Full Length Research Paper

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

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Accepted 22 April, 2010

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 glyceria 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 hepatobiliary toxic effect.

**Key words:** Antidiabetic plant, alkaline phosphatase, biochemical markers, hepatobiliary 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 relinikopathy, 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 (Arkkiila 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...
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 kaempferitin 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 kaempferitin 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 kaempferitin (Fractions 8 - 10; 1.9 g) (Figure 1). The molecular structures of these compounds were identified by comparison with literature data, mainly 1H and 13C NMR chemical shifts (Reuter, 1963; Pizzolatti et al., 2003). The NMR spectra were collected in
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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 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, θ DW versus DG.

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 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.

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
production, may have been destroyed by the STZ treatment, abolishing their capacity for regeneration, given that the treated animals had an initial glyemia 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 glyemia 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 hepatobiliary 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 hepatobiliary alteration provoked by diabetes. However, it can also be deduced that neither the plant extract nor the glycerin had a toxic effect on the hepatobiliary system, since throughout the treatment the diabetic rats treated with water, with extract and with glycerin 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 experimental 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 hepatobiliary 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 organisms 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**

The authors are grateful to the funding agencies FAPESP, FUNDUNESP and PADC-Araquara and to Marcos A. Dangona for his technical help. We would also like to thank Dr. Maria Cláudia Marx Young, who collected the plant material, and Dr. Inês Cordeiro, who was responsible for identifying, authenticating and depositing the specimens in the Botanical Institute in São Paulo. Aline de Souza is an undergraduate in Clinical Biochemistry at the Araraquara School of Pharmaceutical Sciences (UNESP), where she receives a technical training grant (FAPESP) for a work placement in the same department.

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