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BENEFICIAL EFFECTS OF AN HYDROETHANOLIC EXTRACT OF *SMALLANTHUS SONCHIFOLIUS* LEAVES ON THE METABOLIC CHANGES IN DIABETIC RATS

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ABSTRACT

In this study the anti-hyperglycemic effect of the hydroethanolic extract of S*mallanthus sonchifolius* leaves (yacon extract) in streptozotocin-induced type 1 diabetic rats (SZT i.p, 50 mg/kg) was characterized, with emphasis on the metabolic changes found in this experimental model. Diabetic and normal rats received extract (400 mg/kg) or water by the oral route for 30 days. At the end of the treatment, rats were fasted for 15 h and blood samples were collected for the determination of glycemia. The activity of glucose-6-phosphate dehydrogenase, transaminases (ALT and AST), the glycogen contents of the liver and muscle, and glucose release in substrate-free perfused liver were quantified. The treatment with the extract reduced glycemia in diabetic animals, restored the activity of glucose-6-phosphate dehydrogenase and AST, decreased the glycogen content of the liver and skeletal muscle, and decreased glucose release in the perfused liver. The results support the beneficial effects of the extract on some metabolic changes of SZT-induced diabetes in rats.

KEYWORDS: ***Smallanthus sonchifolius,*** **Streptozotocin Diabetes, Hyperglycemia, Yacon Extract, Metabolic Disorders.**

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INTRODUCTION

Diabetes mellitus comprises a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, particularly the eyes, kidneys, nerves, heart and blood vessels[1]. Diabetes mellitus is the most common serious metabolic disorder and is considered to be one of the five leading causes of death in the world[2]. The increase in the incidence of diabetes is due to longevity, changing lifestyle, obesity, sedentary work, changing dietary patterns and low birth weight[3]. The global prevalence of diabetes mellitus for all age groups was estimated to be 2.8% in 2000, and is projected to rise to 4.4% in 2030[4]. Extensive research and development on diabetes have led to a number of synthetic oral hypoglycemic agents, but the disease and its related complications remain uncontrolled. Moreover, most of the synthetic hypoglycemic agents are associated with serious adverse effects. On the other hand, traditional medicinal plants with their diverse phytoconstituents have been successfully employed by communities for a long time to treat diabetes, without producing demonstrable adverse effects[5]. *Smallanthus sonchifolius* (Poepp. & Endl.) H. Robinson, popularly known as as “yacon”, is an herbaceous perennial plant native to South America belonging to the family Asteraceae. The vegetative parts are used as food and for medicinal use. Their roots are juicy, devoid of starch-rich oligofructan that, because they are not metabolized in the digestive tract, and have a relatively low energy value, despite the sweet taste[6,7,8]. For this reason, the consumption of yacon roots does not increase blood glucose levels and is indicated to diabetic, overweight or obese people[8]. Leaves and stems contain phenolic compounds which have strong antioxidant activity, and are considered a potential food supplement in the prevention of chronic diseases involving oxidative stress, particularly type 2 diabetes[9,10,11]. In folk medicine, leaves are dried and prepared as tea as an adjunct in the treatment of diabetes[6], in controlling blood pressure and reducing cholesterol levels[12]. Several studies have shown that leaves of this plant possess different biological effects, among them the inhibition of migration of polymorphonuclear leukocytes[13], immunomodulation[14,15], antioxidant and cytoprotective[15] effects. In addition, studies have reported that yacon leaf extracts reduce glycemia[6,16,17] and increase insulin concentration in the plasma of diabetic rats[6]. Valentová et al.[9,18] observed that an yacon extract reduced the concentration of glucose in hepatocyte cultures, acting similarly to insulin. The exact mechanisms underlying these effects, however, have not yet been elucidated. Nonetheless, there are studies showing that changes in glucose metabolism in diabetes are frequently accompanied by changes in the activities of the enzymes that control glycolysis and gluconeogenesis in the liver[19]. Considering what has been exposed above, this work has two main goals: (a) to characterize the anti-hyperglycemic effect of the hydroethanolic extract from S*mallanthus sonchifolius* leaves (yacon extract), administered for 30 days to streptozotocin-induced type 1 diabetic rats and (b) to investigate if the treatment has beneficial effect on some metabolic changes found in this experimental model.

MATERIALS AND METHODS

(i) Animals

The experiments were developed using male Wistar rats weighing 200 to 220 g. Animals were maintained in controlled temperature (±22 ºC) and 12-hour dark-light cycle, with balanced food and free access to water. The protocol for these experiments was approved by the Ethics Committee on Animal Experimentation of the State University of Maringá.

(ii) Preparation of Yacon Extract

The extracts were prepared from *Smallanthus sonchifolius* leaves acquired from Takashi Kakihara Ltda., Capão Bonito - São Paulo State, Brazil. A voucher specimen

was deposited in the Herbarium of the State University of Maringá under the number HUEM 13021. Hydroethanolic extract from S*mallanthus sonchifolius* was obtained from a 10% (w/w) solution with 70% ethanol (10 g dried leaves/100 mL 70% ethanol), mechanically stirred for 5 hours. Next, the extract was filtered, slowly evaporated to remove the solvent, lyophilized, and stored at -20ºC. Extract yield after freeze-dried was 1.2 g. For the assays, the hydroethanolic extract of S*mallanthus sonchifolius* (yacon extract) was suspended in water immediately before use.

(iii) Induction of Diabetes

Type 1 diabetes was induced in rats fasted for 15 hours by means of a single intraperitoneal injection of streptozotocin (STZ), 50 mg/kg body weight, dissolved in 0.1 M citrate buffer (pH 4.6)[20]. After 12 days the blood was collected from the fed animals from a small incision made at the distal end of the tail to determine glycemia. Rats with fed glycemia above 300 mg/dL were included in the study.

