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Full Length Research Paper

Alcohol extract of *Pterogyne nitens* leaves fails to reduce severity of streptozotocin-induced diabetes in rats

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Two constituents of *Pterogyne nitens* leaves, kaempferitrin, a diglycosylated flavonol, and galegin, a guanidine alkaloid, may be considered likely to exert an antidiabetic effect, on the basis of their chemical structures. Thus, experimentally diabetic rats were treated with *P. nitens* leaf extract, to observe the effects on biochemical and toxicological marker variables. Streptozotocin-diabetic rats (50 mg/kg body weight) were given ethanolic extract of the leaves (76 mg suspended in 0.5 mL of 10% aqueous glycerine per rat) (DP) by gavage, twice a day for 32 days. Diabetic controls were given 0.5 mL of 10% glycerine (DG), insulin (2.5 U in 0.3 mL s. c.) (DI) or 0.5 ml water (DW). Initial glycemia was 537.11 ± 10.35 mg/dL. Each week or fortnight after the treatment glucose, urea and protein contents were determined in the urine and glycemia and alkaline phosphatase activity in the serum. Except for proteinuria, the results for groups DP, DG and DW all differed significantly ($p < 0.05$) from those for group DI, which exhibited reduced values of all the other variables. The plant extract neither improved nor worsened the diabetic state of the rats; nor did it give rise to any hepato-biliary toxic effect.

Key words: Antidiabetic plant, alkaline phosphatase, biochemical markers, hepato-biliary toxicity marker.

INTRODUCTION

According to the World Health Organization, a total of 171 million people suffered from Diabetes mellitus in 2000 and this number is projected to rise to 366 million by 2030 (Setacci et al., 2009). This disease is defined as a complex of metabolic disorders characterized by hyperglycemia resulting from defective insulin secretion, resistance to insulin action or both. As a result of this insulin deficiency, the metabolism of carbohydrates, lipids and proteins is disturbed. Such metabolic changes increase the risk of retinopathy, nephropathy, neuropathy and atherosclerosis (The Expert Committee, 2003). High blood glucose is the main cause of these complications since it leads to excessive nonenzymatic glycosylation of body proteins (Hall, 2003). Changes in protein metabolism include a reduced uptake of amino acids by tissues, a higher rate of proteolysis and a fall in protein synthesis, leading to an increase in the production of urea by the liver (Felig, 1995). The overload of urea, glucose and other compounds in the kidney, together with renal vascular changes arising from the increased glycosylation of blood proteins, can damage the kidney and thus promote a loss of protein in the urine (Viberti et al., 1994).

Enzyme changes are also known to occur in human (Arkkila et al., 2001) and animal (Mori et al., 2003) liver tissues in diabetes, which are reflected in higher blood serum activities of transaminases and alkaline phosphatase (ALP). As part of the effort to combat this condition with material readily available in the tropics, we

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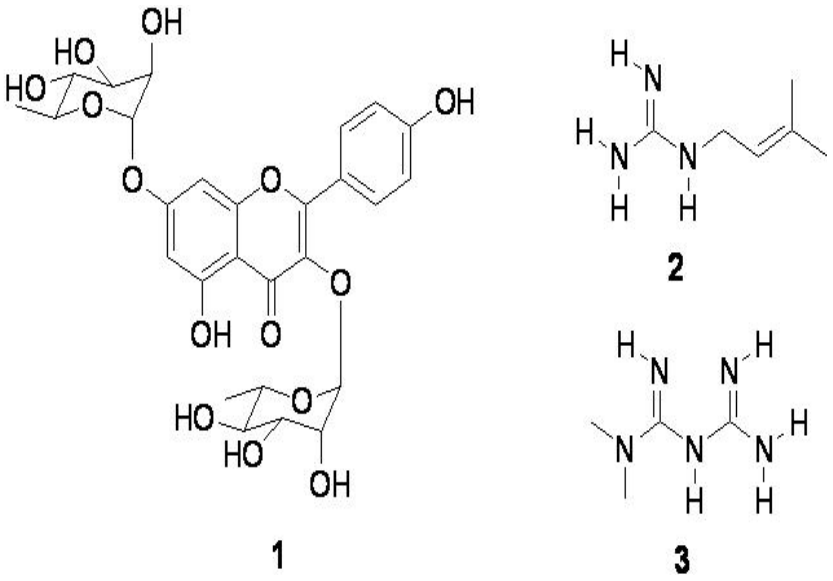

Figure 1. Molecular structures of (1) kaempferitrin, (2) galgegin and (3) metformin, an antidiabetic drug of the biguanide class.

