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Renal toxicity caused by oral use of medicinal plants: The yacon example

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ABSTRACT

Aim of the study: Yacon [*Smallanthus sonchifolius* (Poepp. & Endl.) H. Robinson, Asteraceae] is an Andean species that has traditionally been used as an anti-diabetic herb in several countries around the world, including Brazil. Its hypoglycaemic action has recently been demonstrated in normal and diabetic rats. However, studies about the safety of prolonged oral consumption of yacon leaf extracts are lacking. Thus, this work was undertaken to evaluate the repeated-dose toxicity of three extracts from yacon leaves: the aqueous extract (AE) prepared as a tea infusion; the leaf-rinse extract (LRE), which is rich in sesquiterpene lactones (STLs); and a polar extract from leaves without trichomes, or polar extract (PE), which lacks STLs but is rich in chlorogenic acids (CGAs).
Materials and methods: The major classes of the compounds were confirmed in each extract by IR spectra and HPLC–UV-DAD profiling as well as comparison to standard compounds. The toxicity of each extract was evaluated in a repeated-dose toxicity study in Wistar rats for 90 days.
Results: The PE was rich in CGAs, but we did not detect any STLs. The AE and LRE showed the presence of STLs. The polar extract caused alterations in some biochemical parameters, but the animals did not show signs of behavioural toxicity or serious lesions in organs. Alterations of specific biochemical parameters in the blood (creatinine 7.0 mg/dL, glucose 212.0 mg/dL, albumin 2.8 g/dL) of rats treated with AE (10, 50 and 100 mg/kg) and LRE (10 and 100 mg/kg) pointed to renal damage, which was confirmed by histological analysis of the kidneys.
Conclusions: The renal damage was associated with increased blood glucose levels after prolonged oral administration of the AE. This observation suggested that the hypoglycaemic effect observed after treatment for 30 days in an earlier study is reversible and was likely the result of renal injury caused by the toxicity of yacon. Because STLs were detected in both AE and LRE, there is strong evidence that these terpenoids are the main toxic compounds in the leaves of the yacon. Based on our results, we do not recommend the oral use of yacon leaves to treat diabetes.

1. Introduction

The consumption of medicinal plants without studies of efficacy and safety can result in several side effects that may affect different organs. The liver and kidneys are prime targets because they are involved in the degradation and excretion of a myriad of chemical compounds. Renal damage has been associated with the use of the medicinal plants in the treatment of different disorders, including diabetes mellitus (Mapanga and Musabayane, 2010). Studies using medicinal plants for the treatment of various disorders must be aware of the possibility of renal toxicity.

Smallanthus sonchifolius (Poepp. & Endl.) H. Robinson (Asteraceae), which is known as yacon, is an herb that is native to the Andes. The roots of the herb are used as dietetic food, and the leaves are useful in popular medicine for the treatment of hyperglycaemic disorders of diabetes mellitus (Vilhena et al., 2000; Aybar et al., 2001; Valentová and Ulrichová, 2003; Valentová et al., 2004). In recent decades, the cultivation of this species has expanded to several countries. It was introduced in Brazil in the late 1980s by Japanese immigrants who used the leaves for an anti-diabetic tea (Vilhena et al., 2000). Several commercial teas from the leaves of yacon are found on the Internet, and these teas are sold with the promise of blood glucose reduction. In fact, Aybar et al. (2001) have demonstrated the hypoglycaemic action of an aqueous extract of the yacon leaves in normal and diabetic rats treated for 30 days with tea infusions. Another study by Genta et al. (2010) showed

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that three organic extracts, and the sesquiterpene lactone enhydrin, from yacon leaves had effective hypoglycaemic activity in rats. Acute toxicity has also been evaluated, and the same authors found that the extracts had a high safety margin. Studies about toxicity of these extracts after prolonged consumption, however, need to be performed.

Recent studies have demonstrated that yacon leaves are rich in sesquiterpene lactones (STLs), flavonoids and phenolic compounds related to chlorogenic acids (CGAs) (Simonovska et al., 2003; Valentová et al., 2004; Schorr and Da Costa, 2005; Schorr et al., 2007; Hong et al., 2008; Xie et al., 2008; Xiang et al., 2010). STLs are stored inside glandular trichomes on the foliar surface and are known for their highly toxic properties (Schmidt, 1999). Chlorogenic acids are believed to be located in the mesophyll but not in the trichomes. Chlorogenic acids are believed to have very low toxicity because they are common constituents of plants used in human diets (Zhao and Moghadasin, 2010). Biological activities described for CGAs include antioxidant and anti-inflammatory properties (Peluso et al., 1995; Rastrelli et al., 1998). Further compounds identified from yacon leaves include diterpenes and phenylpropanoids (Qiu et al., 2008; Xie et al., 2008; Xiang et al., 2010).

Because they are toxic (Schmidt, 1999), the simple presence of STLs in trichomes on the foliar surface warns that the analysis of the toxic potential of popular preparations of this plant should be carefully evaluated. Aqueous extracts in the form of tea infusions are the most common preparations of this plant in folk medicine. Thus, the aims of this work were to analyse the long-term toxic potential of the aqueous extract of yacon leaves and compare its toxicity with two other extracts that were rich in either STLs or CGAs. These comparisons will allow us to determine if the STLs are the toxic compounds of the yacon leaves and whether they are present in aqueous extracts similar to those used in popular medicine.

