes. Together, these mechanisms lead to the dysfunction of antioxidant systems throughout the body owing to chronic hyperglycemia [4].

Defense mechanisms against free radical-induced oxidative stress include preventive and repair processes as well as antioxidant defenses. Enzymatic antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), work alongside nonenzymatic antioxidants such as vitamins (ascorbic acid and tocopherol), glutathione, and redox metal chelators [5].

Conventional treatments for diabetes include oral antidiabetic drugs and insulin therapy. However, in the last few decades, several studies have identified novel compounds that may act through mechanisms distinct from those of current drugs. Naturally occurring antioxidant compounds in plants have been found to potentially modulate oxidative stress in humans through their free radical scavenging capacity and subsequent inhibition of oxidative stress-induced cell damage [6, 7]. Despite these advantages, it cannot be ignored that the antioxidant properties of medicinal plants depend on the plant itself, its variety, growing practices, as well as postharvest handling and processing.

Yacon (*Smallanthus sonchifolius* [Poepp & Endl.] H. Robinson), a traditional Andean crop, has multiple pharmacological effects. We demonstrated the efficacy and safety of the aqueous extract of yacon leaves for the treatment of hyperglycemic disorders in a rodent model of induced diabetes [8-12]. In addition, several *in vitro* assays have shown a variety of biological activities, including antimicrobial [13], anti-inflammatory [14], antioxidant and radical scavenging properties [15], although to date they have not been verified by *in vivo* animal tests.

The yacon leaves are rich in phenolic compounds, with a predominance of chlorogenic acid (Chl), ferulic acid, caffeic acid (Caf), and their derivatives, together with the less polar sesquiterpene lactone, enhydrin [12, 15-17]. These natural compounds are of great importance to human health. The determination of the major chemical components in yacon leaf aqueous extracts along with their antioxidant capacity *in vivo* using a streptozotocin-induced diabetes rat model was the focus of this study.

2. Materials and Methods

2.1. Plant material and aqueous extract preparation

Smallanthus sonchifolius (Poepp & Endl.) H. Robinson (Clone LIEY97-1) leaves were collected from plants cultivated in an experimental field at the National University of Tucumán, Horco Molle, Tucumán province, Argentina (26°47′ S, 65°19′ W, 547 m.a.s.l.) between February and April 2022. The plant material was identified by experts from the "Fundación Miguel Lillo", San Miguel de Tucumán, Tucumán, Argentina, and a voucher specimen was deposited in the herbarium of this institution (LIL607173).

The plant material was carefully dried under airflow in an oven (40°C). The aqueous extract was prepared by boiling (100°C) 10 g of dried leaves in 100 mL of distilled water under reflux for 10 min and then allowed to cool to room temperature. The 10% decoction obtained was lyophilized to afford 1.80 g of dry residue (18%, w/w) and then stored in dark glass bottles at -20°C until use.

2.2. Quantification of phenolic compounds

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method [18]. Briefly, dry extract was dissolved in distilled water. Then, serial dilutions were oxidized with 0.1 N Folin-Ciocalteu reagent and the reaction was neutralized with saturated sodium carbonate (75 g/L). After 30 min, the absorbance was measured at 765 nm. Quantification was performed based on a standard curve of gallic acid (1–40 μg). Results were expressed as mg of gallic acid equivalent (GAE)/ g of dry weight (DW).

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2.3. Thin-layer chromatography (TLC)

Caf and Chl were identified by comparison with authentic standards (C0625 and C3878, Sigma-Aldrich). Silica gel 60 F254 aluminum sheets (Merck) were used as the stationary phase, and various solvent systems were applied for development. For Caf identification, plates were developed using *n*-hexane: ethyl acetate: acetic acid (4:6:0.15). For Chl identification, two solvent systems were used: *n*-butanol: acetic acid: water (10:1.75:8) and ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:27). Detection was performed by (i) fluorescence at 366 nm (Mineral Light Lamp, Model UVGL, multi-band UV 254/366, UVP San Gabriel, USA), (ii) spraying with a 1% solution of 2-amino-ethyl-diphenyl-borinate in methanol, followed by a 5% solution of polyethylene glycol in ethanol (NP/PEG), and (iii) spraying with a 1% solution of $FeCl_3$ in methanol.

2.4. HPLC analysis

For HPLC analysis, a Gilson 322 HPLC (binary pump) with a Gilson UV/VIS-152 Detector, Rheodyne injector with a 20 mL loop, and Unipoint software were used. A Grace Smart RP18 analytical column (5 µm; 4.6 mm x 250 mm) and two solvent systems (A: 5% acetic acid-water solution and B: methanol) were employed. Elution was achieved with a linear gradient of 20-33.5% B for 60 min. Flow rate: 0.7 mL/min. UV detection was performed at 326 nm with 0.01 sensitivity. Injection volume 20 µL. Identification was performed by comparing retention times, co-injection with authentic samples, and UV spectra.