have engaged in a series of experimental studies in diabetic rats, to assess the effectiveness of long-term treatment with various local plants, popularly held to have hypoglycemic properties, by testing their effects on a variety of marker variables that are altered in diabetes (Pepato et al., 1993; 2001; 2002; 2003; 2004; 2005; Brunetti et al., 2006). *Pterogyne nitens* Tul. (fam. Fabaceae, subfam. Caesalpinioideae), known in Brazil as forest peanut, is a beautiful leguminous tree and the sole member of its genus, which is distributed mainly in South America and tropical East Africa (Lorenzeti, 1998). Ethnopharmacological data on this species have not frequently been recorded, but aqueous extracts of the bark have been used by Paraguayans to treat parasitic diseases, mainly to eliminate ascarid worms (Crivos et al., 2007).

Among the major constituents that have previously been extracted from *P. nitens* leaves and purified are the compounds kaempferitrin (a diglycosylated flavonol) (Regasini et al., 2008) and galegin (a guanidine alkaloid) (Regasini et al., 2009).

Souza et al. (2004) proposed that kaempferitrin is the main compound in the *n*-butanol fraction of the leaf extract of *Bauhinia forficata* (Fabaceae) and is responsible for the hypoglycemic action of that leguminous tree. Furthermore, early human and animal model experiments with galegin, isolated from *Galega officinalis*, indicated that this alkaloid has a strong hypoglycemic effect (Reuter, 1963; Benigni et al., 1964). The importance of galegin is by no means restricted to herbal medicine. It played a crucial role in the history of modern drugs used by diabetics when it served as the structural model that led to the discovery and development of the biguanide class of oral hypoglycemics (Bailey and Day, 2004), including the still widely-used metformin (Figure 1). Thus, in view of the possibility that the combined presence of the alkaloid galegin and flavonoid kaempferitrin in a single plant extract could give rise to a heightened antidiabetic effect, we monitored the levels of biochemical markers in the urine and the hepato-biliary toxicity marker enzyme alkaline phosphatase (ALP) in the blood serum, during chronic treatment of diabetic rats with extract of *P. nitens.*

MATERIALS AND METHODS

Plant materials

P. nitens leaves were collected from trees in the São Paulo Botanical Garden, Brazil in May, 2003. A voucher specimen of this material (SP 204319) was deposited in the herbarium of the São Paulo State Botanical Institute.

Phytochemical procedures

The shade-dried leaves (2.8 kg) of *P. nitens* were ground and defatted with hexane (2.0 L x 5, at room temperature, for five weeks) and exhaustively extracted by maceration with ethanol (4.0 L x 5) at room temperature. The ethanol extract was concentrated under reduced pressure (< 40°C) to yield 12.7 g of syrup. This ethanol extract (10.0 g) was separated by gel permeation on a Sephadex LH-20 column (10 x 230 cm), eluted with MeOH, into twelve fractions, which were combined after comparison of their TLC analyses to afford galegin (Fractions 2 - 3; 2.8 g) and kaempferitrin (Fractions 8 - 10; 1.9 g) (Figure 1). The molecular structures of these compounds were identified by comparison with literature data, mainly ^{1}H and ^{13}C NMR chemical shifts (Reuter, 1963; Pizzolatti et al., 2003). The NMR spectra were collected in

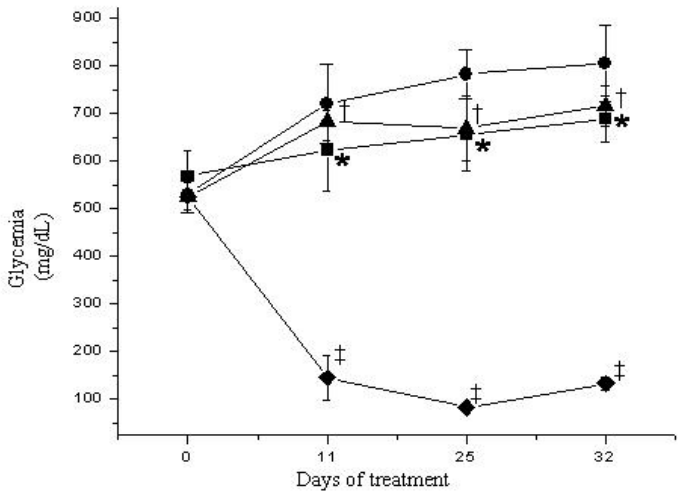

Figure 2. Levels of serum glucose of STZ induced diabetic rats treated with alcoholic extract of *P. nitens* leaves. 0 days is just before start of treatment, which started 7 days after STZ injection; (■) – DW-rats treated with water; (●)- DG-rats treated with 10% aqueous glycerine; (♦) – DI-rats treated with insulin; (▲) – DP-rats treated with *P. nitens* extract. Values are means ± standard error of mean. Intergroup comparisons for same time ($p < 0.05$): *DW versus DI; ‡ DG versus DI; † DP versus DI.