(iv) Treatment of Rats and Determination of Glycemia

Rats were randomly assigned to groups: N and D (normal and diabetic rats that received water) and NY and DY (normal and diabetic rats that received yacon extract). The extract (400 mg/kg body weight) or water were administered by the oral route (gavage), in a single daily dose at 9 a.m., for 30 days. The dose of the extract of yacon was based on the previous works of Aybar et al.[6] and Baroni et al.[16]. At the end of the treatment (30 days), blood samples were collected from the inferior vena cava of the rats fasted for 15 h, centrifuged at 1100 g for 10 min and the plasma was separated. The concentration of glucose was determined in aliquots of plasma (10 µL), using the glucose-oxidase colorimetric enzymatic method (Gold Analisa®). The body weights of the rats were evaluated daily throughout the experimental period.

(v) Enzyme Activities and Glycogen Content

(v.i) Glycogen levels

The glycogen contents of the liver and skeletal muscles were determined in freshly isolated tissues of decapitated rats fasted for 24 hours. Tissue portions of approximately 2 g were freeze-clamped with liquid nitrogen. Samples were homogenized and extracted with 10 ml of 6% $HClO_4$. The supernatant was neutralized with 5 N K_2CO_3 and used for the enzymatic glycogen assay[21]. The amount of glycogen in the tissue sample was expressed in µmoles of glucose per gram of tissue.

(v.ii) Liver perfusion

Hemoglobin-free, non-recirculating perfusion was done according to Scholz & Bücher[2]; Kelmer-Bracht[23]. For the surgical procedure, rats were anesthetized by intraperitoneal injection of sodium thiopental (50 mg/kg). After cannulation of the portal vein and vena cava, the liver was positioned in a Plexiglas chamber. The flow was maintained constant by a peristaltic pump (Minipuls3, Gilson, France) and adjusted between 30 and 35 ml · min^{-1}, depending on the liver weight. The perfusion fluid was Krebs/Henseleit-bicarbonate buffer (pH 7.4), saturated with a mixture of oxygen and carbon dioxide (95:5) by means of a membrane oxygenator with simultaneous temperature adjustment at 36 °C. The composition of the Krebs/Henseleit-bicarbonate buffer was the following: 115 mM NaCl, 25 mM $NaHCO_3$, 5.8 mM KCl, 1.2 mM Na_2SO_4, 1.18 mM MgCl2, 1.2 mM NaH_2PO_4 and 2.5 mM $CaCl_2$. Glucose in the outflowing perfusate was measured enzymatically[24].

(v.iii) Assay of glucose-6-phosphate dehydrogenase activity in the liver

The hepatic glucose-6-phosphate dehydrogenase activity was measured according to the standard protocol[25]. Tissue was homogenized in ice-cold, saline 0.9%/EDTA 0.66 mM. The incubation system in a spectrophotometer cuvette contained 1.3 ml of 0.1 M Triethanolamine buffer, 0.05 ml of 2.92×10^{-2} M glucose-6-phosphate, 0.05 ml of 11.38×10^{-3} M NADP, 0.1 ml of 100 mM $MgCl_2$, and the required volume of ice-cold tissue homogenate (50 mg protein of tissue). The change in absorbance at 340 nm was recorded. The enzymatic activity was

expressed as nmol NADP reduced per min per mg protein (5-12 animals per group).

(v.iv) Assay of glutamate aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the liver tissue

Livers were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.4), centrifuged and the supernatant was used. The kit for the measurement of liver AST and ALT was supplied by Gold Analisa®, and the activities of enzymes were measured. Enzyme activities were expressed as units per milligram of tissue (6-12 animals per group).

(vi) Chromatographic Analysis

The yacon extract (10.0 g) was dissolved in EtOH-H_2O 1:1 and partitioned with *n*-hexane, chloroform and ethyl acetate. The solvent was evaporated to give the hexane (1.0 g), chloroform (1.4 g), ethyl acetate (3.6 g) and hydro-ethanol (4.0 g) fractions. The *n*-hexane fraction (0.7 g) was purified on a silica gel chromatography column, eluting with a mixture of *n*-hexane:ethyl acetate of increasing polarity, to allow the separation of kaurenoic acid (15.5 mg) and 15-α-angeloyloxy-kaur-16-en-19-oic acid (12.0 mg). The ^{1}H (300.06 MHz) and ^{13}C NMR (75.45 MHz) spectra were recorded in a $CDCl_3$ solution on a Varian Mercury spectrometer, with δ (ppm), *J* in Hz, and the spectra referred to $CDCl_3$ (δ 7.27 for ^{1}H and 77.0 for ^{13}C) as an internal standard. Melting points were determined using a Mettler FP-52 apparatus and the column chromatographies were performed using silica gel 60 Merck (70-230 mesh ASTM).

(vii) Statistical Analysis

The statistical analysis of the data was run using GraphPad Prism® (Graphpad Software Inc, Microsoft Corp.). Results were expressed as mean ± standard error of the mean and analyzed using analysis of variance (ANOVA followed by Tukey's test) for multiple comparisons.

RESULTS

(i) Effect of the yacon extract on glycemia in diabetic and normal rats

The glycemia of fed rats at 12 days after the induction of diabetes was D=343±11.5 mg/dL, while the glycemia of normal rats was N=113±6.2 mg/dL. Prior to starting the treatment the glycemia of the different groups in rats fasted for 15 hours were: normal (N) 94.5 ± 3.5, diabetic (D) 200.7 ± 10.5, group destined to become the treated normal (NY) 96.6 ± 3.3 and group destined to become the treated diabetic (DY) 212±7.9 mg/dL. The yacon extract treatment for 30 days did not produce a significant change in the glycemia of normal rats (NY = 105.56 ± 4.1 mg/dL) when compared to untreated normal rats (N = 91.02 ± 2.7 mg/dL). However, the treatment with the extract significantly reduced the glycemia of diabetic rats (DY = 126.2 ± 7.4 mg/dL) when compared to untreated diabetic rats (D = 190.6 ± 11.7 mg/dL). The results are shown in Graph 1. The treatment of animals with the extract for 30 days did not improve the weight gain in diabetic animals (data not shown). The body weight gain by the animals in the diabetic group (weight gain = 45.6 ± 9.4 g) was significantly lower when compared to that of the normal animals (weight gain = 137.8 ± 6.2 g).