2. Materials and methods

2.1. Plant material

Leaves of *Smallanthus sonchifolius* were collected on June 2007 at a cultivated area of the campus of the Universidade de São Paulo, Ribeirão Preto, SP, Brazil. Intact leaves were air-dried at 40 °C and a voucher specimen was deposited at the herbarium SPFR of Universidade de São Paulo under the code R.B. Oliveira 495.

2.2. Extract preparation

Three lyophilised extracts from the air-dried leaves were obtained: the aqueous extract (AE), the leaf-rinse extract (LRE), and the polar extract (PE). The AE was prepared with dried leaves (20 g) in boiling water (1000 mL). This step was repeated 24 more times for a total of 500 g of leaves. The extraction preparation was left to sit for 20 min until the extract reached room temperature (*ca.* 26 °C). The extract was filtered, lyophilised, and frozen at −20 °C until the experiment (1.8 g of extract was acquired from each portion of 20 g of dried leaves for a total of 45 g). Similar aqueous extracts are commonly used in popular medicine. The LRE was prepared from dried leaves (1 kg) rapidly rinsed with acetone. The goal was to extract the glandular trichome content (rich in STLs) from the surface of the leaf. Each intact leaf was individually rinsed in acetone for 10 s with the abaxial surface facing down (where the trichomes are located). Extraction of the glandular content was monitored under stereomicroscope (Stemi 2000C, Carl Zeiss, Germany). The extract was then filtered and evaporated under vacuum. This extract (26.9 g) was resuspended with MeOH:H_2O (7:3, v/v) and submitted to liquid–liquid partition with *n*-hexane to remove fatty material. The hydromethanolic fraction was evaporated under vacuum, lyophilised and frozen at −20 °C until the experiment. The PE was prepared with the leaves from the LRE preparation, which were air-dried and powdered. Extraction was performed by maceration in 70% MeOH three times during 24 h, which resulted in a polar extract. This extract was filtered, the solvent was evaporated under vacuum and the sample was submitted to liquid–liquid partition with *n*-hexane to remove fatty material. The hydromethanolic fraction (95 g) was dried under vacuum, lyophilised and frozen at −20 °C until the experiment.

2.3. Phytochemical analysis of the extracts

The analysis of the extracts was performed by infrared spectroscopy (IR) and reversed-phase HPLC–UV-DAD profiling. The presence of some classes of secondary metabolites (i.e., STLs, flavonoids or CGA derivatives) was deduced from their UV spectra obtained by HPLC as well as by comparison of their retention times with those of authentic standards (Sigma, Germany). The major metabolites of the LRE were identified by comparison of their retention times with those of authentic standards available in our laboratory (Schorr and Da Costa, 2005; Schorr et al., 2007; Gobbo-Neto and Lopes, 2008).

The samples were prepared in KBr tablets and the IR spectra were performed in a Perkin-Elmer 502 spectrometer (USA).

The HPLC system employed consisted of a Shimadzu (Japan) SCL 10Avp liquid chromatograph with a Shimadzu SPD-M10Avp photodiode array detector-DAD. The gradient elution was a binary mobile phase consisting of H_2O (0.5% CH_3COOH) and MeCN (0.5% CH_3COOH) in a linear gradient from 0 to 45% MeCN in 30 min. Isocratic elution occurred with 45% MeCN from 30 to 50 min, and the linear gradient was from 45 to 100% MeCN from 50 to 80 min. The flow rate was 1.3 mL/min using a C-18 column (Shimadzu, ODS Shim-pack 5 μm, 4.6 mm × 250 mm), and the injection volume was 20 μL. The UV data were acquired between 190 and 600 nm and chromatograms were registered at 254 and 325 nm simultaneously. The chromatographic data were processed using Class VP software (version 5.02; Shimadzu, Japan).

2.4. Animals

Male and female Wistar rats weighing 200–250 g from the animal house of the campus of the Universidade de São Paulo, Ribeirão Preto, SP were used in this study. All animals were housed in the animal facility of the Faculdade de Ciências Farmacêuticas de Ribeirão Preto. The temperature in the animal room was kept at 25 ± 2 °C, and the animals were allowed free access to food and water. The study was approved by the Institutional Ethical Animal Committee of the Universidade de São Paulo (protocol number: 07.1.636.53.5), which followed the rules of the Brazilian Committee to Animal Care (COBEA).

2.5. Repeated-dose toxicity study

Wistar rats were housed in plastic cages (three animals of the same sex in each cage) and divided into 13 groups containing six animals (three male and three female) per group. Different doses of the extracts were orally administered using a gastric tube (gavage), and the control group received vehicle (Cremophor® EL 5% Basf, Germany). The maximum volume administered for each rat was 1 mL. All groups were treated daily (six days per week) for a maximum period of 90 days. Body weights and food intake were measured weekly, and animals were observed for signs of abnormalities throughout the study. Two groups receiving the highest doses were treated differently: group (A) was treated for 90 days until the day before the blood collection, and the group (B) was treated for 90 days and then monitored for another 14 days with-

out treatment with the extract. This procedure was performed to investigate if the haematological alterations caused by the extracts were reversible. All other groups received the treatment until one day before blood collection. The administered doses of the AE were 10, 50, 100 (A), and 100 mg/kg (B). The administered doses of the PE and LRE were 10, 100, 1000 (A), and 1000 mg/kg (B).