DMSO-d_6 solution, using a Varian INOVA 500 spectrometer (11.7 T), operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts are given as δ values (ppm) relative to tetramethylsilane (TMS) as internal standard.

Kaempferitrin (1, yield 19%): yellow solid. ^{1}H NMR δ_H (multiplicity; *J* in Hz; position): 13.2 (*br s*, 5-OH), 6.33 (*br s*, H-6 and H-8), 7.73 (*d*, 8.5, H-2' and H-6'), 6.81 (*d*, H-3' and H-5'), 5.29 (*br s*, H-1''), 3.95 (*br s*, H-2''), 3.50 (*dd*, 3.0 and 9.0, H-3''), 3.14 (*t*, 9.0, H-4''), 3.22 (*m*, H-5''), 0.81 (*d*, 6.0, H-6''), 5.49 (*br s*, H-1'''), 3.82 (*br s*, H-2'''), 3.62 (*dd*, 3.5 and 9.0, H-3'''), 3.29 (*t*, 9.0, H-4'''), 3.42 (*m*, H-5'''), 1.11 (*d*, 6.0, H-6'''). ^{13}C NMR δ_C (position): 157.5 (C-2), 134.1 (C-3), 177.4 (C-4), 161.5 (C-5), 99.7 (C-6), 166.9 (C-7), 93.0 (C-8), 156.1 (C-9), 106.3 (C-10), 118.4 (C-1'), 130.5 (C-2' and C-6'), 116.1 (C-3' and C-5'), 163.0 (C-4'), 101.7 (C-1''), 70.1 (C-2''), 69.8 (C-3''), 71.2 (C-4''), 70.6 (C-5''), 17.9 (C-6''), 98.7 (C-1'''), 70.3 (C-2'''), 70.0 (C-3'''), 71.7 (C-4'''), 70.4 (C-5'''), 17.5 (C-6''').

Galegin (2, yield 28 %): yellow oil. ^{1}H NMR δ_H (multiplicity; *J* in Hz; position): 7.57 (*br t*; 5.5, H-3), 3.67 (*t*; 5.5, H-1'), 5.18 (*t*, 5.5, H-2'), 1.61 (*s*, H-4'), 1.63 (*s*, H-5'). ^{13}C NMR δ_C (position): 156.9 (C-2), 39.0 (C-1'), 119.1 (C-2'), 136.3 (C-3'), 17.9 (C-4'), 25.4 (C-5').

Preparation of plant extract suspension

Before each administration of plant material to the rats, 760 mg of ethanol extract of *P. nitens* leaves was added to 5 mL of 10% aqueous glycerine (Allen et al., 2007) and the mixture treated in an ultrasound bath at 27°C for 25 min. This procedure yielded a fine suspension which was used to treat the animals.

Continuous treatment of rats

Following approval of the experimental protocol (CEP/FCF/CAr. 21/ 2006) by the Research Ethics Committee of the Araraquara School of Pharmaceutical Sciences, 50 male Wistar rats weighing 111.86 ± 1.72 g were adapted to metabolic cages for 2 days, after which they were fasted for 14 - 16 h, and injected with streptozotocin (STZ) at 50 mg/kg body weight (bw) in 0.01M citrate buffer (pH 4.5) *via* the jugular vein. All rats were returned to their metabolic cages where they had free access to food and water. After 4 days, blood was collected from the caudal vein of each animal, to measure the serum levels of glucose and alkaline phosphatase (ALP). Animals with a serum glucose content higher than 300 mg/dL were placed in 4 groups of 10, respectively treated with water (DW) (n = 10), with 10% aqueous glycerine (extract vehicle) (DG) (n = 10), with insulin (DI) (n = 10) and with the plant extract suspension (DP) (n = 10). Rats with serum levels of glucose below 300 mg/dL were not used. Treatment started 3 days later and continued for 32 days (i.e. until 39 days after STZ). During this period, each rat in the experimental group (DP) was given 0.5 mL of the plant suspension (containing 76 mg *P. nitens* extract and 10% aqueous glycerine) by gavage twice a day (8am and 6pm); while the controls were given the same volume of water (group DW) or 10% aqueous glycerine (group DG), at the same times, by gavage, and group DI received 0.3 mL injections s.c. of insulin (8.33 U/mL, Humulin NPH, Lilly, SP, Brazil), also twice a day. At intervals of 1 or 2 weeks, the following biochemical variables were measured: levels of glucose, urea and protein in the urine and glucose and ALP activity in blood serum.