2.5. Standard solutions

Chl and Caf standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). Standard solutions were prepared by dissolving 10 mg of each acid in 100 mL of methanol (100 ppm, w/v solution).

2.6. Acid hydrolysis

To determine other caffeoylquinic acid derivatives, acid hydrolysis was performed using a haemolysis tube. One millilitre of the dry extract was dissolved in distilled water, and a drop of concentrated HCl was heated in a water bath for three hours. Then, it was extracted with AcOEt (2 x 1 mL), the organic phase was dried, and the residue was resuspended in MeOH: H_2O (20:80) and analyzed by HPLC using the same solvent system described above.

2.7. Infrared (IR) spectroscopy

The 10% decoction was analyzed by infrared (IR) spectroscopy. The samples were prepared as KBr pellets, and spectra were recorded using a Perkin-Elmer 1600 FT-IR spectrophotometer.

2.8. Antioxidant activity: in vitro assays

2.8.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method

The free radical scavenging capacity of the 10% yacon leaf decoction was evaluated based on the decrease in 2,2-diphenyl-1-picrylhydrazyl (DPPH) absorbance, according to Manian et al. [19]. Aliquots of 10% decoction (25, 50 and 100 µL), positive controls butylated hydroxytoluene (BHT, 1000 ppm) and quercetin (Q, 200 ppm), or distilled water (negative control) were mixed with 500 µL of DPPH methanolic solution (120 mg/L). The volume was adjusted to 2 mL with methanol and allowed to react at room temperature in the dark. After 20 min, the absorbance values were measured at 520 nm using a microplate reader (Biotek EL 808, BioTek Instruments Inc., Shoreline, WA). The IC_{50} values were calculated using a regression equation prepared from different extract concentrations. All assays were performed in triplicates.

2.8.2. β-carotene bleaching (BCB) test

The antioxidant activity of the 10% yacon leaf decoction was determined according to the β-carotene bleaching method described by Kumar et al. [20], with slight modifications. β-carotene (1 mg) was added to a flask together with linoleic acid (20 µl) and Tween 40 (200 mg), all dissolved in chloroform (0,5 mL). After evaporation to dryness under vacuum at 50 ºC, oxygenated distilled water (40 mL) was added and the mixture was emulsified for 1 min to form a yellowish emulsion. A volume of 200 µL of a 10% yacon leaf decoction, containing 1000 µg of soluble solids per mL of water, was mixed with 4 mL of a β-carotene/linoleic acid emulsion. As negative and positive controls, 200 µL of distilled water, 200 µL of BHT solution (1000 ppm), or 200 µL of Q solution (200 ppm) were mixed with 4 mL of the β-carotene/linoleic acid stock solution, respectively. The absorbance was measured at 470 nm using a spectrophotometer Shimadzu UV-240PC, Shimadzu Corporation, Kyoto, Japan) immediately (t = 0) and at 20 min intervals for 120 min.

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The vials containing the reaction mixture were placed in a water bath at 50°C between measurements. All measurements were performed in triplicates.

The % antioxidant activity (AA) was measured in terms of bleaching of β-carotene using the following formula:

$$\%AA = [1- (A_{0Dec} - A_{tDec} / A_{0Positive\ control} - A_{tPositive\ control})] \times 100$$

A_{0Dec} and $A_{0Positive\ control}$ are the absorbance values before incubation for the decoction and positive control, respectively.

A_{tDec} and $A_{tPositive\ control}$ are the respective absorbances of the decoction and the positive control after incubation for 120 min.

This formula expresses the antioxidant activity as a percentage decrease in absorbance. A higher proportion of β-carotene suggests better inhibition of β-carotene oxidation, indicating stronger antioxidant capabilities. The results were expressed as % of the prevention of β-carotene bleaching.

2.9. Antioxidant activity: in vivo assays

2.9.1. Experimental animals and diabetes induction

Male Wistar rats (3 months old) weighing 200±20 g were obtained from a colony bred at INSIBIO (CONICET-UNT), Tucumán, Argentina, and acclimated for 7 days before the start of the experimental procedures. The animals were housed in cages in a room with a 12 h day-night cycle, temperature of 24 ± 2°C, and humidity of 45-64%. Throughout the experimental period, the animals were fed a powdered certified rodent diet (Standard Food-Association of Argentinean Coop.-S.E.N.A.S.A. N° 2706), and water was provided *ad libitum*. No known contaminants were present in the food or water that could have interfered with the results of this study. All animal procedures were approved by the Committee on Bioethics in Research of the National University of Tucumán (Protocol No. 0015-2017) and conducted in accordance with the International Ethical Guidelines for the Care of Laboratory Animals (United States Food and Drug Administration). All efforts were made to minimize both the number of animals used and their suffering.