For the haematological and biochemical analysis, the animals were fasted for 12 h before blood collection. On the day of blood collection, the animals were anaesthetised (xylazine and ketamine 1:1 v/v, 1 μL/g of the body weight). We measured total and differential (%) leukocytes (neutrophils, lymphocytes, monocytes, eosinophils and basophils). These haematological analyses were performed manually in a Neubauer chamber for the total leukocyte count (diluted in Turk solution), and blood smears stained with Panotic® solution (Laborclin, Brazil) were used for the differential leukocyte count. Biochemical analyses of the plasma (glucose) and serum samples were performed using an automatic chemistry analyser (ABBA VP, Abott®) and commercial kits for biochemical analyses (Labtest, Brazil). We measured the following biochemical parameters: uric acid, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), amylase, cholesterol, triglyceride, total protein, creatinine and glucose.

After blood collection the animals were euthanised by decapitation followed by necropsy. The position, shape, size and colour of internal organs (e.g., heart, kidneys, adrenal glands, lungs, stomach, liver, intestine, spleen, urinary bladder, testis in male rats, or ovary and uterus in female rats) were observed for any signs of lesions. These organs were collected and weighed to determine relative organ weights. Small pieces of these organs were fixed in 10% buffered formaldehyde solution for 48 h and then processed for histopathological studies. These pieces of organs were dehydrated by increasing alcohol grade (70, 80, 90, 95% and absolute), further cleared in xylene and embedded in paraffin. We cut 7-μm thick sections using a rotator microtome. Micrometer sections were stained with haematoxylin–eosin and examined under a light microscope where photomicrographs of the samples were recorded.

2.6. Statistical analysis

The differences among experimental and control groups were determined using the statistical software GraphPad Prism 5 (GraphPad Software, Inc., USA). For the analysis of the biochemical parameters, the data were expressed as mean ± S.E.M., and treated and control groups were compared using a one-way analysis of variance (ANOVA), followed by Dunnett's test. Pearson two-tailed analyses were performed to verify the correlations among the different biochemical analyses. The R^2 value was multiplied by 100 and expressed as percentage of correlation. Statistical treatment for weight increase and water and food intake was performed by a two-way ANOVA followed by Bonferroni's post-test. In all analyses, a 95% confidence level was used to determine statistically significant differences between treated and control groups.

3. Results

3.1. Phytochemical analysis

Infrared spectroscopy analysis was performed to verify the presence of STLs in the extracts. The STLs display a typical intense stretch band around 1760 cm^{-1} due to the α,β-unsaturated γ-lactone. This band was intense in the LRE (1768 cm^{-1}), which demonstrated that this extract was rich in STLs. The lack of the characteristic IR band for STLs in AE and PE demonstrated that these extracts did not contain STLs or that these substances were present in very low concentrations. The phenolic acids showed –OH stretching bands between 3400 and 3230 cm^{-1} and C=C stretching between 1000 and 1700 cm^{-1} depending on the positions of their substituent groups. The AE and PE showed intense stretching bands around 1600 and 3300 cm^{-1}.

The HPLC analysis demonstrated that AE and PE had polar compounds and many peaks with low retention times (Fig. 1). In both extracts, the UV curves of most peaks indicated aromatic compounds. The UV of each peak suggested that CGAs [UV max: 298 and 325 nm (Gobbo-Neto and Lopes, 2008)] were predominant in the AE and PE, including the major peaks 1 and 4 at 325 nm (Fig. 1B). The comparison of retention times of the main peaks for the AE and PE with those of authentic standards available in our laboratory identified peaks 2 and 3 as coumaric acid and caffeic acid, respectively. The chromatogram of the LRE showed compounds with intermediate retention times and therefore intermediate polarity, most of them visible at 254 nm (Fig. 1A). The LRE UV profile, as well as the comparison with authentic compounds, suggested the presence of STLs [UV max: 254 nm (Schorr and Da Costa, 2005)] and flavonoids [II band UV max: 260–280 nm, I band UV max: 320–370 nm (Gobbo-Neto and Lopes, 2008)]. The comparison of retention times of the main peaks for the LRE with those of authentic standards available in our laboratory identified peak 5 as the flavonoid 3-*O*-methylquercetin, peak 6 as the flavonoid 3,4′-di-*O*-methylquercetin, and peaks 7 and 8 as the STLs enhydrin and uvedalin, respectively. Peaks with lower intensity and with the same retention times and UV profiles as those of enhydrin and uvedalin were also observed in the AE at 254 nm (Fig. 1A), which was then confirmed by co-injection with authentic compounds. It was also possible to observe coincident peaks of minor STLs (peaks 9–11) in the AE and LRE at 254 nm (Fig. 1A). Thus, the HPLC–UV-DAD analyses suggested that AE and PE were rich in CGA derivatives, and LRE mainly contained STLs. Although STLs were not detected in the PE, they were found in lower concentrations in the AE, which was not observed in the IR analysis.

3.2. Repeated-dose toxicity study

3.2.1. Clinical signs

The rats that received the PE did not present noticeable signs of toxicity at any of the doses. The rats that received AE at doses of 100 mg/kg and LRE at doses of 10 mg/kg presented hypoactivity and asthenia. The groups that received the AE at lower doses (10 and 50 mg/kg) did not show noticeable signs of toxicity. The rats treated with 100 mg/kg LRE showed piloerection, asthenia, ataxia, anorexia, respiratory difficulty and respiratory arrest. Nevertheless, all animals of this group (LRE 100 mg/kg) died in the fourth week of treatment. It was not possible to collect the blood of these animals because they died during the night. Then, a new group that received the LRE at the dose of 100 mg/kg was started. Blood was collected from this new group of rats treated with 100 mg/kg LRE after the first signs of toxicity (*ca.* 14 days after beginning the oral administration). The animals from both groups that received LRE 1000 mg/kg (1000 mg/kg A and 1000 mg/kg B) showed the same symptoms as the 100 mg/kg group described above and died between the second and third days. We did not attempt to administer 1000 mg/kg LRE to any more rats because of the toxicity of the extract. The animals were euthanised by decapitation and submitted to necropsy for histopathology analysis.

