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Improvement of biochemical parameters in type 1 diabetic rats after the roots aqueous extract of yacon [*Smallanthus sonchifolius* (Poepp.& Endl.)] treatment

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ABSTRACT

The aim of this study was to evaluate the effect of yacon (*Smallanthus sonchifolius*) (Poepp.& Endl.) on clinical parameters under diabetic conditions. The aqueous extract of yacon tuberous roots (YRAE; 0.76 g fructan kg^{-1} body weight) was prepared at the moment of each administration. Thirty-two male rats were divided into four groups ($n = 8$): control group (C); group that received YRAE (Y); untreated diabetic group (DM1); and diabetic group treated with YRAE (Y-DM1). The diabetes mellitus was induced by streptozotocin (60 mg kg^{-1} body weight). The animals from Y2 and Y-DM1 received YRAE by gavage, at 7-day intervals, for 30 days. The aqueous extract of yacon roots decreased ($p < 0.05$) the water and food intake in diabetic rats (Y-DM1). YRAE treatment reduced ($p < 0.05$) glycaemia, total cholesterol, VLDL-c, LDL-c and triacylglycerol levels in diabetic rats (YRAE). HDL, urea and creatinine levels did not differ ($p > 0.05$) between the Y and Y-DM1 groups. YRAE normalised alanine aminotransferase (ALT) activity, when comparing DM1 and Y-DM1 rats, but had no effect on lactate dehydrogenase activity (LDH). In conclusion, YRAE was sufficient for controlling water and food consumption, hyperglycaemia and dyslipidaemia, and promote the reduction of the ALT, suggesting a hepatoprotective effect in rats with STZ-induced DM1.

1. Introduction

Type 1 diabetes (DM1) is a multifarious group of disorders characterized by progressive and chronic hyperglycaemia. It is associated with the inhibition of the secretion of insulin from pancreatic ß-cells, thus the tissues do not adequately use the energetic substrates, resulting in metabolic abnormalities and tissue injury (WHO, 2009), which been implicated in the pathogenesis of complications.

It is a disease that interferes with the quality and style of life, leading to a reduction in life expectancy in the diabetic population (Lyra et al., 2006). Diabetes currently affects approximately 171 million individuals worldwide and is projected to reach 366 million by the year 2030. Estimate that the worldwide mortality attributed to diabetes in 2000 was 2.9 million, which was equivalent to 5.2% of the total mortality (Wild et al., 2004).

Anti-diabetogenic agents of naturals products, used both in modern and traditional medicine, are recommended by the World Health Organization (WHO). Alternative therapies are being studied in order to glycemic control and prevent diabetic complications. In this context, the medicinal plant *Smallanthus sonchifolius* (Poepp.& Endl.), popularly known as yacon, is frequently used due of its possible anti-diabetic properties. It is a native plant to South America (Lachman et al., 2003; Valentova et al., 2006) rich in polymers of fructose considered to be inulin-type fructans (Valentova and Ulrichova, 2003; Santana and Cardoso, 2008) in the form of oligosaccharides accumulated in the tuberous roots (Delzenne and Kok, 2001). These fructans also occur in other foods, such as leeks, Jerusalem artichoke, chicory roots and onions (Roberfroid, 2005).

Fructan is the general term used for any carbohydrate in which one or more fructosyl-fructose linkages constitute the majority of glycosidic groups. The inulin-type fructans from natural foods have lower caloric values due to the β-bonds (2,1) that link fructosyl-fructose molecules (Roberfroid, 2000). These β-configurations of glycosidic linkages are resistant to enzymatic hydrolysis in the human gastrointestinal tract. Thus, fructans are fermented by Bifidobacteria saccharolytic, resulting in end-products, such as short chain carboxylic acid (mainly acetate), propionate and butyrate.

This inulin-type fructans and oligofructose have been classified as prebiotics (Roberfroid, 2000) and show promise as functional foods (Valentova and Ulrichova, 2003) because they are able to

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stimulate the growth and/or activity of intestinal flora bacteria (Santana and Cardoso, 2008).