Analytical methods

Plasma glucose and serum activity of ALP were determined in a Technicon RA-XT autoanalyser (Bayer, Dublin, Ireland). Urinary glucose was measured by the *o*-toluidine method of Dubowski (1962), urea by the urease method (Bolleter et al., 1961; Bergemeyer, 1985) and proteinuria by the modified Bradford method (Bradford, 1976), with a Femto 600S spectrophotometer (São Paulo, Brazil). The animal data were assessed by one-way ANOVA, in conjunction with the Student-Newman-Keuls test. These statistical tests were carried out with the computer program Sigma Stat 2.03.

RESULTS AND DISCUSSION

In this study we assessed the effects of treating STZ-induced diabetic rats by gavage with *P. nitens* leaf extract for 32 days. To this end, we monitored the biochemical variables that are frequently altered in diabetes, as well as a serum marker for liver toxicity, the enzyme ALP.

The effects of this oral treatment with the *P. nitens* extract on the levels of serum and urinary glucose are shown in Figures 2 and 3, respectively, and it can be seen that, from day 11, group DI exhibited significantly lower levels than all other groups. However, comparisons between groups DP, DG and DW showed no significant differences at any time. For urinary urea (Figure 4), no differences were found between DW or DG and group DP and, while there was a tendency for levels to be lower in group DI, this effect only became significant on day 25, between groups DI and DG, and on day 18, between groups DI and DW. Lastly, there was no significant difference between any of the groups in the urinary protein content (Figure 5), except between groups DP and DI on day 4 of treatment. In an attempt to explore the above results further, we analyzed the sum of the data obtained over the whole treatment period (Table 1). This analysis demonstrated significantly lower values for all

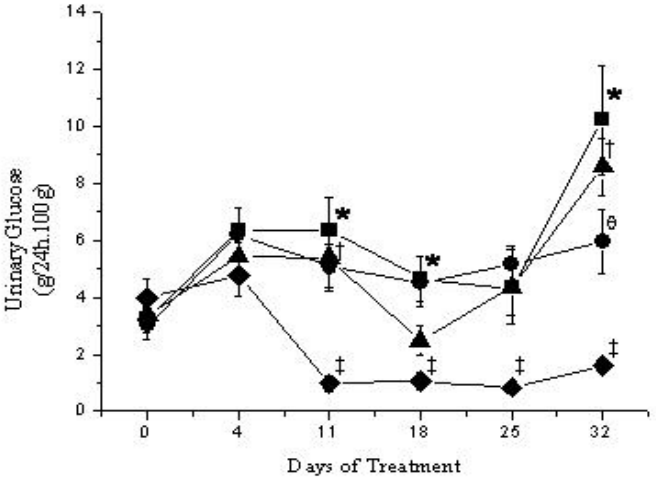

Figure 3. Levels of urinary glucose of STZ induced diabetic rats treated with alcoholic extract of *P. nitens* leaves. 0 days is just before start of treatment, which started 7 days sfter STZ injection; (■) – DW-rats treated with water; (●)- DG-rats treated with 10% aqueous glycerine; (♦) – DI-rats treated with insulin; (▲) – DP-rats treated with *P. nitens* extract. Values are means ± standard error of mean. Intergroup comparisons for same time ($p < 0.05$): *DW versus DI; ‡ DG versus DI; † DP versus DI, θ DW versus DG.

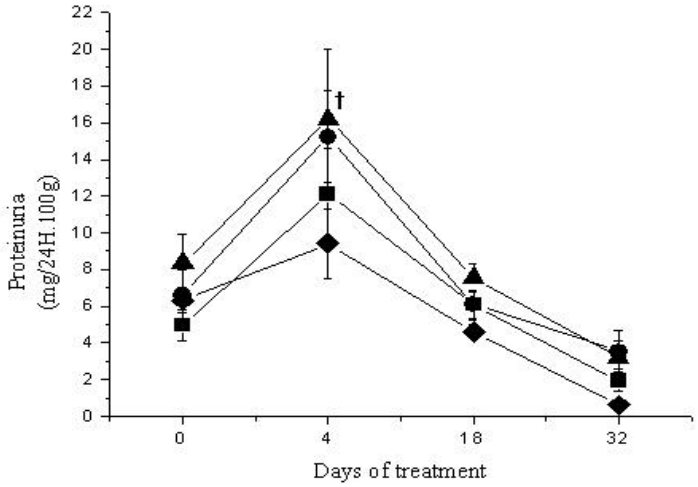

Figure 5. Levels of proteinuria of STZ induced diabetic rats treated with alcoholic extract of *P. nitens* leaves. 0 days is just before start of treatment, which started 7 days sfter STZ injection; (■) – DW-rats treated with water; (●)- DG-rats treated with 10% aqueous glycerine; (♦) – DI-rats treated with insulin; (▲) – DP-rats treated with *P. nitens* extract. Values are means ± standard error of mean. Intergroup comparisons for same time ($p < 0.05$): † DP versus DI.