Diabetes was induced in overnight-fasted rats by a single intraperitoneal injection of streptozotocin (STZ, Sigma Chemical Company, St. Louis, MO, USA) at a dose of 45 mg/kg body weight (b.w.) and dissolved in 10 mM sodium citrate buffer (pH 4.5). The rats in the normal control group received only citrate buffer (vehicle). Diabetes was achieved within 48 h in most animals. Only animals with fasting blood glucose levels ≥ 350 mg/dL and glucosuria were included in the diabetic control or diabetic treatment groups.

2.9.2. Experimental design

Diabetic rats were randomly divided into two groups: a diabetic control group (DC, n=6) and a diabetic treated group (DT, n=6). Age-matched non-diabetic rats were included in the normoglycemic rats group used as a control group (NC, n=6).

Yacon decoction was administered orally to DT at a dose of 140 mg /kg b. w./day, dissolved in distilled water (0.5 mL). This dose was selected based on the effective hypoglycemic and safe doses previously tested in our laboratory [12]. The treatment was carried out for 4 weeks, at 6:00 pm before feeding, using an intragastric tube. The DC and NC groups were administered the same volume of distilled water as the decoction administrated to the treatment group. All animals received a standard diet and water *ad libitum*. Body weight, urine, and plasma glucose levels were recorded weekly. Food intake was recorded daily. At the end of the experimental period, the animals were fasted overnight and euthanized with an intraperitoneal injection of ketamine/xylazine (50:5 mg/kg b. w.). Blood samples were collected via an intracardiac needle, and then the liver and kidneys were surgically removed, washed with ice-cold saline solution, and stored immediately at -70 °C until used for biochemical assays.

2.9.3. Biochemical assay

Blood glucose was measured using an Accu-Chek® Active (Roche Diagnostics, Mannheim, Germany), based on the glucose dye oxide-reductase mediator reaction. Urine glucose was determined using a reagent-based glucose oxidase–peroxidase enzymatic method (Bayer S.A. Diagnostics). Plasma insulin levels was determined using an Enzyme-linked immunosorbent assay (rat/mouse Insulin ELISA kit, Linco Research, Inc.). Haemoglobin A1c (HbA1c) was separated by a chromatographic method using a cationic exchange resin (Hemoglobin A1c Kit,

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BioSystem S.A, Barcelona, Spain) and quantified by spectrophotometric reading at 415 nm. A fully automated BS-200-Chemistry Analyzer Mindray® was used to measure the following parameters: triglycerides, total cholesterol, HDLc, and LDLc. The atherogenic index (HDLc/total cholesterol) was also calculated.

2.9.4. Tissue homogenate preparation

Weighed kidney and liver tissue samples were homogenized on ice using a sonic dismembrator VibraCell (Sonios & Materials Inc, USA) in an appropriate 0,1 M phosphate buffer, pH 7,4, containing 1 mM EDTA (1:40 parts (w/v)). The samples were centrifuged at 18.000 x *g* for 15 minutes at 4 °C, and the supernatants were subjected to the protein measurement.

2.9.5. Estimation of endogenous antioxidants in liver and kidney tissues

Determination of non-enzymatic antioxidants and antioxidant enzymes**:** Non-enzymatic antioxidants (reduced glutathione, GSH) and endogenous antioxidant enzymes- catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were determined as previously described by Habib et al. [21].

2.9.6. Determination of malondialdehyde

The level of lipid peroxidation in tissues was assessed by determining the level of malondialdehyde (MDA) in liver and kidney homogenates by the spectrophotometric method of Beuge and Aust [22].

2.10. Data analysis

The results from three independent experiments were presented as the mean ± S.D. The significance of differences was evaluated using one-way analysis of variance (ANOVA). A p-value <0.05 was considered statistically significant.

3. Results

3.1. Phytochemical analysis

According to our previous studies, extraction of yacon leaves using the decoction method with water yielded an extract with valuable hypoglycemic activity in diabetic rats [8, 9, 11]. In the present study, phytochemical analysis showed an interesting composition associated with this biological activity. Firstly, this aqueous extract contained a significant amount of total phenolic compounds, as measured at 77.47 ± 1.23 mg GAE/g of dry extract. Their IR spectrum (KBr) displayed strong absorptions at 3300 cm^{-1} (corresponding to -O-hydrogen bonding, polymeric association) and 1600 cm^{-1} (alkenyl C=C stretch), indicating the presence of phenolic acids. Absorption bands corresponding to γ-lactone carbonyl (1755–1780 cm^{-1}) were also observed, indicating the presence of sesquiterpene lactones, as stated by Barcellona et al. [12]. Caf and Chl were detected as constituents of the 10% yacon leaf decoction by TLC (Fig. 1) and HPLC (Fig. 2).