In dietary terms, fructan has a low energy value (1.5 kcal/g), which becomes relevant for patients with both DM1 and those with excessive obesity (Oliveira and Nishimoto, 2004). This supports the possibility that this functional food can exert hypolipidaemic effects, reducing atherosclerotic cardiovascular disease risk factors in diabetic patients (Stanley et al., 2004)

Moreover, a diet containing fructans increased the production of peptides by endocrine cells present in the gastrointestinal mucosa. These substances have been described as modulators of appetite (Druce et al., 2004; Delzenne et al., 2005), as well as being involved in the regulation of pancreatic insulin secretion and the differentiation of the β-cells (Brubaker and Drucker, 2004).

Dyslipidaemia represents a risk factor for cardiovascular disorders and contributes to the pathogenesis and complications in patients with DM1 (Lehto et al., 1997). Typically the atherogenic profile in the diabetic population includes hypertriglyceridaemia, low levels of HDL-c and elevated LDL-c levels (Purnell et al., 1998; Thorn et al., 2005).

Experimental studies have demonstrated that the supplementation of oligofructose normalised lipidaemia and glycaemic levels (Delzenne and Williams, 2002; Pamell and Reimer, 2009), and decrease serum triacylglycerol in both fasting and postprandial rats fed (Trautwein et al., 1998).

Since it is known that fructans are carbohydrates found in the storage tuberous roots of yacon, and that they demonstrate a beneficial effect on diabetic disturbances, it represents new possibilities for the treatment of metabolic disorders, with an improvement in the quality of life for diabetic individuals (Delzenne and Kok, 2001).

Therefore, the aim of the present study was to evaluate the possible protective effects of the aqueous extract of yacon roots on some biochemical parameters of clinical importance, as well as on the evaluation of body weight and consumption of food and water in rats with experimentally-induced diabetes mellitus type 1 (DM1).

2. Materials and methods

2.1. Plant material

The tuberous roots of yacon (*S. sonchifolius*) were collected in June 2010 from eight month-old plants (Jenkins et al., 1999), cultivated in Capão Bonito, São Paulo, Brazil. The tuberous roots were washed and packed in plastic bags, and then transported and stored at a temperature of 4 °C (Seminario et al., 2003) during the experimental period of 30 days. The plants were identified and deposited at the Herbarium BOTU/São Paulo State University, Botucatu, São Paulo (accession no. 23408).

2.2. Quantitative analysis of fructan in tuberous roots

The concentration of fructan was determined in the tuberous roots of yacon using High Performance Liquid Chromatography (HPLC) analysis, with a HPX 87p – BIO-RAD column (85 °C, flow 0.6 mL/min and purified water as a mobile phase), as described by Kaneko et al. (1990), followed by 4 weeks of administration of the aqueous extract of yacon roots (Table 1).

2.3. Preparation of the yacon roots aqueous extract (YRAE)

The aqueous extract of yacon tuberous roots was prepared at the moment of each administration to the animals. Since the concentration of fructan declines during storage (Table 1), the quantity in the tuberous roots used to prepare fresh aqueous extracts was recalculated at the moment of each administration, in order to maintain the same dose of fructan (0.76 g fructan kg^{-1} body weight) given to the animals, at intervals of 7 days (Delzenne and Williams, 2001). Root samples were homogenised using a blender, filtered and immediately administrated to the animals.

Table 1
Results obtained for the concentration of fructans (%) in tuberous roots of yacon plants in the four experimental weeks.

	Week 1	Week 2	Week 3	Week 4
Fructan (%)	5.03	4.09	2.85	1.75

2.4. Experimental animals and groups

The experiment was conducted at the Laboratory of Animal Experiments of the Department of Chemistry and Biochemistry/IB – UNESP, Botucatu/São Paulo, Brazil. Adult male wistar rats (200–250 g) were housed in polypropylene cages in a room maintained at 23 ± 2 °C, 50–66% relative humidity with a 12 h light–dark cycle. The rats were fed a standard chow diet (Purina, Campinas, SP, Brazil) and water ad libitum during the experimental period (30 days). The Ethical Committee for Animal Research the Bioscience Institute, São Paulo State University/UNESP approved this study.