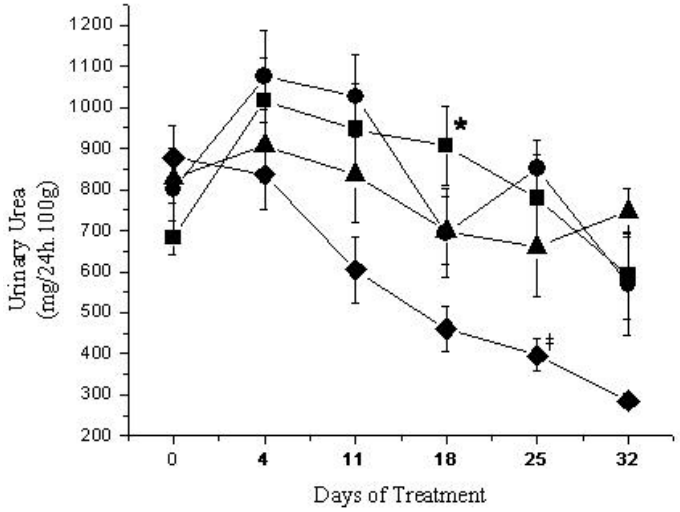

Figure 4. Levels of urinary urea of STZ induced diabetic rats treated with alcoholic extract of *P. nitens* leaves. 0 days is just before start of treatment, which started 7 days sfter STZ injection; (■) – DW-rats treated with water; (●)- DG-rats treated with 10% aqueous glycerine; (♦) – DI-rats treated with insulin; (▲) – DP-rats treated with *P. nitens* extract. Values are means ± standard error of mean. Intergroup comparisons for same time ($p < 0.05$): *DW versus DI; ‡ DG versus DI.

variables measured in group DI than in groups DP, DG and DW, except for proteinuria. Even proteinuria showed a tendency to follow the same pattern. This type of thus, rendered the effect of insulin treatment, relative to groups DW and DG, even clearer, for all the parameters tested, demonstrating that the experimental model used was appropriate for this study, in which a plant was tested for antidiabetic properties. The analysis of summed data did show that the plant extract-treated group had just significantly less glucose in the urine than group DW (Table 1). However, examination of the data for glucosuria in Figure 1 suggests that this result was entirely due to a single data point (on day 18), which could represent a random variation.

Corroborating the apparent lack of effect of this extract on glucosuria, earlier results obtained in similar conditions showed that *P. nitens* leaf extract did not alter either serum glucose levels or the physiological variables, water and food intake and body weight (Souza et al., 2009). These results partially disagree with those obtained by Jorge et al. (2004), in that those authors observed that kaempferitrin caused acute lowering of blood glucose in diabetic rats and stimulated glucose uptake by soleus muscle from normal rats as efficiently as insulin. However, they also reported that this compound had no effect on glucosuria or on protein synthesis in muscle from normal and diabetic rats.

When this study was planned, the extract of *P. nitens* was seen as a very promising candidate as a natural hypoglycemic product, yet both types of analysis of the results (time profile and global sum) have demonstrated its ineffectiveness against diabetes. Some comments can be made on this result. One possibility is that the great majority of the pancreatic β cells, responsible for insulin

Table 1. Biochemical marker variables for diabetic rat groups during 32 day treatments.

	DW	DI	DG	DP
Glycemia (mg/dL)	657.50 ± 44.25	120.87 ± 8.47 * ‡	750.85 ± 53.62	690.25 ± 42.00 †
Glucosuria (g/24 h.100 g)	6.32 ± 0.59	1.85 ± 0.29 *‡	5.39 ± 0.38	4.94 ± 0.48 †#
Urinary urea (mg/24 h.100 g)	867.84 ± 51.63	521.94 ± 39.06 *‡	863.83 ± 54.19	795.91 ± 45.18 †
Proteinuria (mg/24 h.100 g)	7.28 ± 1.40	4.88 ± 0.93	9.00 ± 1.49	10.42 ± 1.97 †

Treatment initiated on day 7 after STZ injection. Test groups: DW - treated with water; DG - treated with 10% aqueous glycerine; DI - treated with insulin; DP - treated with extract of *Pterogyne nitens*. Values are means ± standard error of the mean of 46 - 50 determinations. Intergroup comparisons: *($p < 0.001$) DW versus DI; ‡ ($p < 0.001$) DG versus DI; † ($p < 0.001$) DP versus DI; # ($p < 0.05$) DP versus DW.