3.2.2. Body and organs weight, water and food consumption

Rats treated with PE did not show differences in weight gain or water and food intake when compared with the control group (Table 1). Rats from the groups that received 100 mg/kg AE or LRE presented lower weight gain when compared with control animals (Table 1). These rats also had lower water and food intake. Thy-

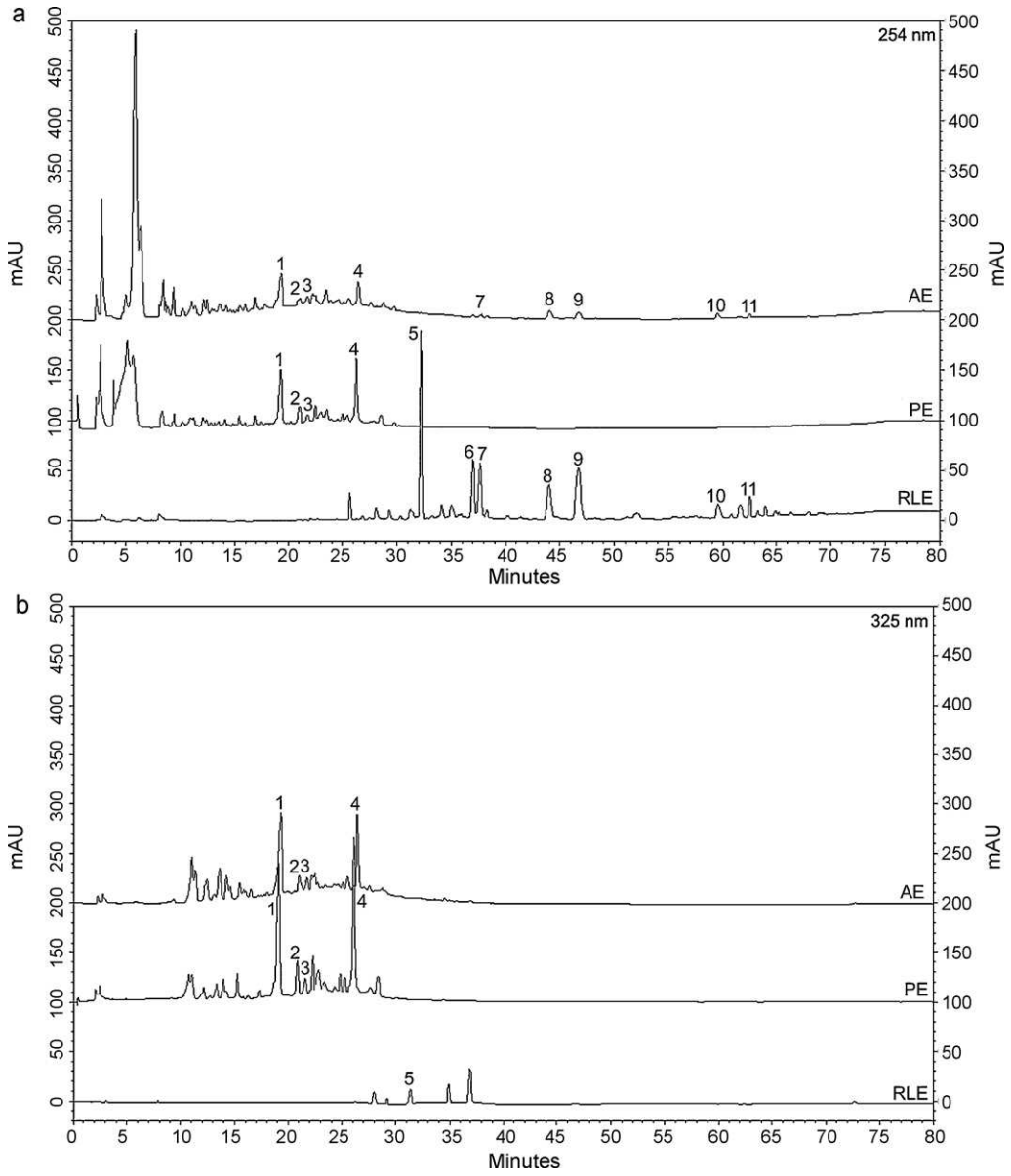

Fig. 1. HPLC–UV-DAD chromatograms of the yacon extracts. (A) 254 nm and (B) 325 nm. HPLC gradient elution was a binary mobile phase consisting of H_2O (0.5% CH_3COOH) and MeCN (0.5% CH_3COOH) in a linear gradient of 0–45% of MeCN in 30 min. Isocratic elution occurred with 45% MeCN from 30 to 50 min followed by a linear gradient of 45–100% MeCN from 50 to 80 min. The flow rate was 1.3 mL/min using a C-18 column (Shimadzu, Shimpack 5 μm, 4.6 mm × 250 mm). Peak 1 is a CGA derivative (UV max: 298 and 325 nm), peak 2 is coumaric acid, peak 3 is caffeic acid, peak 4 is a CGA derivative (UV max: 298 and 325 nm), peak 5 is 3-*O*-methylquercetin, peak 6 is 3,4′-di-*O*-methylquercetin, peak 7 is enhydrin, peak 8 is uvedalin and peaks 9–11 are unidentified STLs.