The animals were randomly distributed into four groups ($n = 8$): control group (C); group that received YRAE (Y); untreated diabetic group (DM1); and diabetic group treated with YRAE (Y-DM1).

2.5. Induction of diabetes and treatment with YRAE

Diabetes mellitus type 1 (DM1) was induced by intraperitoneal administration of streptozotocin (STZ; Sigma, St. Louis, MO.), at a single dose of 60 mg kg^{-1} body weight, dissolved in citrate buffer (0.1 M, pH 4.5). After 48 h blood samples were obtained from the tail vein and glycaemia was determined using a glucometer (Boehringer Mannheim, Eli Lilly Ltda, São Paulo, Brazil). The STZ-induced rats with fasting blood glucose concentrations above 220 mg/dL were considered diabetic and included in the experiment (Babu and Srinivasan, 1999). With the establishment of the diabetic state, the animals of the Y and Y-DM1 groups received YRAE (0.76 g fructan kg^{-1} body weight) by gavage at 7-day intervals for 30 days. The food (g) and water (mL) intake was measured daily. Body weight (g) of the rats was measured weekly.

2.6. Biochemical determination

At the end of the experimental period (30 days), the animals were deprived of food overnight (12 h), anaesthetised with sodium pentobarbital 3%, 0.1 mL, i.p. and euthanised by cervical fracture and decapitation. The blood was collected and the serum was separated by centrifugation (14,000*g*/15 min) and used for biochemical analysis.

The concentration of glucose was measured according to the enzymatic colorimetric method, after incubation with glucose oxidase. The triacylglycerol concentration was determined through the enzymatic hydrolysis and release of fatty acids and glycerol, with the final production of H_2O_2 (Soloni, 1971). Total cholesterol levels were determined enzymatically by cholesterol ester/oxidase, according to Moura (1982). The fractions of high density lipoprotein-cholesterol (HDL-c) were measured through precipitation by phosphotungstic acid (Lopes-Virella et al., 1977). Low density lipoprotein-cholesterol (LDL-c) and very low density lipoprotein-cholesterol (VLDL-c) were calculated using the Friedewald equation.

The serum urea level was determined in the presence of urease, resulting in CO_2 and ammonia production. The addition of phenol-hypochloride led to an indophenol-blue complex with an absorbance at 600 nm, which was a method adapted from Fawcett and Scoth (1960). Creatinine was assayed by reaction with picric acid in alkaline buffer to form a yellow-orange complex, with the colour intensity, determined at 500 nm, being proportional to the creatinine concentration in the sample.

The serum enzymatic activities of alanine aminotransferase (ALT – E.C. 2.6.1.2) and lactate dehydrogenase (LDH – E.C. 1.1.1.27) were measured by the rate of oxidation of NADH to NAD, which is directly proportional to the ALT and LDH activities, according to Wilkinson (1972).

The spectrophotometric analyses were performed using a Pharmacia Biotech spectrophotometer (UVvisible/Ultrospec with Swift II application software to computer system control, Cambridge, UK).

2.7. Statistical analysis

Results are presented as the mean ± SEM. Significant differences among the groups were determined using the one-way analysis of variance (ANOVA). The means of groups were compared using the Tukey test at a 5% probability and a value of $p < 0.05$ was considered significant (Zar, 1996).

3. Results and discussion

Untreated diabetic animals (DM1 group) were characterized by classic symptoms of insulin-dependent diabetes mellitus (type 1),

i.e., persistent hyperglycaemia, excessive fasting, polydipsia and severe loss of body weight compared to the control group (Table 2). These results are in agreement with previous studies by Robert (2001).