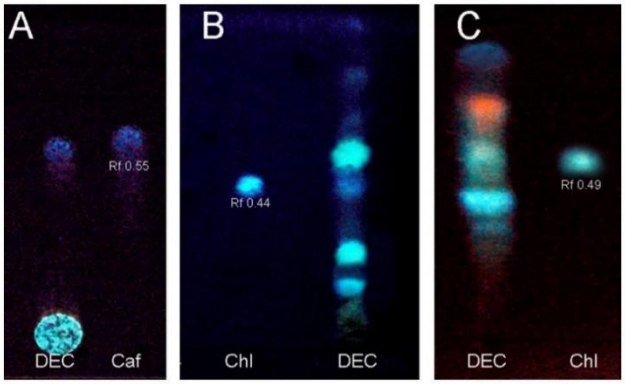

Figure 1. TLC analysis. (A) For caffeic acid identification, plates were developed with n-hexane: ethyl acetate: acetic acid (4:6:0.15). For chlorogenic acid identification, plates were developed with (B) ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:27) or (C) n-butanol: acetic acid: water (10:1.75:8). Detection was performed by spraying with NP/PEG before observation under UV at 366nm. Caf: caffeic acid, Chl: chlorogenic acid, DEC: 10% yacon leaves decoction.

Caf and Chl were readily detected in the aqueous extract by TLC using an authentic standard as reference and three different detection systems. As shown in Fig. 1, some undetermined caffeoylquinic acid derivatives were also identified as constituents of the extract.

HPLC chromatograms of yacon leaf decoction showed peaks corresponding to Caf and Chl (UV detection at 326 nm) (Fig. 2A), demonstrated by co-injection with authentic samples (Fig. 2B). The other peaks may correspond to caffeoylquinic acids, previously identified in yacon leaves and roots [9, 12, 23]. This was corroborated by acid hydrolysis. After hydrolysis, a peak (Fig. 2C) was observed as the Caf.

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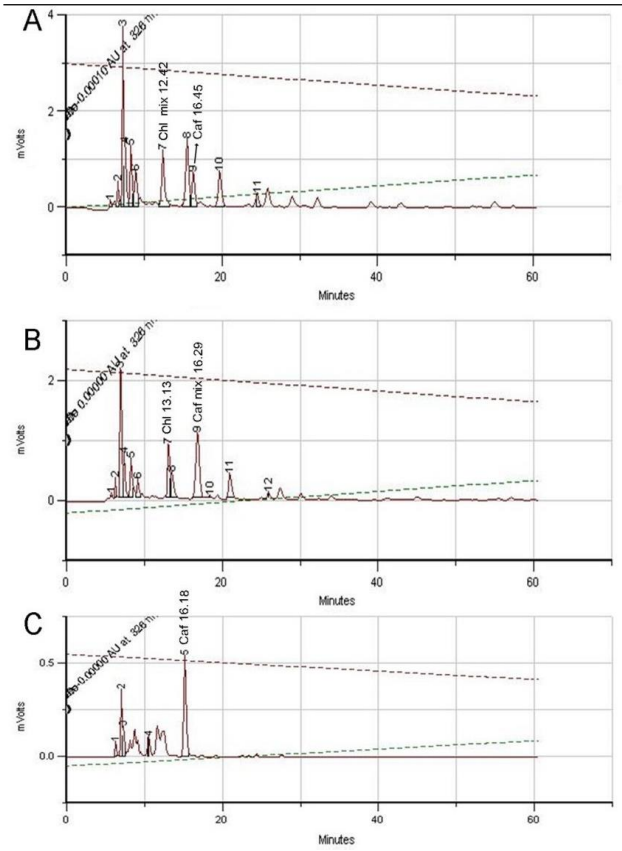

Figure 2. HPLC chromatograms. (A) 10% yacon leaf decoction extract. (B) 10% yacon leaf decoction extract co-injected with caffeic and chlorogenic acid. (C) 10% yacon leaf decoction extract after acid hydrolysis. Grace Smart RP 18 analytical column (5 µm; 4.6 mm x 250 mm). Mobile phase I: solvent (a), 5% acetic acid-water solution; solvent (b), methanol. Linear gradient: 20 to 33.5% (b) in 60 min. Flow rate: 0.7mL /min. UV detection: 326 nm. Caf: caffeic acid, Chl: chlorogenic acid.

3.2. In vitro radical scavenging and antioxidant activity of the 10% yacon leaf decoction

The antioxidant activity of the 10% yacon leaf decoction was evaluated using two *in vitro* assays. The decoction showed lower DPPH free radical scavenging activity compared to the positive controls, BHT and Q. Indeed, the sample concentration necessary to decrease the initial DPPH concentration by 50% (IC_{50} values) was 64.00±2.32 µg/mL, whereas BHT and Q displayed IC_{50} values of 104.1±5.2 and 1.7±0.1 µg/mL, respectively. According to Setha et al. [24], an IC_{50} between 50-100 ug/mL indicates strong antioxidant activity, suggesting that this yacon decoction is a promising antioxidant option. Additionally, the antilipoperoxidative capacity of the decoction was assessed using the β-carotene/linoleic acid bleaching test, which measures the extract's to inhibit β-carotene oxidation in the presence of linoleic acid. The standard antioxidant Q showed a high inhibition capacity (84.96±4.25), while BHT and decoction exhibited a lower inhibition (43.00±1.87 and 48.8±1.30%, respectively). Both *in vitro* assays indicate that the yacon leaf decoction has moderate antioxidant activity. These tests provide valuable insights and serve as predictive tools for further *in vivo* studies on antioxidant effects in experimentally induced diabetic models.