Table 1
Body and organ weight ratios of treated animals compared with the controls.

Group	Body weight (g)	Thymus (%)	Liver (%)	Spleen (%)	Kidney (%)
Control	303.0 ± 23.0	0.35 ± 0.06	4.63 ± 0.30	0.32 ± 0.03	0.60 ± 0.03
AE 10 mg/kg	299.2 ± 59.8	0.38 ± 0.08	5.99 ± 1.08	0.53 ± 0.12	0.88 ± 0.14
AE 50 mg/kg	295.8 ± 65.4	0.37 ± 0.05	6.92 ± 1.95	0.48 ± 0.09	1.04 ± 0.27
AE 100 mg/kg (A)	149.8 ± 31.1*	0.65 ± 0.24	7.99 ± 1.38	0.87 ± 0.27	1.39 ± 0.40*
AE 100 mg/kg (B)	177.3 ± 52.2*	1.05 ± 0.39*	7.87 ± 1.03	1.09 ± 0.37*	1.68 ± 0.54*
PE 10 mg/kg	328.2 ± 50.6	0.25 ± 0.06	4.65 ± 0.81	0.35 ± 0.06	0.66 ± 0.10
PE 100 mg/kg	310.3 ± 55.0	0.31 ± 0.04	5.70 ± 1.08	0.43 ± 0.05	0.77 ± 0.10
PE 1000 mg/kg (A)	326.7 ± 39.9	0.22 ± 0.03	4.09 ± 0.53	0.33 ± 0.06	0.57 ± 0.09
PE 1000 mg/kg (B)	333.8 ± 51.0	0.27 ± 0.04	4.01 ± 0.56	0.33 ± 0.05	0.59 ± 0.09
LRE 10 mg/kg	279.3 ± 30.2	0.25 ± 0.07	4.74 ± 0.67	0.37 ± 0.04	0.72 ± 0.08
LRE 100 mg/kg	154.8 ± 20.0*	0.54 ± 0.07	9.07 ± 1.60*	0.72 ± 0.12	1.20 ± 0.24*

Organ weight ratios were calculated as: (organ weight/terminal body weight) × 100 and expressed as percentage of organ weight. Value are expressed as mean ± S.E.M., $n = 6$. Experimental results of different treatments were analysed using analysis of variance (ANOVA) followed by Dunnett's test for comparison with the control group.
(A) Rats were treated until the day before the blood collection (90 days treatment).
(B) Rats were observed for an additional 14 days after 90 days of treatment before the blood collection.
* $P < 0.05$.