The lower ($p < 0.05$) body weight in the DM1 group highlights the catabolic state of a poorly controlled glycaemic level. Under these conditions, the metabolic processes, lipolysis and the oxidative degradation of amino acids are increased, it degrade the greatest energy and tissue reserves in the organism, decreasing the body weight of diabetic rats, as verified in the present study (Table 2).

The high ($p < 0.05$) food intake, detected for the DM1 group, may be due to the incapability of the cell to use glucose as an energetic substrate and also due to the excessive loss of glucose through the urine, which causes intense diuresis and increased water intake (Table 1).

The glycaemic level in the DM1 group was significantly increased compared with the control group (Table 2). The change in glucose homeostasis under diabetic conditions can be as a consequence of the low glucose intake by peripheral tissues, as well as high hepatic glycogenolysis and gluconeogenesis.

STZ-induced diabetic rats treated with YRAE (Y-DM1) showed decreased ($p < 0.05$) glycaemic levels, when compared with DM1 group. It caused a significant improvement in food and water intake.

Improved glycaemic levels can be as a result of higher glucose utilization by cells, which start to oxidize glucose as a source of metabolic energy, thereby preserving adipose and muscular tissue and contributing to body weight recovery in YRAE-treated diabetic animals (Y-DM1).

These results are consistent with Valentova and Ulrichova (2003) since the anti-hypoglycaemic effect of the yacon plant may be due the decreased hepatic glucose synthesis via metabolic processes, gluconeogenesis and glycogenolysis. Moreover, these metabolic pathways were controlled in the propionate. This has also been reported for other indigestible carbohydrates, which may alter the kinetics of the absorption of monosaccharides, thus improving the hyperglycaemic state (Leclere et al., 1994). In addition, Aybar et al. (2001) showed that a 10% leaf extract of yacon reduced glucose levels in diabetic rats.

The administration of the aqueous extract of the yacon roots decreased ($p < 0.05$) serum levels of cholesterol and LDL-c and increased HDL-c levels in diabetic rats (Y-DM1) when compared to the non-treated rats (DM1). Kim and Shin (1998) reported that chicory extract and inulin diets increased the excretion of cholesterol and bile acid, which probably contributed to a decrease in serum levels of cholesterol and, consequently, reduced serum LDL-c. Fiordaliso et al. (1995) found that the administration of 10% oligofructose in the diet for 16 weeks reduced serum levels of total cholesterol in rats.

Others studies showed a beneficial effect of soluble fibres on the metabolism of lipids. The supplementation of soluble fibres has been shown to increase the excretion of bile acids (Stout, 1993). Since greater amounts of cholesterol are converted into bile acids in the liver and are subsequently excreted, the supplementation of soluble fibres possibly elevates the catabolism of cholesterol (Fiordaliso et al., 1995; Kim and Shin, 1998; Chen and Huang, 2009). Consequently, lower concentrations of intracellular cholesterol stimulate the expression of LDL receptors on the plasma membrane, increasing the internalisation of LDL-c, resulting in decreased serum levels of these lipoproteins.

In addition, the products of bacterial fermentation of fructans, i.e. short-chain fatty acids, are absorbed in the digestive tract into enterohepatic circulation, and suppress the synthesis of the cholesterol in the liver (Levrat et al., 1994).

The concentration of HDL-c decreased significantly ($p < 0.05$) in diabetic animals both treated and untreated with YRAE (DM1 and Y-DM1) compared to the C and Y groups. Although there was no statistically significant difference, the serum concentration of HDL-c was more often increased in Y-DM1 group. Russo et al. (2008) reported that an inulin-enriched diet elevated serum levels of HDL-c by approximately 35%. HDL-c has an important role in the removal and transportation of cholesterol from peripheral tissues to the liver for metabolic degradation in bile acids (Voet et al., 2000). Evidence suggests that HDL-c removes cholesterol and apolipoproteins during the normal catabolism of chylomicrons and VLDL-c (Baynes and Dominiczak, 2000). This is possibly explained as a result of the increase in HDL-c leading to a reduction of other lipoproteins, total cholesterol and triacylglycerol. However, since the YRAE showed a tendency to elevate the level of HDL-c, this may suggest an appropriate profile for the treatment and prevention of atherosclerosis in diabetic individuals.