(ii) Glycogen and glucose levels in tissues

The glycogen levels in liver (Graph 2A) and skeletal muscle (Graph 2 C) in rats fasted for 24 hours was increased in the diabetic compared with the normal group. The treatment of the diabetic rats with the yacon extract caused a significant decrease in both liver and skeletal muscle glycogen levels compared with the diabetic group, but without restoring the control levels. Glucose levels in liver and muscle of the diabetic group were higher than in the normal group (Graph 2B, 2D). Glucose levels in liver and skeletal muscle of diabetic treated rats were lower when compared with the diabetic group.

(iii) Glucose release in the perfused liver

The glucose released from the livers of normal rats was low and almost constant over the 60-minute perfusion time. However livers from diabetic animals initially showed very high rates of glucose release, with a diabetes/normal ratio equal to 19.28 at time zero. These high rates diminished progressively during the subsequent time and

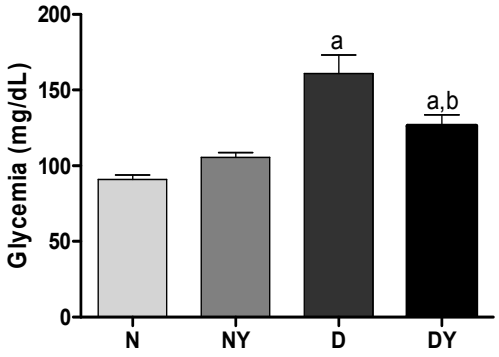
the diabetes/normal ratio was reduced to 2.62 at 60 minutes perfusion time. This decrease most likely represents the progressive reduction of the glycogen stores as the result of glycogenolysis. Livers from diabetic rats treated with yacon extract showed reduced glucose release compared to livers from treated diabetic rats; the treated diabetes/normal ratio was equal to 13.42 at time zero and 1.67 at 60 minutes perfusion time. The total amount of glucose released, computed from the areas under the curves, of diabetic treated rats was significantly lower throughout the test (Graph 3).

(iv) Liver glucose-6-phosphate dehydrogenase, AST and ALT activities

The liver glucose-6-phosphate dehydrogenase activity was decreased while the AST and ALT activities were significantly increased in diabetic rats compared with normal rats ($P<0.05$). After 30 days of treatment with the yacon extract the glucose-6-phosphate dehydrogenase activity was normalized and there was a significant reduction in the AST activity of diabetic rats. However, there was no significant reduction in the ALT activity in diabetic rats treated with the extract (Graph 4 and Table 1).

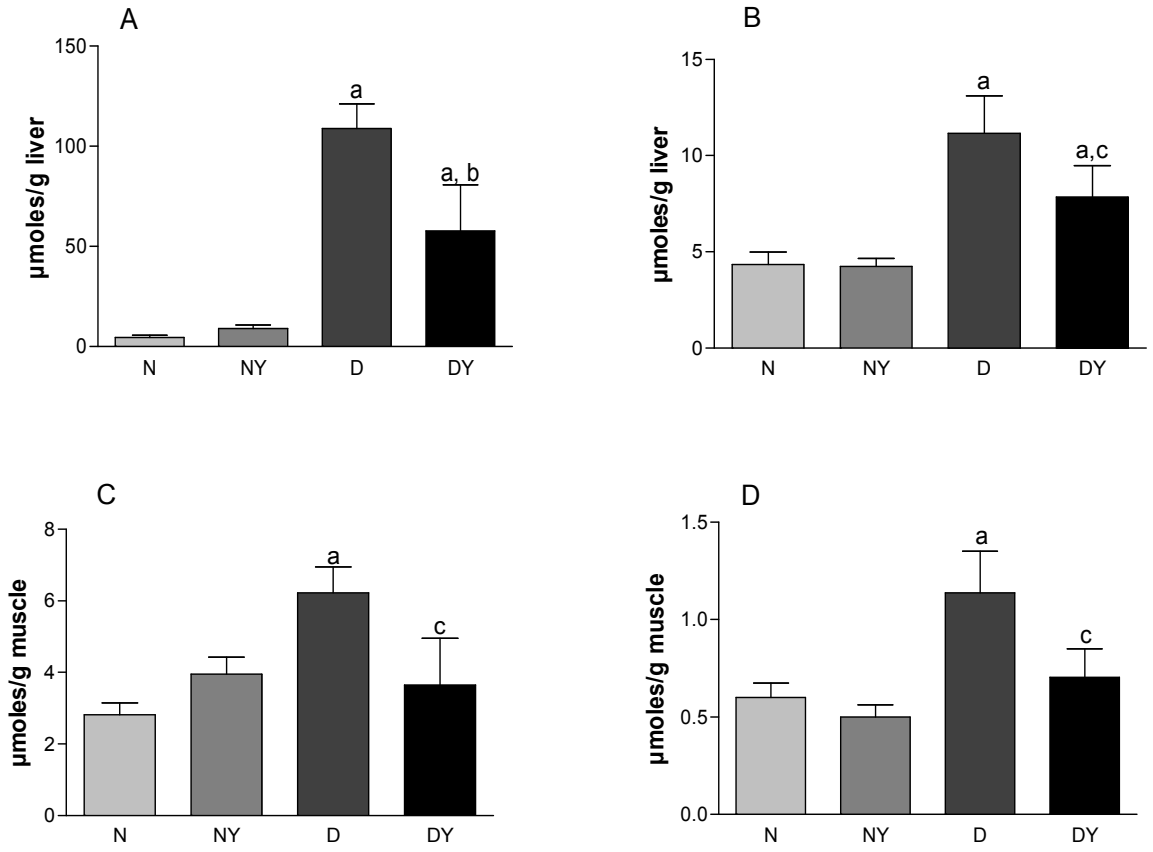
(v) Chromatographic analysis of the yacon extract

Chromatographic separation of the dichloromethane fraction resulted in the isolation of the kaurenoic and 15-α-angeloyloxy-kaur-16-en-19-oic acids (Graph 5). Kaurenoic and 15-α--angeloyloxy-kaur-16-en-19-oic acids were characterized by ^{1}H and ^{13}C NMR spectra (Graph 5a, 5b, 5c, 5d). Characteristic signals were observed for the hydrogens of an exocyclic double bond (1, δ 4.73, d, J = 17.4 Hz; 2, δ 5.08, d, J = 15.9 Hz) and of the methyl groups attached to C-4 (1, δ 1.22; 2, δ 1.23) and C-10 (1, δ0.93; 2, δ 0.94) of the kaurane skeleton. All other spectral data for both compounds matched those previously reported in the literature. The ^{1}H and ^{13}C NMR spectra of the ethyl acetate and hydro-ethanol fractions showed characteristic signals of saccharide mixtures (sucrose, fructose and glucose).