3.3. In vivo assessment of diabetic state and effects of treatment with 10% yacon leaf decoction

Particularly, the potential health benefits of 10% yacon leaf related to its antioxidant capacity, which were evaluated in STZ-induced diabetic Wistar rats. To assess the diabetic state, several key parameters were first analyzed.

As shown in Table 1, the fasting blood glucose levels in DC were significantly higher than those in the NC, confirming the induction of diabetes In concordance with previous results from our laboratory [8, 11], 30-day treatment with 10% yacon leaf decoction (140 mg/kg/day) significantly reduced fasting blood glucose levels in DT compared to DC, although normal glycemia was not fully restored. In concordance, HbA1c levels at the end of the experimental period were significantly lower in DT compared to DC, indicating improved glycemic control after one month of treatment (Table 1**).** Notably, treatment for 30 days also significantly improved the glucose/insulin index, although it did not increase the plasma insulin levels, (Table 1). Alterations in the plasma lipid profile are other remarkable characteristics of diabetic. Under our experimental conditions, diabetic rats showed significantly increased levels of triglycerides, total cholesterol, HDLc and LDLc compared to non-diabetic rats (Table 2). Interestingly, daily treatment for 30 days at 140 mg/kg/day significantly reduced serum triglyceride levels to normal levels. In addition, total cholesterol and LDLc levels also decreased, approaching normal values. Although HDLc levels were reduced by 27.3% following treatment, the atherogenic index (total cholesterol/HDLc) showed a significant decrease, although it did not return to the level observed in the NC (Table 2). These findings

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Table 1. Effect of yacon leaf decoction treatment on the fasting blood glucose, insulin and HbA1c levels in diabetic rats at the end of experimental period

Parameters	DT	DC	NC
Glucose (mg/dL)	333.17 ± 16.65 [a]	514.00 ± 25.70 [b]	84.33 ± 4.21 [c]
Insulin (ng/mL)	0.37 ± 0.05 [a]	0.35 ± 0.02 [a]	2.39 ± 0.09 [b]
Glucose/insulin	915.53 ± 45.78 [a]	1294.03 ± 64.70 [b]	46.04 ± 2.30 [c]
Hb A1c (%)	11.00 ± 0.05 [a]	15.00 ± 0.09 [b]	6.00 ± 0.06 [c]

Results are expressed as mean±standard deviation of six determinations. Values with the same superscripts in a row are not significantly (p≥0.05) different. DT: diabetic group treated with 10% yacon leaf decoction (140 mg/kg b.w.); DC: diabetic control group; NC: normal control group.

Table 2. Effect of yacon leaf decoction treatment on the lipid profile in diabetic rats at the end of experimental period

Parameters	DT	DC	NC
Triacylglycerol (mg/dL)	68.50 ± 13.18 [a]	148.17 ± 9.21 [b]	69.37 ± 2.40 [a]
Total cholesterol (mg/dL)	68.00 ± 4.24 [a]	78.50 ± 21.72 [a,b]	57.15 ± 2.69 [b]
HDLc (mg/dL)	44.00 ± 0.56 [a]	60.50 ± 3.28 [b]	47.00 ± 0.24 [c]
LDLc (mg/dL)	14.50 ± 0.02 [a]	15.00 ± 0.52 [a]	11.50 ± 0.02 [b]
Atherogenic index (Chol/HDLc)	1.54 ± 0.08 [a]	1.90 ± 0.09 [b]	1.22 ± 0.11 [c]

Results are expressed as mean±standard deviation of six determinations. Values with the same superscripts in a row are not significantly (p≥0.05) different. DT: diabetic group treated with 10% yacon leaf decoction (140 mg/kg b.w.); DC: diabetic control group; NC: normal control group.

demonstrate the efficacy of 10% yacon leaf decoction in improving the lipid profile of diabetic rats within a 30-day period, contributing to better metabolic control.

3.4. Effects of 10% yacon leaf decoction treatment on lipid peroxidation in liver and kidney

As shown in Fig. 3, kidney and liver homogenates of diabetic rats exhibited significantly higher MDA levels compared to non-diabetic rats. These increases were 30.03% in the kidney and 57.22% in the liver, indicating enhanced lipid peroxidation due to diabetes. The treatment with 10 % yacon leaf decoction (140 mg/kg/day for 30 days) significantly reduced renal and hepatic MDA levels, with decreases of 30.97% and 24.3%, respectively.