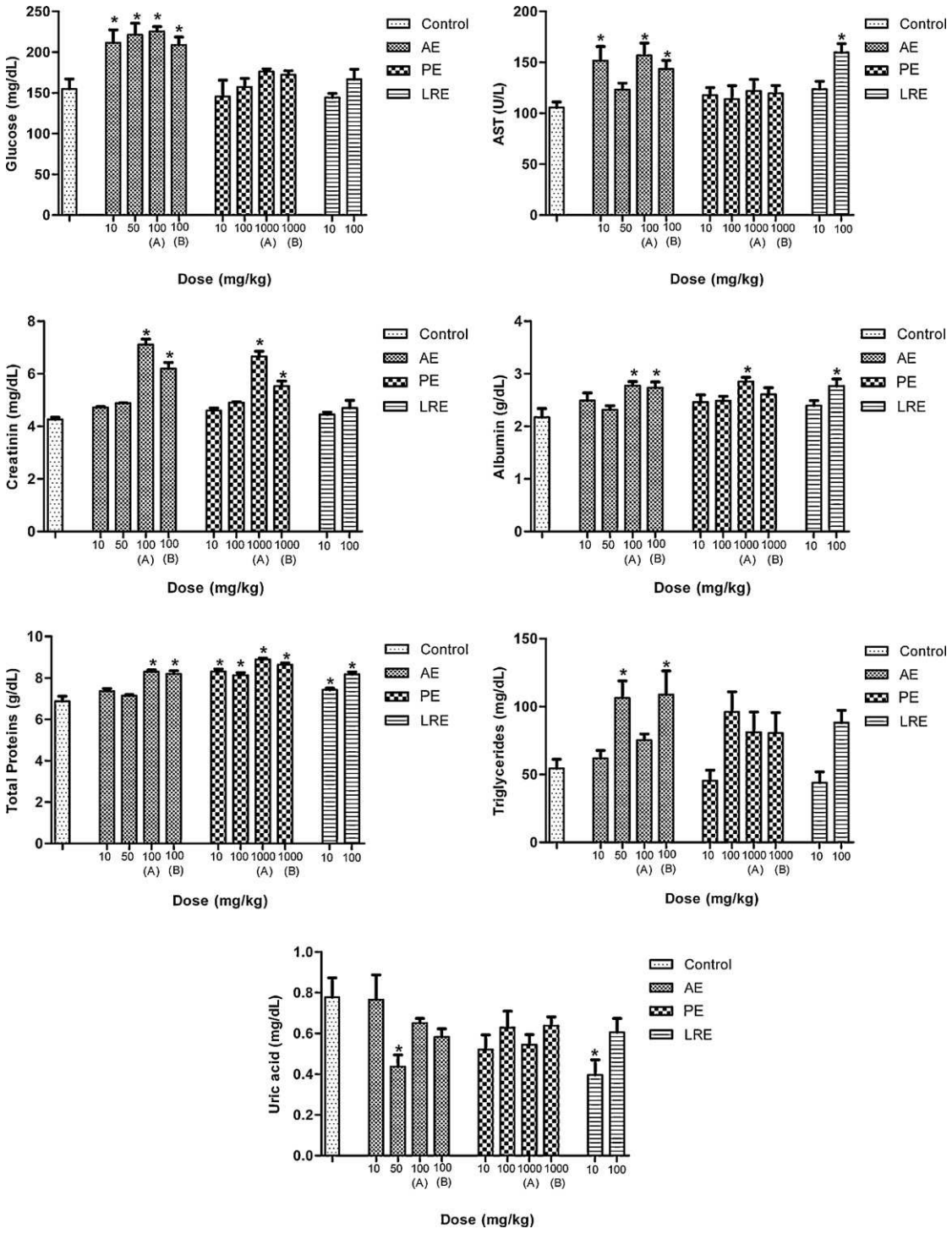

Fig. 2. The effects of oral administration of yacon extracts on biochemical parameters in rats. Data are expressed as mean ± S.E.M.; $^{*}P \leq 0.05$ compared to control (ANOVA).

mus and spleen weights were increased in the rats that received 100 mg/kg AE, and liver weight was increased in the rats that received 100 mg/kg LRE (Table 1). Kidney weight was increased in the rats treated with 50 and 100 mg/kg AE as well as the rats treated with 100 mg/kg LRE (Table 1).

3.2.3. *Haematological and biochemical parameters*

Statistically significant alterations in leukocytes were not observed in any group. However, the total leukocyte count and the differential monocyte count were slightly increased in the groups that received AE and PE (Table 2).

The effects of oral administration of the three extracts on all biochemical parameters analysed are presented in Fig. 2. All extracts triggered some changes in biochemical parameters. Compared with control rats, we observed the following increases in the biochemical parameters: a marked and significant increase in blood glucose levels [AE 10 mg/kg, 50 mg/kg and 100 mg/kg (A)], AST [AE 10 mg/kg and 100 mg/kg (A); LRE 100 mg/kg], triglycerides [AE 50 mg/kg and 100 mg/kg (B)], albumin [AE 100 mg/kg (A) and 100 mg/kg (B); PE 1000 mg/kg (A); LRE 100 mg/kg], total proteins [AE 100 mg/kg (A) and 100 mg/kg (B); PE 10 mg/kg, 100 mg/kg, 1000 mg/kg (A) and 1000 mg/kg (B); LRE 10 mg/kg and 100 mg/kg] and creati-

Table 2
Total and differential white blood cell (WBC) count of rats following treatment with various yacon extracts.[a]

Group	WBC (10^3/mL)	Neutrophils (%)	Mononuclear cells (%)	Eosinophils (%)
Control	9.47 ± 1.80	32.77 ± 3.90	65.39 ± 5.55	1.84 ± 1.84
AE 10 mg/kg	12.91 ± 2.08	20.22 ± 6.78	85.19 ± 6.44	1.33 ± 0.48
AE 50 mg/kg	12.07 ± 1.30	14.64 ± 3.78	84.91 ± 3.84	0.45 ± 0.29
AE 100 mg/kg (A)	8.96 ± 0.46	24.38 ± 5.44	73.09 ± 5.81	2.53 ± 1.19
AE 100 mg/kg (B)	8.37 ± 0.84	31.00 ± 2.00	66.41 ± 2.34	2.59 ± 1.12
PE 10 mg/kg	10.03 ± 0.70	18.15 ± 2.23	81.72 ± 2.23	0.13 ± 0.13
PE 100 mg/kg	8.44 ± 1.05	24.18 ± 5.57	74.84 ± 5.90	0.98 ± 0.65
PE 1000 mg/kg (A)	10.19 ± 1.39	23.63 ± 5.74	75.92 ± 5.76	0.45 ± 0.21
PE 1000 mg/kg (B)	8.38 ± 1.69	50.72 ± 14.24	48.60 ± 14.40	0.54 ± 0.40
LRE 10 mg/kg	9.10 ± 1.05	36.03 ± 7.68	63.84 ± 7.65	0.13 ± 0.13
LRE 100 mg/kg	10.88 ± 0.97	16.46 ± 4.11	82.55 ± 4.27	0.99 ± 0.26

Value are expressed as mean ± S.E.M., $n = 6$. Experimental results of different treatments were analysed using analysis of variance (ANOVA) followed by Dunnett's test. Probability values <0.05 were considered significant.
(A) Rats were treated until the day before the blood collection (90 days treatment).
(B) Rats were observed for an additional 14 days after 90 days of treatment before the blood collection.
[a] Basophils were counted but the value was zero in all doses and extracts.

nine [AE 100 mg/kg (A) and 100 mg/kg (B); PE 1000 mg/kg (A) and 1000 mg/kg (B)] (Fig. 2).

Correlation analyses indicated that there was a positive relationship between the glucose levels and other biochemical parameters analysed, especially with albumin and creatinine levels in the groups treated with AE (Table 3), the extract of interest in this analysis due do its use as tea. The Pearson two-tailed correlation was greater at doses of 10 and 50 mg/kg.

3.2.4. Necropsy and histopathological analysis

Histological features of kidneys from control rats showed normal structures (Fig. 3A and B). We did not observe any negative effects in the kidneys of rats treated with low doses of PE (10 and 100 mg/kg), and the glomeruli, distal and proximal tubules appeared to be normal. However, in the kidneys of the rats treated with 1000 mg/kg PE, an inflammatory reaction with polymorphonuclear cells was observed.