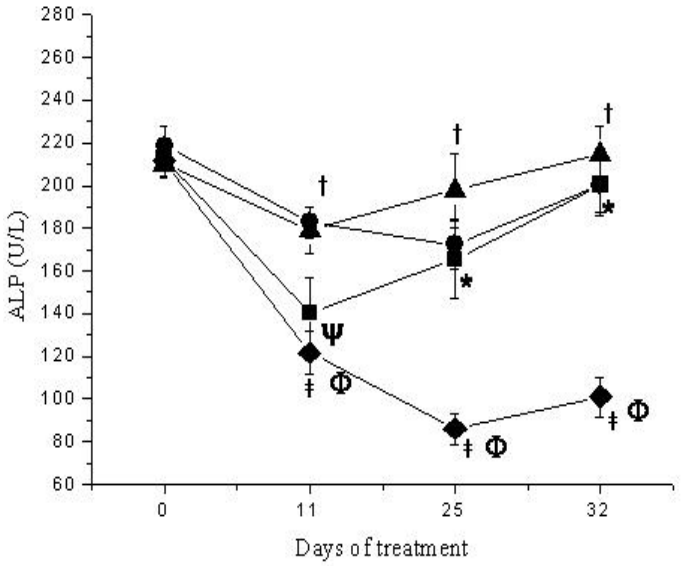

Figure 6. Levels of serum alkaline phosphatase of STZ induced diabetic rats treated with alcoholic extract of *P. nitens* leaves. 0 days is just before start of treatment, which started 7 days sfter STZ injection; (■) – DW-rats treated with water; (●)- DG-rats treated with 10% aqueous glycerine; (♦) – DI-rats treated with insulin; (▲) – DP-rats treated with *P. nitens* extract. Values are means ± standard error of mean. Intergroup comparisons for same time ($p < 0.05$):*Dw versus DI; ‡ DG versus DI; † DP versus DI. Intragroup comparisons between days ($p<0.01$): ψ DW versus day 0; Φ DI wersus day 0.

production, may have been destroyed by the STZ treatment, abolishing their capacity for regeneration, given that the treated animals had an initial glycemia of 537.11 ± 10.35 mg/dL. Many of the beneficial effects of plant products on diabetes are seen more clearly in milder cases of the disease, in which the mean initial glycemia is around 180 - 250 mg/dL (Viana et al., 2004; Oliveira et al., 2008; Hamden et al., 2009; Rauter et al., 2009). Under such conditions, the β cells have a high capacity for regeneration. The intention in the present study was to test the effects on really severe diabetes (Grover et al., 2000), since in the course of our experimental work we have found that animals with blood glucose levels between 150 and 300 mg/dL often revert spontaneously to normal levels. It is also likely, judging by the present results, that the extract did not exert any peripheral effect. Secondly, it is not impossible that another route of administration could lead to a better therapeutic response.

Thirdly, the high concentration of extract used might be responsible for the failure of the treatment. It has been reported that an extract prepared from *Eugenia jambolana* seeds and given to diabetic rats at 2.5 - 5.0 g/kg b.w. was capable of reducing their blood glucose level, whereas the same extract at a dose of 7.5 g/kg b.w. failed (Prince et al., 1998). This possibility should be given serious consideration in the present case, in view of the fact that 4 rats in group DP died during the treatment, perhaps because of toxic effects of the extract. One way to follow up this hypothesis is to test hepato-biliary toxicity markers, such as serum ALP activity. We know that diabetes itself provokes a rise in this activity and that insulin treatment counteracts this effect (Mori et al., 2003). Indeed, in the present study, group DI rats developed a lower mean ALP activity than those in groups DG or DW, as expected (Figure 6). The ALP activity in group DP also remained higher than that of the insulin-treated rats and not lower than that of any group, showing that the extract failed to reverse this hepato-biliary alteration provoked by diabetes. However, it can also be deduced that neither the plant extract nor the glycerine had a toxic effect on the hepato-biliary system, since throughout the treatment the diabetic rats treated with water, with extract and with glycerine all showed similar ALP profiles.