3.5. Effects of 10% yacon leaf decoction treatment on enzymatic and non-enzymatic endogenous antioxidants in liver and kidney

A significant increase ($p<0.05$) in CAT activity in both the kidney and liver was observed in diabetic animals compared to non-diabetic animals (23.8% and 10.7%, respectively). This alteration was effectively reversed by the daily administration of yacon leaf decoction (140 mg dry extract/kg) for 30 days. As shown in Fig. 4A and Fig. 5A, CAT activity was significantly

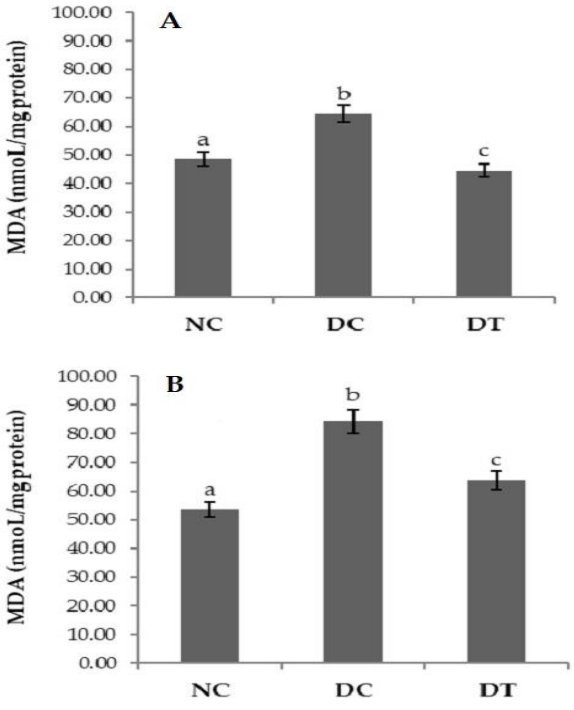

Figure 3. Effect of 10% yacon leaf decoction (140 mg/kg b.w.) on malondialdehyde (MDA) concentrations in (A) kidney and (B) liver homogenates. Results are expressed as mean±standard deviation of six determinations. Values with the same superscripts are not significantly (p≥0.05) different. DT: diabetic group treated with 10% yacon leaf decoction (140 mg/kg b.w.); DC: diabetic control group; NC: normal control group.

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reduced (p<0.05) in both organs after treatment, with a greater reduction in the kidney (59.4%) compared to the liver (19.5%). SOD activity was significantly increased in the kidney (41.3%) and liver (12.6%) of untreated diabetic rats compared to normal control rats. Interestingly, treatment of diabetic animals with 10% yacon leaf decoction significantly decreased (p<0.05) SOD activity in the kidney (66.1%), while no significant difference was observed in the liver of treated diabetic rats (Figs. 4B and 5B).

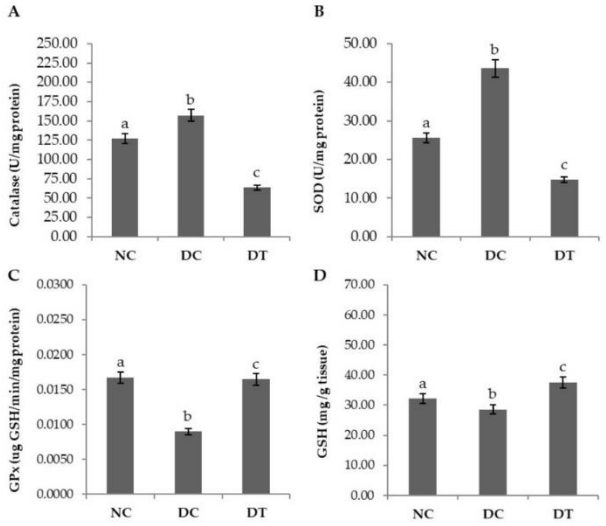

Figure 4. Effect of 10% yacon leaf decoction (140 mg dry extract/kg) on enzymatic and non-enzymatic antioxidants in the kidney of diabetic rats. A) Catalase (CAT), B) Superoxide dismutase (SOD), C) Gluthatione peroxidase (GPx) and D) Gluthatione reduced (GSH). Results are expressed as mean±standard deviation of six determinations. Values with the same superscripts are not significantly (p≥0.05) different. DT: diabetic group treated with 10% yacon leaf decoction (140 mg/kg b.w.); DC: diabetic control group; NC: normal control group.