In the kidneys of the rats treated with 100 mg/kg AE, we observed a high influx of cells in the cortical region, mostly close to the glomeruli, as well as the medullar region (Fig. 3C and D). The glomeruli morphology was altered in this group of rats. We observed alterations in the mesangial cells, and the urinary space was increased, which resulted from a loss of glomeruli. The remaining glomeruli increased their filtration capacity to try to keep a normal renal filtration rate.

In the kidneys of the rats treated with 100 mg/kg LRE, we observed histological alterations in the glomeruli as well as the proximal and distal tubules (Fig. 3E and F). The glomeruli presented atrophy and marked degeneration of the mesangial matrix, and amorphous material was deposited in the inner part of the tubules. In addition, we also observed a decrease in the affinity to eosin colouring. Because eosin has affinity to acid compounds in the cytoplasm, this decrease in the colouring suggested that the deposited amorphous material had basic characteristics.

4. Discussion

In the present study, phytochemical analyses were carried out to confirm the major chemical classes of the compounds present in each of the three extracts. The combined IR spectroscopy analyses and chromatographic profiling by HPLC–UV-DAD demonstrated that the LRE was rich in STLs, and the PE and AE were rich in CGAs. Although STLs were also observed in the AE, the chromatographic profiling of the PE and AE was very similar (Fig. 1). STLs were observed in small amounts in the AE only by HPLC–UV-DAD profiling. Therefore, it can be stated that such compounds are present in the infusion used in folk medicine.

In the present repeated-dose toxicity study, rats were treated with the extracts (10, 50, 100 or 1000 mg/kg) for a period of 90 days. Although triglycerides, albumin and total protein levels were increased in rats treated with PE (1000 mg/kg), these animals did not present signs of toxicity. The histological analysis of the organs of animals treated with this extract did not present serious abnormalities. This suggested that repeated oral administration of the yacon leaf extract without STLs (PE) was safe.

The animals treated with AE showed signs of toxicity as hypoactivity and asthenia when compared with control rats. The biochemical analyses demonstrated increased levels of AST, triglycerides, albumin, total proteins, creatinine, and glucose.

The albumin and total protein levels are related because albumin corresponds to 50% of the total protein in the serum (Doweiko and Nompleggi, 1991). Thus, the high levels of total proteins that we observed may simply be a reflection of high levels of albumin. The most important function of albumin is its role in the maintenance of plasma volume; it is responsible for 80% of the colloid osmotic pressure. Albumin is also critical for the transport of several substances in the blood. However, the causes of increased levels of albumin are poorly understood. One study reported a relationship between elevated levels of albumin and dehydration, which may be related to renal function (Willard et al., 1994).

Table 3
Percentage of correlation between glucose levels and biochemical parameters with statistical significant difference between control and groups treated with AE.

Group	AST (%)	Triglycerides (%)	Albumin (%)	Total proteins (%)	Creatinine (%)
AE 10 mg/kg	40*	0	75*	37*	44*
AE 50 mg/kg	39*	49*	44*	12	51*
AE 100 mg/kg	0	6	16	0	13
Average	26	18	45	16	36

Values are represented as percentage of the correlation ($R^2 \times 100$). Experimental results of different treatments were analysed using a Pearson two-tailed analyses.
* Indicates significant correlation ($\alpha = 0.05$).