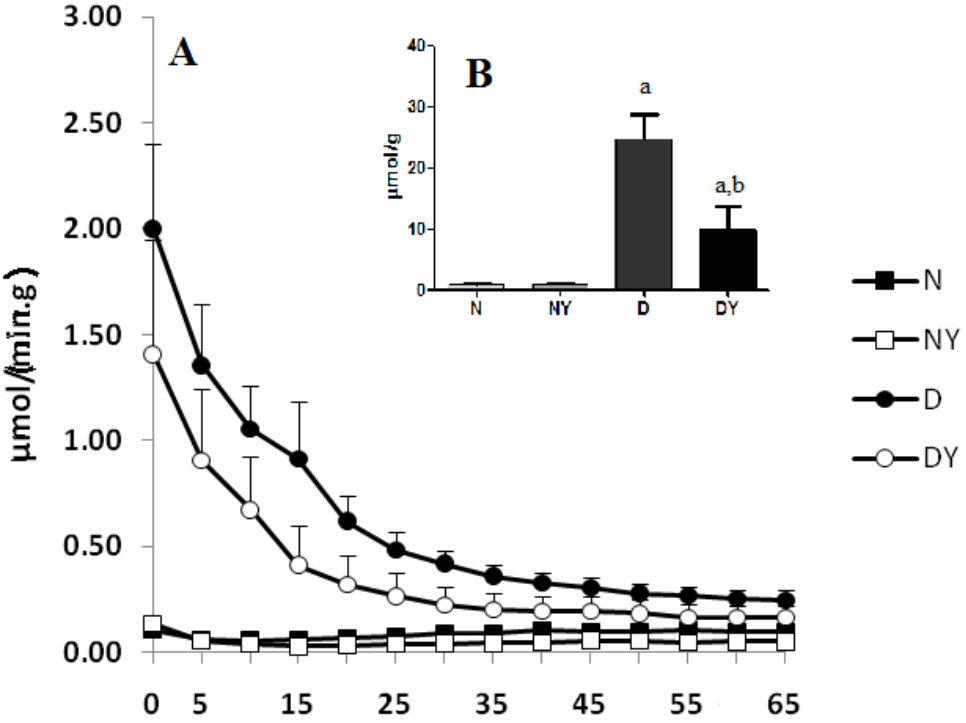
Graph 1

Effect of yacon extract on glycemia of fasting rats. The animals were treated orally with yacon extract in a single daily dose (400 mg/kg body weight) for 30 days. The results represent the mean ± S.E.M. of 5-10 animals per group. [a]P < 0.05 compared to non-diabetic rats (N); [b]P < 0.05 compared to diabetic animals (D) (ANOVA followed by Tukey's test).



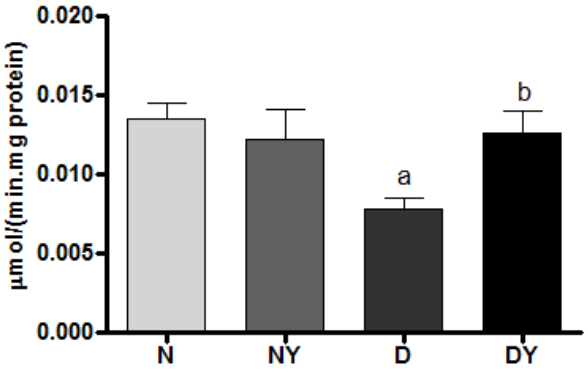
Graph 2

Effect of yacon extract on glycogen level of liver (A) and skeletal muscle (C) and free glucose on liver (B) and skeletal muscle (D) of 24-hour fasting rats. The animals were treated orally with the extract with a single daily dose (400 mg/kg body weight) for 30 days. The results represent the mean ± S.E.M. of 6-11 animals per group. $^{a}P < 0.05$ compared to non-diabetic rats (N); $^{b}P < 0.001$ compared to diabetic animals (D); $^{c}P < 0.05$ compared to diabetic animals (D). (ANOVA followed by Tukey's test).



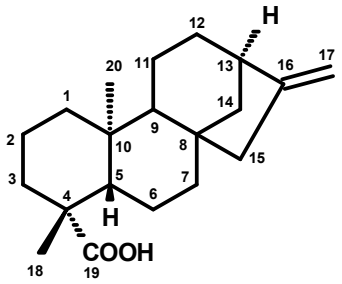
Graph 3

Rates of glucose release by rat livers as a function of time after connecting the liver to the perfusion apparatus. Livers were perfused with Krebs/Henseleit-bicarbonate buffer (pH 7.4). Sampling of the outflowing perfusate was initiated immediately after connecting the liver to the perfusion apparatus. Glucose in the perfusate was measured enzymatically. The glucose release is shown in A and the areas under curves are shown in B. The results represent the mean ± S.E.M. of 5-8 animals per group. [a]P < 0.001 compared to non-diabetic rats (N); [b]P < 0.05 compared to diabetic animals (D) (ANOVA followed by Tukey's test).



Graph 4

Effect of the yacon extract on the liver glucose-6-phosphate dehydrogenase activity in non-diabetic rats (N) and diabetic rats (D) fasted for 24 hours. The animals were treated with the yacon extract for 30 days, orally, with a dose of 400 mg/kg body weight. Each bar represents the mean ± S.E.M. of 5-12 animals. [a]P < 0.05 compared to non-diabetic rats; [b]P < 0.05 compared to diabetic rats (ANOVA followed by Tukey's test).