These findings suggest that the effect of the decoction on SOD activity in the liver was not significant. On the other hand, a significant reduction in GPx activity was observed in the kidney and liver homogenates of diabetic rats compared to normal rats, with decreases of 46.1 % and 54.3 %, respectively. Treatment of diabetic rats with a 10% yacon leaf decoction for 30 days resulted in a significant increase (83.3%) in renal GPx activity. A remarkable effect was also observed in the liver, where GPx activity increased by 177% compared to untreated diabetic animals (Figs. 4C and 5C). Regarding the endogenous non-enzymatic antioxidants, liver homogenates had higher GSH

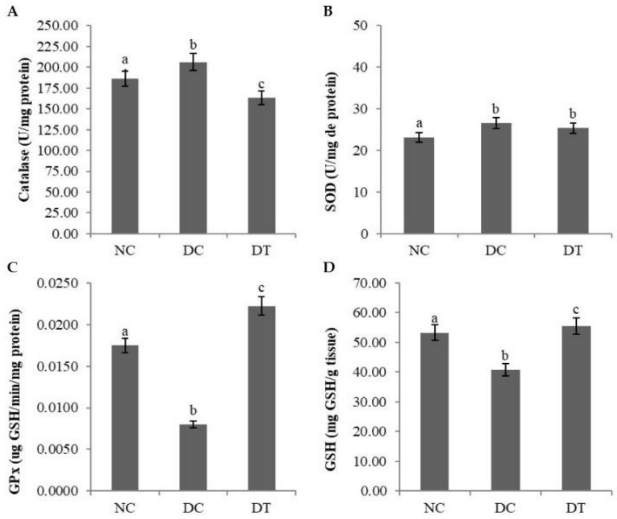

Figure 5. Effect of 10% yacon leaf decoction (140 mg/kg b.w.) on enzymatic and non-enzymatic antioxidants in the liver of non-diabetic and diabetic rats. A) Catalase (CAT), B) Superoxide dismutase (SOD), C) Gluthatione peroxidase (GPx) and D) Gluthatione reduced (GSH). Results are expressed as mean±standard deviation of six determinations. Values with the same superscripts are not significantly (p≥0.05) different. DT: diabetic group treated with 10% yacon leaf decoction (140 mg/kg b.w.); DC: diabetic control group; NC: normal control group.

levels than the kidney in normal animals, as shown in Figs. 4D and 5D.

Induction of the diabetic state in rats led to significant reduction in GSH content in both organs, with a decreases of 11.9% in the kidney and 23.5% in the liver (p<0.05) compared to normal rats. However, daily treatment with 10% yacon leaf decoction (140 mg dry extract/kg) for 30 days resulted in a significant increase (p<0.05) in GSH concentrations in both kidney and liver homogenates, bringing levels close to normal values. This increase likely contributes to oxidative stress control, particularly in susceptible organs such as the kidneys.

4. Discussion

Oxidative stress, defined as an imbalance between reactive species production and antioxidant defenses, plays a central role in the pathogenesis of chronic diseases such as diabetes [25]. Mitochondrial-derived reactive oxygen species (ROS) can damage cellular components, and chronic hyperglycemia exacerbates their production, contributing to the progression of diabetic complications [26, 27]. Thus, plant-based

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preparations rich in antioxidant compounds may be a valuable therapeutic strategy.

In this study, we investigated the antioxidant and antidiabetic effects of 10% yacon leaf decoction (dose: 140 mg/kg/day), administered for 30 days to STZ-induced diabetic rats. The treatment significantly reduced glycemic levels in diabetic rats and effectively alleviated marked oxidative stress in both the liver and kidneys.

This preparation, standardized in our laboratory [12], mirrors the traditional Andean method and yields a good extraction rate (18%) compared to other reports [28]. It is well established that the decoction method, which uses water as an environmentally friendly solvent, yields extracts rich in phytochemicals, such as phenolic acids and flavonoids, with antioxidant and free radical scavenging activities [17, 29]. Under our experimental conditions, we obtained a decoction rich in phenolic compounds (77.47 ± 1.23 mg GAE/g of dry extract), particularly caffeic acid, chlorogenic acid, and its derivatives, with a chemical composition that remained consistent across different batches. This approach adds value to local plantations by promoting their use as a source of raw materials for the production of preparations with potential therapeutic activity.

The decoction showed good radical scavenging capacity *in vitro*, as evidenced by DPPH and β-carotene bleaching assays, though its activity was lower than that of synthetic antioxidants like BHT. According to Setha et al. [24], an IC_{50} value between 50-100 µg/mL indicates strong activity, thus positioning this yacon extract as a promising antioxidant. These tests, while useful for preliminary screening [30, 31], do not fully reflect *in vivo* bioefficacy, which depends on absorption, metabolism, and cellular interactions that occur in a living organism [32].

Caf has long been recognized for its *in vivo* antioxidant properties [33]. Chl enhances endogenous antioxidant defenses by activating the Nrf2 pathway and neutralizes excess cellular free radicals [34]. Additionally, these bioactive compounds promote *in vivo* glucose and lipid metabolic homeostasis [35-37].