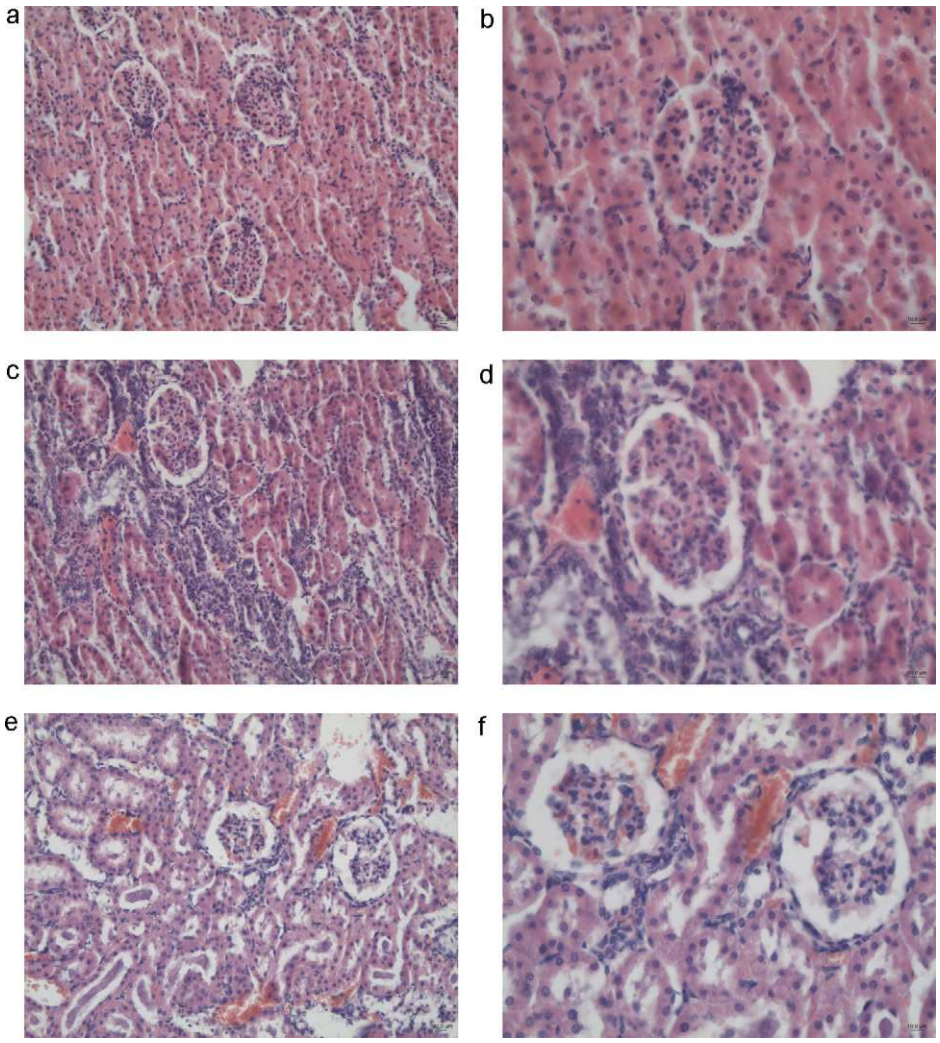

Fig. 3. Photomicrographs of the longitudinal sections of the renal cortex region of control and treated animals. (A) Renal cortex of a control rat showing three glomeruli and distal tubules (20×). (B) Detail of the same region shown in (A) showing intact glomeruli (40×). (C) Renal cortex of a rat treated orally with 100 mg/kg AE showing cell influx around the glomeruli and distal tubules (20×). (D) A detailed image of the same region shown in (C) showing cell influx and an increase in the urinary space (40×). (E) Renal cortex of a rat treated with 100 mg/kg LRE, which shows degenerated glomeruli and amorphous material deposited inside the distal tubules (20×). (F) A detailed image of the same region (40×) shown in (E).

In this study the increased creatinine level after 100 mg/kg AE suggested renal damage as an alteration in serum creatinine levels is an indicator for such a damage (Bishop et al., 2000; Muntner et al., 2000). In addition, alterations in fatty acid metabolism have been related in patients with chronic renal disease (Muntner et al., 2000). These alterations in fatty acid metabolism were shown to be characterised by increased levels of total cholesterol and triglycerides (Muntner et al., 2000). Increased triglyceride levels and a high level of creatinine were observed in the group that was observed for 14 days after treatment with 100 mg/kg AE. Muntner et al. also reported that serum glucose levels were increased in cases of chronic renal disease. In the present study, glucose levels were increased in all of the rats that were treated with AE regardless of dose. Interestingly, chronic renal disease results in a blockage of insulin degradation (Chen et al., 2003). In the early phase of the disease, a reduction of the glucose levels occurs due to the increase of insulin levels in the blood. Later, insulin levels remain high, but insulin resistance develops and the glucose is not transported to the inner cells anymore, which results in the increase of glucose levels in the blood (Chen et al., 2003).

In the present study, there was a positive correlation between glucose levels and other biochemical parameters associated with chronic renal disease. This result corroborated the hypothesis that the oral administration of AE results in chronic renal disease.

In a study performed by Aybar et al. (2001), the oral administration of the aqueous extract of yacon for 30 days resulted in decreased blood glucose levels in diabetic rats. The aqueous extract used in our study was prepared the same way as it was in the Aybar et al. (2001) study, but the extract was administered for 90 days in the present study. Our biochemical analyses suggested that the prolonged oral administration of the yacon infusion reversed the decreased plasma glucose levels observed when the extract was administered during a short period of time. This reversion was likely due to renal damage. In fact, a careful reading of the data from the Aybar et al. study suggested that the glucose levels decreased until the fourth day and then began to increase until the thirtieth day, even with increased levels of insulin. This observation corroborated our hypothesis that the hypoglycaemic effect is reversible after several days of treatment with tea infusion.

The LRE was highly toxic at a dose of 1000 mg/kg and caused all of the rats to die after only a few days of treatment. At a dose of 100 mg/kg, the LRE caused serious signs of toxicity, including anorexia and difficulty breathing. Biochemical analyses of the blood of these rats indicated an increase in the levels of the albu-

min, creatinine and total proteins, which also pointed to renal damage.

To show the renal damage observed in the biochemical tests, we performed histological analyses of the kidneys. The rats that were treated with 100 mg/kg LRE showed serious renal damage characterised by the degeneration of the glomeruli and amorphous material deposited in the inner part of the renal tubules. The presence of this amorphous material was reported in the literature as a deposit of immune globular proteins of the light chains κ or λ (Lin et al., 2001). The rats that were treated with 10 mg/kg LRE showed less intense morphological alterations. Similar renal damage was reported in a study describing the toxicology of the ethanolic extract of *Tithonia diversifolia* (Elufioye et al., 2009). In addition, *Tithonia diversifolia* also has secretory trichomes on the foliar surface, and the major compounds in the trichomes are STLs (Ambrósio et al., 2008). This suggests that STLs may play an important role in the toxicity of this species.

Morphological alterations observed in our study corroborated the biochemical alterations found in the blood. Taken together, our results confirmed that repeated doses of an AE from yacon leaves resulted in renal damage. In addition, our results confirmed the high level of toxicity elicited by treatment with LRE, which is rich in STLs.

5. Conclusions

Sesquiterpene lactones were present in the AE and LRE, which were toxic extracts in this study. Although there is evidence that aqueous and organic extracts, as well as secondary metabolites, from yacon leaves exert hypoglycaemic effects in animals, we propose that yacon leaves are not safe. Furthermore, it should be pointed out that due the broad array of *in vivo* toxic effects displayed by STLs, special care should be taken if it is administered orally, even at small doses. Based on our results, we do not recommend the oral use of yacon leaves.

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