Finally, analyzing the ALP data at different times within each group, it can be seen that the insulin treatment promoted a fall in the ALP activity in each period (Figure 6), as would be expected. Both within-group and between-group comparisons indicate that the experi-mental model used here was appropriate for the aims of this study. The significant drop in ALP levels in DW on day 11, relative to day 0, was a random fluctuation. There is no apparent hepato-biliary toxic effect due to the plant extract and this should not be responsible for the death of

the 4 animals between days 20 and 25 of the treatment with the extract. There remains the possibility that a toxic effect occurred in organs other than the liver. For example, the increase detected in proteinuria (Table 1) in group DP could be indicative of kidney damage.

Conclusion

Having used a reliable experimental model to assess relief from induced diabetes in rats, we conclude that the alcoholic extract of *Pterogyne nitens* leaves, administered as described, had no detectable therapeutic effect on the diabetic state. It is still possible that this plant might wholly or partially reverse experimental diabetes if the dose of the extract, treatment route or severity of induced diabetes were altered.

ACKNOWLEDGMENTS

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REFERENCES

Allen Jr LV, Popovich NG, Ansel HC (2007). Formas farmacêuticas e sistemas de liberação de fármacos. 8 ed, Artmed, Porto Alegre pp. 372.

Arkkila PET, Koskinen PJ, Kantola IM, Ronnemaa T, Seppanen E, Viikari JS (2001). Diabetes complications associated with liver enzyme activities in people with type 1 diabetes mellitus. Diabetes Res. Clin. Pract. 52: 113-118.

Bailey CJ, Day C (2004). Metformin: its botanical background. Pract. Diab. Int. 21: 115-117.

Benigni R, Capra C, Cattorini PE (1964). Plante medicinali, chimica farmacologia e terapia. Inverni Della Beta, Milão, pp. 626-632.

Bergemeyer HU (1985). Methods of Enzymatic Analysis. Volume 9, VHC, Florida, pp. 449-453.

Bolleter WT, Bushman CJ, Tidwell PW (1961). Spectrophotometric determination of ammonia as indophenol. Anal. Chem. 33: 592-594.

Bradford MM (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72: 248-254.

Brunetti IL, Vendramini RC, Januário AH, França SC, Pepato MT (2006). Effects and toxicity of *Eugenia punicifolia* extracts in streptozotocin-diabetic rats. Pharm. Biol. 44: 35-43.

Crivos M, Martinez MR, Pochettino MI, Remorini C, Si A, Teves A (2007). Pathways as "signatures in landscape": towards an ethnography of mobility among the Mbya-Guarani (Northeastern Argentina). J. Ethnobiol. Ethnomed. 3: 1-12.

Dubowski KM (1962). An *o*-toluidine method for body fluid glucose determination. Clin. Chem. 8: 215-235.

Felig P, Bergaman M (1995). The endocrine pancreas: diabetes mellitus. In Felig P, Baxter JD, Frohman LA (eds) Endocrinolgy and Metabolism, McGraw-Hill, New York pp. 1107-1250.

Grover JK, Vats V, Rathi SS (2000). Anti-hyperglycemic effect of *Eugenia jambolana* and *Tinospora cordifolia* in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism. J. Ethnopharmacol. 73: 461-470.

Hall M (2003). Carbohydrates. In Anderson SC, Cockayne S (eds) Clinical Chemistry - concepts & applications, McGraw-Hill, New York pp. 153-178.

Hamden K, Allouch N, Damak M, Elfeki A (2009). Hypoglycemic and antioxidant effects of phenolic extracts and purified hydroxytyrosol from olive mill waste *in vitro* and in rats. Chem. Biol. Interact. 180: 421-432.

Jorge NA, Horst H, De Souza E, Pizzolatti MG, Silva FRMB (2004). Insulinomimetic effects of kaempferitrin on glycaemia and on $_{14}$C-glucose uptake in rat soleus muscle. Chem. Biol. Interact. 149: 89-96.

Lorenzi H (1998). Árvores Brasileiras: Manual de Identificação e Cultivo de Plantas Arbóreas do Brasil. Plantarum, Nova Odessa.

Mori DM, Baviera AM, Ramalho LTO, Vendramini RC, Brunetti IL, Pepato MT (2003). Temporal response pattern of biochemical analytes in experimental diabetes. Biotechnol. Appl. Bichem. 18: 183-191.

Oliveira DM, Freitas HS, Souza MFF, Arçari DP, Ribeiro MI, Carvalho PO, Bastos DHM (2008). Yerba Maté (*Ilex paraguariensis*) aqueous extract decrease intestinal SGLT1 gene expression but does not affect other biochemical parameters in alloxan-diabetic Wistar rats. J. Agric. Food. Chem. 56: 10527- 10532.

Pepato MT, Baviera AM, Vendramini RC, Brunetti IL (2004). Evaluation of toxicity after one-months treatment with *Bauhinia forficata* decoction in streptozotocin-diabetc rats. BMC Complement Altern. Med. 4: 1-7.