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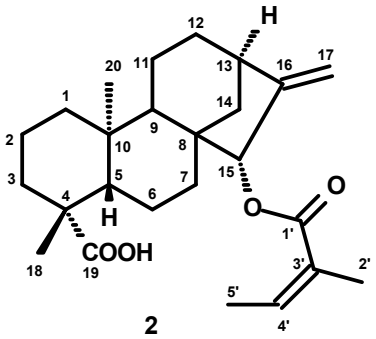
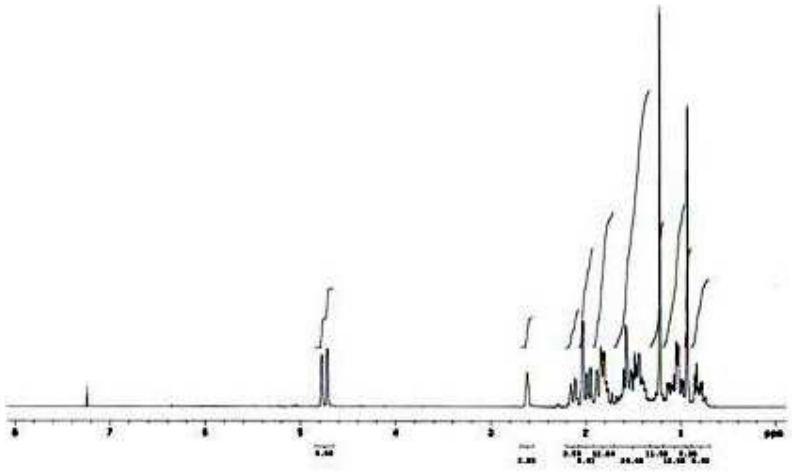


2

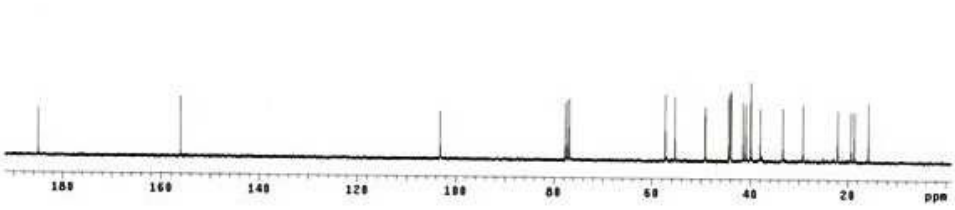
Graph 5a

Diterpenes isolated from the fraction of the extract of Smallanthus sonchifolius. (1) Kaurenoic acid and (2) 15-α-angeloyloxy-kaur-16-en-19-oic acid.



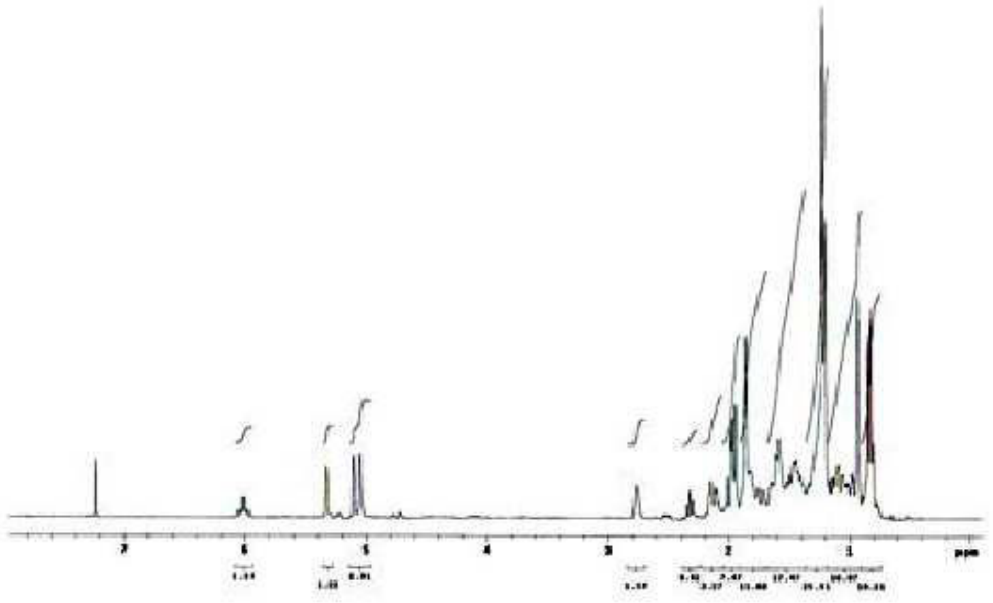
Graph 5b

^{1}H-NMR spectrum (300.06 MHz, $CDCl_3$) of kaurenoic acid

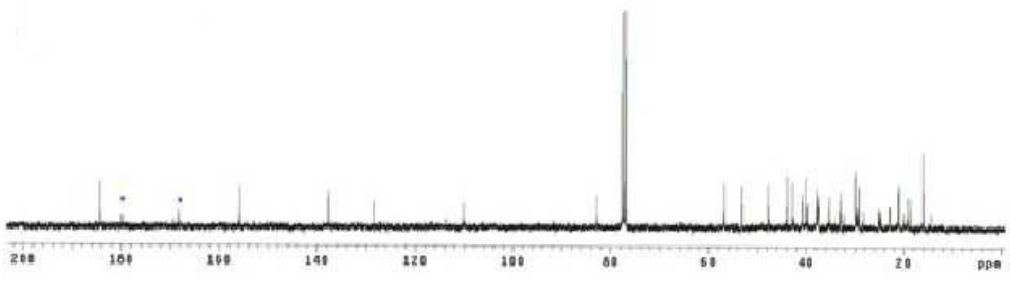


Graph 5c

^{13}C-NMR spectrum (75.45 MHz, $CDCl_3$) of kaurenoic acid



Graph 5d
^{1}H-NMR spectrum (300.06 MHz, $CDCl_3$) of 15-α-angeloyloxy-kaur-16-en-19-oic acid.



Graph 5e
^{13}C-NMR spectrum (75.45 MHz, $CDCl_3$) of 15-α-angeloyloxy-kaur-16-en-19-oic acid.