Considering the abundance of phenolic acids (particularly Caf and Chl) in the 10% yacon leaf decoction, we propose that it represents a promising candidate for therapeutic use in diseases associated with oxidative stress and hyperglycemia, such as diabetes. Diabetes was induced in rats using STZ [21], which causes rapid, irreversible β-cell damage and persistent hyperglycemia. Some studies have suggested that the antidiabetic effects of plant extracts depend on the degree of β-cell destruction [38]. In our study, insulin deficiency and hyperglycemia persisted for 30 days, likely due to partial β-cell preservation, which is crucial for assessing the potential therapeutic effects of yacon decoction.

The treatment of diabetic rats with 10% decoction for 30 days significantly reduced fasting blood glucose levels. These findings were consistent with a significant decrease in HbA1c levels at the end of the experimental period, a parameter widely used to evaluate treatment efficacy [39 Notably, neither glucose nor HbA1c levels reached normal values, suggesting that a higher dose of the decoction might enhance its antidiabetic effect. While insulin levels remained unchanged, the improved glucose/insulin ratio indicates possible insulin-mimetic activity at the cellular level and/or α-glucosidase inhibitory actions in the small intestine, as previously proposed [10].

The treatment also improved lipid profiles, significantly reducing triglycerides, total cholesterol, and LDL-c, in line with the known hypolipidemic effects of polyphenol-rich extracts [40, 41]. Notably, it decreased the atherogenic index, a relevant finding considering the cardiovascular risk in diabetes [42].

We evaluated the *in vivo* antioxidant properties of a 10 % yacon leaf decoction in organs commonly affected by diabetes, such as the liver and kidneys [43, 44]. Treatment with the decoction significantly reduced MDA levels in both liver and kidney homogenates of diabetic rats, suggesting decreased lipid peroxidation. The elevated hepatic MDA levels observed in diabetic animals are likely associated with increased free fatty acid content in the liver, as described by Habib et al. [21]. The absence of increased plasma insulin levels after treatment suggests that the phenolic compounds in decoction, particularly those with hydroxyl groups, may have direct antioxidant effects independent of insulin, possibly by scavenging ROS and preventing lipid peroxidation. The significant reduction in kidney lipid peroxidation could help prevent or reduce

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diabetic nephropathy.

Treatment with 10% decoction significantly increased the activity of GPx and restored GSH levels in liver and kidney organs, with a more pronounced effect in liver homogenates. Given that GSH is the substrate for GPx and its depletion impairs enzyme function [45, 46], this recovery could explain the increase in GPx activity and the observed decrease in oxidative stress. Moreover, elevated GSH levels could exert a cumulative antioxidant effect over time, contributing to the overall reduction in oxidative stress in treated animals.

It is well established that hyperglycemia disrupts antioxidant defense systems, and variations in both the levels and activities of antioxidant enzymes are likely influenced by the duration of diabetes [47]. In our study, diabetic animals showed increased CAT and SOD activities in liver and kidney homogenates of diabetic rats, likely as an early adaptive response to oxidative stress. Treatment with 10% decoction significantly reduced CAT activity in both organs, below control levels, whereas SOD activity was normalized only in the renal tissue. It is likely that treatment with yacon decoction reduces oxidative stress in the kidney, with a consequent decrease in SOD activity, as was suggested by Ugochukwu and Cobourne in other plant extracts [48]. In contrast, SOD activity in the liver remained elevated in treated animals, possibly due to persistent superoxide production from hepatic xanthine oxidase in diabetic state [49], suggesting a compensatory response to increased ROS production.

5. Conclusions

Despite the limited bioavailability of polyphenols, the administered dose of yacon decoction (140 mg/kg/day) delivered a sufficient amount of phenolic compounds to exert both direct and indirect effects on redox status modulation. The *in vivo* model used in this study highlights the importance of early intervention in diabetes to reduce oxidative damage, as evidenced by the overall reduction in oxidative markers and modulation of antioxidant enzyme activities. Taken together, the findings support the therapeutic potential of a 10% yacon leaf decoction as a multitarget preparation capable of improving oxidative homeostasis and glycemic control during the early stages of diabetes mellitus.

Authors' contributions

Responsible for methodology, investigation, and formal analyses, C.S.B.; Conceptualization, responsible for methodology and original draft writing, N.C.H.; Responsible for phytochemical methodology and formal analyses, M.I.M.; Conceptualization, design and coordination, manuscript review, supervision, and funding acquisition, S.S.S.; Conceptualization, design and coordination, manuscript writing-review and editing, supervision, and funding acquisition, S.B.G.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical statement

All animal handling and procedures in this study complied with the current research standards in Argentina, based on the "Ethical Framework of Reference for Biomedical Research in Laboratory, Farm and Wild Animals" (Resol. D No. 1047 Annex II, 2005, National Council for Scientific and Technical Research-Argentina), and ARRIVE guidelines 2.0. The study protocol was approved by the Institutional Committee of Animal Care and Use of the National University of Tucumán (Approval N°0015-2017).

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