Treatment with YRAE markedly decreased ($p < 0.05$) both triacylglycerol and VLDL-c in diabetic rats (Y-DM1), in comparison to the untreated diabetic rats (DM1).

Experimental data indicated that a diet supplemented with fructans lowered the serum concentration of triacylglycerol. Diets containing 10% oligofructose showed a reduction of these lipids Roberfroid and Delzenne (1998). Roberfroid (2000) and Letexier et al. (2003) reported that inulin inhibited the lipogenesis in liver. This effect is probably a consequence of the decrease of the de novo hepatic synthesis of triacylglycerol, which can contribute to the reduction of triacylglycerol and VLDL-c in the presence of fructooligosaccharides (Van Loo, 2004; Venter, 2006) (see Table 3).

Other possible mechanisms may be involved in the triacylglycerol-lowering effect, for example the production of propionate from the intestinal fermentation of fructans has been shown to act as an inhibitory agent of hepatic lipogenesis (Nishina and Freeland, 1990; Kuryl et al., 2006). Poor glycaemic control in the diabetic state increased fatty acid levels due to a higher of lipolysis in the adipose tissue. This results in an elevated re-esterification of the fatty acids in triacylglycerol, which are incorporated and secreted in VLDL-c. Daubioul et al. (2000) observed that fructan intake is associated with the reduction of hepatic steatose in Zucker rats, which possibly reduces the availability of free fatty acids from adipose tissue for the liver. Thus, the decrease in serum VLDL-c levels observed in the group Y-DM1, may be attributed to the decrease in the re-esterification of fatty acids and, consequently, the reduction in triacyglycerol secretion by the liver.

It is known that serum urea and creatinine are markers of renal and hepatic dysfunction in the diabetic state (Burtis et al., 2008). As

Table 2
General characteristics of animals.

Parameters	Groups			
	C	Y	DM1	Y-DM1
Food consumption (g/day)	24.85 ± 1.04a	25.86 ± 1.09a	37.84 ± 4.45b	35.08 ± 1.45b
Water intake (mL/day)	31.78 ± 2.00a	35.71 ± 3.01a	145.26 ± 25.32c	110.51 ± 10.73ba
Body weight (g)	373.86 ± 19.13c	378.59 ± 25.15c	260.29 ± 23.06a	335.79 ± 17.62b

Values are given as the as mean ± standard deviation. [a,b,c] Means followed by different letters refer to statistical difference ($p < 0.05$). Control rats (C); group received YRAE (Y); untreated diabetic group (DM1) and diabetic group treated with YRAE (Y-DM1).

Table 3
Results obtained for glicemia and lipid profile.

Parameters	Groups			
	C	Y	DM1	Y-DM1
Glucose (mg/dL)	94.79 ± 4.19a	93.54 ± 2.11a	373.51 ± 45.05c	230.22 ± 18.80b
Cholesterol (mg/dL)	62.18 ± 7.32a	65.72 ± 4.99a	134.72 ± 13.70c	88.99 ± 6.49b
Triaglycerides (mg/dL)	105.18 ± 7.56a	111.49 ± 7.18a	172.86 ± 4.18c	134.02 ± 4.48b
HDL-c (mg/dL)	39.23 ± 7.30b	36.05 ± 3.79b	23.15 ± 4.36a	28.9 ± 3.52a
VLDL-c (mg/dL)	21.03 ± 1.51a	22.49 ± 1.55a	34.57 ± 0.83cb	26.80 ± 0.89b
LDL-c (mg/dL)	7.61 ± 2.64a	8.06 ± 4.13a	76.99 ± 15.03c	33.29 ± 7.90b

Values are given as the as mean ± standard deviation. [a,b,c] Means followed by different letters refer to statistical difference ($p < 0.05$). Control rats (C); group received YRAE (Y); untreated diabetic group (DM1) and diabetic group treated with YRAE (Y-DM1).