Table 1
Effects of the yacon extract on the activity of liver AST and ALT of normal and diabetic rats.

	AST µmoles/(min.mg)	ALT µmoles/(min.mg)
N	0.065±0.004	0.057±0.001
NY	0.068±0.004	0.064±0.001
D	0.249±0.044[a]	0.193±0.031[a]
DY	0.166±0.048[b]	0.204±0.049[a]

Yacon extract (Y) was administered orally in a dose of 400 mg/kg body weight for 30 days. Each value represents the mean ± S.E.M. of 6-12 animals per group. [a]P < 0.05 compared to normal rats (N) [b]P < 0.05 compared to the diabetic group (D).

DISCUSSION

Streptozotocin (STZ) injection in rats has been described as a good experimental model to study the effects of drugs on diabetes and some changes occurring in this state. In this model there is destruction of the β-cells of the islet of Langerhans of the pancreas[26], what results in hyperglycemia, polyphagy, polydipsia, and loss of body weight of the animals[27]. The fundamental basis of increased glycemia in diabetic state is an overproduction (excessive hepatic glycogenolysis and gluconeogenesis) and/or decreased use of glucose by the tissues[28]. Furthermore, the diabetes leads to metabolic changes caused by an absolute or relative lack of insulin and/or reduced insulin activity, which results in hyperglycemia and abnormalities in carbohydrate, protein, and fat metabolism[29,30]. The present study provided evidence that the hydroethanolic extract from S*mallanthus sonchifolius* (yacon extract) has an antihyperglycemic activity, with a beneficial effect on the metabolic changes found in streptozotocin-induced diabetic rats. In our study, the hepatic and muscle glycogen content in diabetic rats was increased compared with normal rats. It has been previously demonstrated that glycogen deposition from glucose is modified in diabetic animals[31] in proportion to the severity of insulin deficiency and long-standing diabetes[33,34,35]. Although some studies have shown that in diabetic rats the hepatic glycogen content is decreased[36,37,38], other studies, as well as ours, have demonstrated that the hepatic glycogen content is increased in these animals[38,39]. We have no explanation for these observations, but according to some studies[38,39,40] the mechanism leading to the persistence of the liver glycogen stores in 24-hour fasted diabetic rats are probably the consequence of the high ratio of glycogen synthase activity to glycogen phosphorylase activity. Also, there are reports that the glucose uptake in skeletal muscle is the major site of whole body insulin-mediated glucose disposal. It appears that a high glycogen content in muscle is associated with a state of insulin resistance. Moreover, glycogen has also been proposed as a possible regulator of muscle insulin action[41].

Treatment of diabetic rats with the yacon extract for 30 days significantly decreased both liver and muscle glycogen content indicating that the defective glycogen storage of the diabetic state was partially restored by the extract. In perfusion studies, glucose release in the liver of diabetic rats was high, even in the absence of exogenous gluconeogenic substrates in the perfusion fluid. The same had already been observed in other studies[42], which attributed this effect to glycogenolysis or gluconeogenesis from endogenous substrates. The treatment of diabetic rats with the yacon extract for 30 days caused a significant decrease in glucose release in the perfusion experiment. In the liver, the lower glycogen content in rats treated with the yacon extract could be responsible for the lower glucose release as the result of glycogenolysis. Thus, these findings suggest that this could, at least partially, contribute to the lower fasting glycemia observed in diabetic rats treated with the extract. Furthermore, it was observed that the treatment of diabetic rats with the yacon extract caused a reduction in hepatic AST activity, which was markedly increased in these animals. This effect is important once the increased activity of AST and ALT in diabetic rats has been associated with the increased glyconeogenesis and ketogenesis and/or of hepatic lesions, which may contribute to the increased level of blood glucose[30,43,44]. Also, diabetic rats treated with yacon extract presented a significant improvement in the glucose-6-phosphate dehydrogenase (G-6-PDH) activity that was reduced in streptozotocin-induced diabetic rats. This is consistent with published reports in which the decreased glucose-6-phosphate dehydrogenase activity has also been observed in the liver from diabetic animal models[45,46,47,48,49], suggesting a decrease in the metabolism via the phosphogluconate oxidation pathway[50].

It is known that G-6-PDH catalyzes the first and rate-limiting step of the hexose monophosphate shunt and produces NADPH needed for the maintenance of reduced

glutathione[51,52]. Previous reports have indicated that the NADPH produced by glucose-6-phosphate dehydrogenase participates in both the production of reactive oxygen species, such as superoxide anions and nitric oxide, and in the elimination of these radicals via glutathione peroxidase and catalase in both hepatic and extrahepatic tissues[53]. Increased expression of glucose-6-phosphate dehydrogenase has been associated with increased glutathione levels and resistance to oxidative stress. Increased oxidative stress, in turn, plays a key role in the pathogenesis of diabetes and its complications[54]. In conclusion, this study has advanced our understanding of the beneficial effects of the yacon extract on the hyperglycemia in this experimental model of diabetes, which may be related to: (a) decreased glycogen content in liver and skeletal muscle, b) reduced glucose release in the substrate-free perfused liver, (c) decreased AST activity and (d) increased glucose-6-phosphate dehydrogenase activity. Although this study has not conducted the complete identification of all the compounds present in the extract of yacon, a phytochemical analysis utilizing column chromatography showed the presence of kaurenoic acid, which could be responsible for the anti-hyperglycemic activity of the extract, already shown in another study[55]. As far as we know, this is the first study showing the beneficial effects of yacon extract on some metabolic changes of streptozotocin-induced type 1 diabetes in rats.

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Conflict of interest statement

Conflict of interest declared none.

REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care; 30: S42–S47, (2007).
2. Gipsen WH, Biessels GJ, Cognition and synaptic plasticity in diabetes mellitus. Trends Neurosci; 23: 542–549, (2000).
3. Pavkov ME, Hanson RL, Knowler WC, Bennett PH, Krakoff J, Nelson RG, Changing patterns of type 2 diabetes incidence among Pima Indians. Diabetes Care; 30: 1758–1763, (2007).
4. Wild S, Roglic G, Green A, Sicree R, King H, Global prevalence of diabetes estimates for the year 2000 and projections for 2030. Diabetes Care; 27: 1047–1053, (2004).
5. Dewanjee S, Maiti A, Das AK, Mandal SC, Dey SP, Swietenine: A potential oral hypoglycemic from *Swietenia macrophylla* seed. Fitoterapia; 80: 249–251, (2009).
6. Aybar MJ, Riera AS, Grau A, Sanches SS, Hypoglicemic effect of the water extract of *Smallanthus sonchifolius* (Yacon) leaves in normal and diabetic rats. J Ethnopharmacol; 74: 125-132, (2001).
7. Graefe S, Hermann M, Manrique I, Golombek S, Buerkert A, Effects of postharvest treatments on the carbohydrate composition of yacon roots in the Peruvian Andes. Field Crop Res; 86: 157-165, (2004).
8. Fernandez CE, Viehmannova I, Bechyně M, Lachman J, Milella L, Martelli G, The cultivation and phenological growth stages of Yacon [*Smallanthus sonchifolius* (Poepp. Et Endl.) H. Robinson. Agric. tropica et subtrop.; 40 (3): 71-74, (2007).
9. Valentová K, Moncion A, Wazier I, Ulrichova J, The effect of *Smallanthus sonchifolius* leaf extract on rat hepatic

metabolism. Cell Biol Toxicol; 20: 109-120, (2004).
10. Valentová K, Sersen I, Ulrichová J, Radical scavenging and anti lipoperoxidative activities of *Smallanthus sonchifolius* leaf extracts. J. Agric. Food Chem.; S3: 5571-5582, (2005).
11. Terada LS, Specificity in reactive oxidant signalling: think globally, act locally. J. Cell Biol.; 174: 615–623, (2006).
12. Vignale ND, Gurni AA, Identificación micrográfica de las hojas de S*mallanthus sonchifolius* (Poepp. & Endl.) H. Robinson (Asteraceae). Acta Farm Bonaer; 24: 96-98, (2005).
13. Schorr K, *Smallanthus sonchifolius* (Asteraceae) estudo fitoquímico, controle de qualidade e ensaios biológicos. Ribeirão Preto, 2005. 263p. [Tese de doutorado. Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto. Universidade de São Paulo].
14. Lebeda A, Dolezavola L, Valentová K, Dziechcarkava M, Gleplova M, Opatová H, Ulrichova J, Biological and chemical variability of maca and yacon. Chem Listy; 97: 548-556 (2003).
15. Valentová K, Ulrichová J, *Smallanthus sonchifolius* and *Lepidium meyenii* – Prospective Andean Crops for the prevention of chronic diseases. Biomed Pap; 147: 119-130, (2003).
16. Baroni S, Suzuki-Kemmelmeier F, Caparroz-Assef SM, Cuman RKN, Bersani-Amado CA, Effect of crude extracts of leaves of S*mallanthus sonchifolius* (yacon) on glycemia in diabetic rats. Braz. J. Pharm. Sci.; 44: (3) 521-530, (2008).
17. Genta SB, Cabrera WM, Mercado MI, Grau A, Catalán CA, Sánchez SS, Hypoglycemic activity of leaf organic extracts from *Smallanthus sonchifolius*: Constituents of the most active fractions. Chem-Biol Interact; 185: 143–152, (2010).
18. Valentová K, Cvak L, Muck A, Ulrichova J, Simancj V, Antioxidant effect of extracts from leaves of *Smallanthus sonchifolius.* Eur J Nutr; 47: 61-65, (2003).
19. Levinthal GN, Tavill AS, Liver Disease and Diabetes Mellitus. Clini Diabetes; 17: 2, (1999).
20. Bronsky G, Logothelopoulos, J, Streptozotocin diabetes in the mouse and guinea pig. Diabetes; 18: 606- 609, (1969).
21. Kepler D, Decker K, Glycogen: Determination with amyloglucosidase. In- *Methods of Enzymatic Analysis,* ed. H.U. Bergmeyer, Verlag Chemie-Academic Press, 1974, pp. 1126-1131.
22. Scholz R, Bücher T, Hemoglobin-free perfusion of rat liver. In: Control of Energy Metabolism, ed. B. Chance, R.W. Estabrook, and J. R. Williamson, Academic Press, 1965, pp. 393- 414.
23. Kelmer-Bracht AM, Fedatto-Júnior Z, Ishii- Iwamoto EL, Caparroz-Assef SM, Bracht A, The influence of Ca^{2+} on gluconeogenesis stimulation by glucagon in the liver of arthritic rats. Braz Arch Biol Techn; 45: 309-315, (2002).
24. Bergmeyer HU, Bernt E, Determination of glucose with glucose oxidase and peroxidase. In- Methods of Enzymatic Analysis*,* ed. H.U. Bergmeyer, Verlag Chemie-Academic Press, 1974, pp 1205-1215.
25. Langdon RG, Glucose-6-phosphate dehydrogenase from erythrocytes. In: Wood WA, (ed.) Methods in Enzymology, v. IX. Academic Press, 1966, pp. 126-131.
26. Fedosova NF, Alisievich SV, Lyadov KV, Romanova EP, Rudko IA, Kubatiev AA, Mechanisms underlying diquertin-mediated regulation of neutrophil function in patients with non-insulin-dependent diabetes mellitus. B Exp Biol Med+; 137: 143–146, (2004).
27. Cuman RKN, Bersani-Amado CA, Fortes ZB, Influence of type 2 diabetes on the inflammatory response in rats. Inflamm Res; 50: 460–465, (2001).
28. Pereira B, Costa-Rosa LFBP, Bechara ELK, Newsholme P, Changes in the TBARS content and superoxide dismutase, catalase and glutathione peroxidase activities in the lymphoid organs and skeletal muscles of adrenomedullated rats. Braz J Med Biol Res; 31: 827–33, (1998).