Pepato MT, Baviera AM, Vendramini RC, Perez MPMS, Kettelhut IC, Brunetti IL (2003). *Cissus sicyoides* (princess vine) in the long-term treatment of streptozotocin-diabetic rats. Biotechnol. Appl. Biochem. 37: 15-20.

Pepato MT, Folgado VBB, Kettelhut IC, Brunetti IL (2001). Lack of antidiabetic effect of a *Eugenia jambolana* leaf decoction on rat streptozotocin diabetes. Braz. J. Med. Biol. Res. 34: 389-395.

Pepato MT, Keller EH, Baviera AM, Kettelhut IC, Vendramini RC, Brunetti IL (2002). Anti-diabetic activity of *Bauhinia forficata* decotion on streptozotocin-diabetic rats. J. Ethnopharmacol. 81: 191-197.

Pepato MT, Mori DM, Baviera AM, Harami JB, Vendramini RC, Brunetti IL (2005). Fruit of the jambolan tree (*Eugenia jambolana* Lam.) and experimetnal diabetes. J. Ethnopharmacol. 96: 43-48.

Pepato MT, Oliveira JR, Kettelhut IC, Migliorini RH (1993). Assessment of the antidiabetic activity of *Myrcia uniflora* extracts in streptozotocin diabetic rats. Diab. Res. 22: 49-57.

Pizzolatti MG, Cunha Jr A, Szpoganicz B, Souza E, Braz-Filho R, Schripsema J (2003). Flavonoids glycosides from leaves and flowers of *Bauhinia forficata* (Leguminosae). Química Nova 26: 466-469.

Prince PS, Menon VP, Pari L (1998). Hypoglycaemic activity of *Syzigium cumini* seeds: effect on lipid peroxidation in alloxan diabetic rats. J. Ethnopharmacol. 61: 1-7.

Rauter AP, Martins A, Lopes R, Ferreira J, Serralheiro LM, Araújo ME, Borges C, Justino J, Silva FV, Goulart M, Thomas-Oates J, Rodrigues J, Edwards E, Noronha JP (2009). Bioactivity studies and chemical profile of the antidiabetic plant *Genista tenera*. J. Ethnopharmacol. 122: 384-393.

Regasini LO, Castro-Gamboa I. Silva DHS, Furlan M, Barreiro EJ, Ferreira PMP, Pessoa C, Lotufo LVC, Moraes MO, Young MCM, Bolzani VS (2009). Cytotoxic guanidine alkaloids from *Pterogyne nitens*. J. Nat. Prod. 72: 473-476.

Regasini LO, Vellosa JCR, Silva DHS, Furlan M, Oliveira OMM, Khalil NM, Brunetti IL, Young M.M, Barreiro EJ, Bolzani VS (2008). Flavonols from *Pterogyne nitens* and their evaluation as myeloperoxidase inhibotors. Phytochemistry 69:1739-1744.

Reuter G (1963). Arginin als Vorstufe von Galegin in *Galega officinalis*. Arch. Pharm. 296: 516-522.

Setacci C, Donato G, Setacci F, Chisci E (2009). Diabetic patients: epidemiology and global impact. Cardiovas. Sur. (Torino) 50: 263-273.

Souza A, Vendramini RC, Brunetti IL, Regasini LO, Bolzani VS, Silva SHS, Pepato MT (2009). Continuous treatment with alcohol extract from *Pterogyne nitens* leaves does not alter typical variables of experimental diabetes. Braz. J. Pharmacol. 19: 412-417.

Souza E, Zanatta L, Seifriz I, Creczynski-Pasa TB, Pizzolatti MG, Szpoganicz B, Silva FRMB (2004). Hypoglycemic effect and antioxidant potential of kaempferol-3,7-O-(α)-dirhamnoside from Bauhinia forficata leaves. J. Nat. Prod. 67: 829-832.

The Expert Committee on the Diagnosis and Classification of Diabetes mellitus (2003). Report of the Expert Committee on the Diagnosis and Classification of Diabetes mellitus. Diab. Care 26: S5- S20.

Viana GSB, Medeiros AC, Lacerda AMR, Leal LKAM, Vale TG, Matos JA (2004). Hypoglycemic and anti-lipemic effects of the aqueous extract from *Cissus sicyoides*. BMC Pharmacology 4: 9. About 7 p.

Viberti G, Wiseman MJ, Pinto JR, Messent J (1994). Diabetic nephropathy. In Kahn CR, Weir GC (eds) Joslin' Diabetes mellitus, Lea and Febiger - Waverly Company, Malvern pp. 691-637.