Table 4
Results obtained for serum urea, creatinine, protein and activity of lactate dehydrogenase (LDH) and alanine aminotransferase (ALT).

Parameters	Groups			
	C	Y	DM1	Y-DM1
Urea (mg/dL)	62.17 ± 2.65a	62.12 ± 1.71a	63.87 ± 1.92a	63.71 ± 3.09a
Creatinine (mg/dL)	1.18 ± 0.18b	1.03 ± 0.04a	1.37 ± 0.16c	1.17 ± 0.26b
LDH (U/L)	207.69 ± 16.61a	204.22 ± 11.53a	246.74 ± 13.72b	240.29 ± 15.04b
ALT (U/L)	96.84 ± 10.70a	92.25 ± 11.17a	147.27 ± 11.45c	131.48 ± 10.46b

Values are given as the as mean ± standard deviation. [a,b,c] Means followed by different letters refer to statistical difference ($p < 0.05$). Control rats (C); group received YRAE (Y); untreated diabetic group (DM1) and diabetic group treated with YRAE (Y-DM1).

shown in Table 4, the serum level of both urea and creatinine were not significantly changed in STZ-diabetic rats (DM1), when compared to C and Y-DM1 groups. These results showed that the urea produced from ammonia (NH4) in the liver during the catabolism of amino acids is excreted adequately to the kidney. In contrast, studies have reported elevated serum levels of urea and creatinine and renal dysfunction under diabetic conditions (Saeed et al., 2008; Steinke and Mauer, 2008).

In several forms of hepatic and cardiac toxicity, the serum levels and/or activity of a number of cytosolic, mitochondrial and membrane-associated enzymes are higher in the plasma. The activity of ALT and LDH always increases when the pathological process affects the cell integrity and raises the membrane permeability of the enzymes (Burtis et al., 2008). Thus, understanding the activity of such enzymes in the plasma is important for the diagnosis of cell lesions, especially in hepatic and cardiac tissues, respectively, since the change in the cell membrane is followed by the release of enzymes into the bloodstream.

There were no significantly different serum activities of LDH and ALT between the experimental groups C and Y (Table 4). However, the serum activities of both LDH and ALT in the DM1 group were significantly higher than those in the C group, suggesting that the diabetes mellitus increased the tissue injury. The high activity of ALT and LDH in the serum, detected for diabetic animals DM1, proved that STZ-induced diabetes might have caused a certain degree of hepatic and cardiac toxicity. Therefore, according to Kumar et al. (2006), these lesions cause the overflow of ALT and LDH from the intracellular medium into the bloodstream.

Studies have reported that an increased activity of ALT in the blood of diabetic individuals is due to hepatic dysfunction involving changes in the integrity of cell membranes, as well as because of tissue injury caused by the oxidative stress established under this pathological condition (Packer et al., 2001; Takaike et al., 2004; Arkkila et al., 2011).

Treatment with YRAE did not suppress the activity of LDH in STZ-induced diabetic rats (Y-DM1), while the serum activity of ALT decreased in Y-DM1 group. The reduction in the enzyme catalysis observed for diabetic animals that received YRAE revealed its hepatoprotective potential, probably because it maintains plasmatic membrane integrity and recovers hepatic tissue.

4. Conclusion

Through the results of this study it was concluded that the aqueous extract of the roots of the yacon plant acted beneficially on the biochemical parameters analysed and was shown to be effective in reversing pathological changes, especially the dyslipidaemia and hyperglycaemia caused by diabetes mellitus induced by STZ. It was also found that the administration of the aqueous extract of yacon roots promoted liver protection by reducing the activity of ALT and resulted in an improvement of symptoms commonly associated with diabetes mellitus type 1, such as hyperphagia, polydipsia and weight loss.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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