29. Leelavinothan P, Subramani S, Antihyperglycemic effect of diosmin on hepatic key enzymes of carbohydrate metabolism in streptozotocin-nicotinamide-induced diabetic rats. Biomed Pharmacother; 64: 477-481, (2010).
30. Banerjee S, Singh H, Chatterjee TK, Evaluation of anti-diabetic and anti-hyperlipidemic potential of methanolic extract of Juniperus communis (L.) in streptozotocin-nicotinamide induced diabetic rats. Int J Pharm Bio Sci; 4: 10-17, (2013).
31. Bollen M, Keppens S, Stalmans W, Specific features of glycogen metabolism in the liver. Biochem J; 336: 19–31, (1998).
32. Gannon MC, Nuttall FQ, Effect of feeding, fasting, and diabetes on liver glycogen synthase activity, protein, and mRNA in rats. Diabetologia; 40: 758-763, (1997).
33. Stalmans W, Cadefau J, Wera S, Bollen M, New insight into the regulation of liver glycogen metabolism by glucose. Biochem Soc T; 25: 19–25, (1997).
34. Grover JK, Vats V, Yadav S, Effect of feeding aqueous extract of *Pterocarpus marsupium* on glycogen content of tissues and the key enzymes of carbohydrate metabolism. Mol Cell Biochem; 241: 53–59, (2002).
35. Chandramohan G, Ignacimuthu S, Pugalendi KV, A novel compound from *Casearia esculenta* (Roxb.) root and its effect on carbohydrate metabolism in streptozotocin-diabetic rats. Eur J Pharmacol; 590: 437–443, (2008).
36. Jagtap AG, Patil PB, Antihyperglycemic activity and inhibition of advanced glycation end product formation by *Cuminum cyminum* in streptozotocin induced diabetic rats. Food Chem Toxicol; 48: 2030–2036, (2010).
37. Belfiore F, Rabuazzo AM, Iannello S, Anabolic response of some tissues to diabetes. Biochem Med Metab B; 35: 149–155 (1986).
38. Akatsuka A, Singh TJ, Huang KP, Comparison of the liver glycogen synthase from normal and streptozotocin-induced diabetic rats. Arch Biochem Biophys; 220: 426-434, (1983).
39. Rao PV, Pugazhenthi S, Khandelwal RL, The effects of streptozotocin-induced diabetes and insulin supplementation on expression of the glycogen phosphorylase gene in rat liver. J Biol Chem; 270: 24955-24960, (1995).
40. Gannon MC, Nuttall FQ, Effect of feeding, fasting, and diabetes on liver glycogen synthase activity, protein, and mRNA in rats. Diabetologia; 40: 758-763, (1997).
41. Derave W, Hansen BF, Lund S, Kristiansen S, Richter EA, Muscle glycogen content affects insulin-stimulated glucose transport and protein kinase B activity. Am J Physiol Endocrinol Metab; 279:E947-E955, (2000).
42. Oliveira DS, Bersani-Amado CA, Martini MC, Suzuki-Kemmelmeier F, Bracht A, Glycogen Levels and Energy Status of the Liver of Fasting Rats with Diabetes Types 1 and 2. Braz Arch Biol Techn; 50: 785-791, (2007).
43. Ramesh B, Karuna R, Sreenivasa RS, Haritha K, Sai Mangala D, Sasi Bhusana Rao B, Saralakumari D, Effect of *Commiphora mukul* gum resin on hepatic marker enzymes, lipid peroxidation and antioxidants status in pancreas and heart of streptozotocin induced diabetic rats. Asian Pac J Trop Biomed; 895-900, (2012).
44. Baynes JW, Thorpe SR, Role of oxidative stress in diabetic complications. Diabetes; 48: 1–9, (1999).
45. Gupta BL, Nehal N, Baquer NZ, Effect of experimental diabetes on the activities of hexokinase, glucose-6-phosphate dehydrogenase and catecholamines in rat erythrocytes of different ages. Indian J Exp Biol; 35: 792–795, (1997).
46. Zhang Z, Apse K, Pang P, Stanton RC, High glucose inhibits glucose-6-phosphate dehydrogenase via camp in Aortic Endothelial Cells. J Biol Chem, 275: 40042–40047, (2000).
47. Xu Y, Osborne BW, Stanton RC, Diabetes causes inhibition of glucose-6-phosphate dehydrogenase via activation of PKA, which contributes to oxidative

stress in rat kidney cortex. Am J Physiol Renal Physiol; 289: F1040–F1047, (2005).
48. Panneerselvam RS, Govindaswamy S, Effect of sodium molybdate on carbohydrate metabolizing enzymes in alloxan induced diabetic rats. J Nutr Biochem; 13: 21–26, (2002).
49. Kumar G, Murugesan AG, Rajasekara M, Effect of *Helicteres isora* bark extract on blood glucose and hepatic enzymes in experimental diabetes. Pharmazie*;* 61: 353–355, (2006).
50. Wagle A, Jivraj S, Garlock GL, Stapleton SR, Insulin regulation of glucose-6-phosphate dehydrogenase gene expression is rapamycin-sensitive and requires phosphatidylinositol 3-kinase. J Biol Chem; 273: 14968–14974, (1998).
51. Ugochukwu NH, Babady NE, Antioxidant effects of *Gongronema latifolium* in hepatocytes of rat models of noninsulin dependent diabetes mellitus. Fitoterapia; 73: 612–618, (2002).
52. Park J, Choe SS, Choi AH, Kim KH, Yoon MJ, Suganami T, Ogawa Y, Kim JB, Increase in glucose-6-phosphate dehydrogenase in adipocytes stimulates oxidative stress and inflammatory signals. Diabetes; 55: 2939–2949, (2006).
53. Salvemini F, Franzé A, Iervolino A, Filosa S, Salzano S, Ursini MV, Enhanced glutathione levels and oxidoresistance mediated by increased glucose-6-phosphate dehydrogenase expression. J Biol Chem; 274: 2750–2757, (1999).
54. Oberley LW, Free radicals and diabetes. Free Radical Bio Med; 5: 113-24, (1988).
55. Breaciani LF, Yunes RA, Bürger C, Oliveira LE, Bóf KL, Cechinel-Filho VZ, Seasonal variation of kaurenoic acid, a hypoglycemic diterpene present in *Wedelia paludosa (Acmela brasiliensis)* (Asteraceae). Z Naturforsch C; 59c: 229-232, (2004).