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Full Length Research Paper

Evaluation of the spouted bed dried leaf extract of *Bauhinia forficata* for the treatment of experimental diabetes in rats

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Previously, we have demonstrated that treatment of experimental diabetes with a decoction of *Bauhinia forficata* leaves is beneficial. In this study, we prepared a two-fold concentrate of this extract and tested its effects on physiological, biochemical and toxicity markers in streptozotocin-diabetic rats. Dried and ground leaves were extracted with warm 70% hydroethanol and the filtrate concentrated by evaporation at 50°C. This solution was mixed with colloidal silicon dioxide (Tixosil-333®) and dried in a spouted bed (BfT). Rats were treated with water, insulin and Tixosil particles at low or high doses, alone or coated with dried BfT. Animals were periodically weighed and monitored for water and food intake; urinary volume, glucose, urea and protein; blood glucose, serum lipids, liver toxicity markers transaminase and phosphatase and masses of adipose tissue and skeletal muscle. Insulin treatment gave best rat growth and lowest values for all other markers. No other treatment affected any diabetic marker, but the enzyme activities were changed by diabetes and BfT. Thus, BfT toxicity could arise from secondary products of plant constituents or Tixosil interaction. Therefore, BfT prepared in the spouted bed as described, is unsuitable for treatment of diabetes, which implies that the method of preparation of any medicine is critical for its efficacy and toxicity.

Key words: *Bauhinia forficata*, enzymes, physiological variables, plasma glucose, serum lipids, serum toxicity markers, spouted bed drying.

INTRODUCTION

Bauhinia forficata, a tropical tree of the Leguminosae family, known in Brazil as "cow's hoof" on account of the shape of the leaves (Viana et al., 1999; Panda and Kar, 1999), is used popularly in Brazil as a leaf decoction to treat diabetes. Several methods of preparation of the leaves, studied on normal and diabetic rats as well as diabetic patients have given contradictory results regarding its hypoglycemic effects (Juliani, 1930, 1931, 1941; Costa, 1945; Caricati-Neto et al., 1985; Russo et al., 1990; Viana et al., 1999; Panda and Kar, 1999; Volpato et al., 1999; Silva and Cechinel-Filho, 2002; Lino et al., 2004).

In earlier work in our laboratory, diabetic rats were treated chronically for about 1 month with a decoction of 150 g/L of *B. forficata* leaves in water, ingested at a daily

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Abbreviations: DW, Water; **DI,** insulin; **DT<,** Tixosil at lower concentration (0.0469 g/mL); **DT> ,** Tixosil at higher concentration (0.0938 g/mL); **DBfT<,** Bf T at lower concentration (0.125 g/mL); **DBfT>,** BfT at higher concentration (0.25 g/mL); **STZ,** streptozotocin; **AST,** aspartate aminotransferase; **ALT,** alanine aminotransferase; **ALP,** alkaline phosphatise; D, diabetic; s.c., subcutaneously; **EDL,** *extensor digitorum longus* (EDL); **HDL,** high-density lipoprotein.

rate of 36 mL/kg body weight (bw), in place of normal drinking water. The treated rats exhibited significant falls in the levels of blood glucose and urinary glucose and urea, without any change in liver glycogen. However, these beneficial effects on diabetes were not as significant as those seen in the rats treated with insulin (Pepato et al., 2002).

Considering the acute toxicity of the *B. forficata*, it is known that the crude extract injected intraperitoneally caused the death of 50% of the animals at 2.85 g/kg b.w. whereas 0.5 to 5.0 g/kg b.w. of the same extract given orally had no toxic effect (Silva and Cechinel-Filho, 2002). The toxic side effects of *B. forficata* extract were also assessed in our laboratory when it was administered to normal and diabetic rats by mouth for 33 days in the form of a decoction prepared from 150 g of fresh leaves per liter of water. Toxicity was determined by assaying tissue toxicity markers such as pancreatic amylase, muscle creatine kinase, lactate dehydrogenase from muscle and liver, bilirubin from bile duct and liver and angiotensin-converting enzyme from the kidney and renal microcirculation. None of these enzymatic toxicity markers was altered by the treatment with decoction (Pepato et al., 2004).

In view of the beneficial effects on carbohydrate and protein metabolism observed when diabetic rats were treated continuously with extract of *B. forficata*, it is reasonable to argue that the hypoglycemic effects of the leaf extract could be enhanced by treating the rats with a more concentrated solution. One technique that enables a higher concentrated solution of water-soluble bioactive compounds is the spouted bed drying, which has already been employed successfully to dry biological products and to produce dried extracts of medicinal plants, including *B. forficata* extracts (Cordeiro and Oliveira, 2005; Souza and Oliveira, 2005). However, the processing conditions used in the production of the dried extract can affect the product properties, causing changes in the composition of bioactive compounds originally present in the vegetable material. Thus, evaluation of the presservation of the biological activity of the spouted bed dried extract is of fundamental importance. Therefore, the aim in this study is to investigate the effects of the spouted bed dried extract of *B. forficata* on the physiological and biochemical variables usually modified in diabetic animals and assaying the toxic effects of the concentrated extract on several liver toxicity markers.

MATERIALS AND METHODS

Plant material

Leaves were collected from a *B. forficata* tree between April and May in the Medicinal Plants Garden of the School of Pharmaceutical Sciences, UNESP at Araraquara, São Paulo State, Brazil. An exsiccate from this tree was authenticated and deposited in the Herbarium of the Department of Industrial Pharmacy, Federal University of Santa Maria, Rio Grande do Sul, Brazil (Voucher number 119) by Dr. Gilberto Dolejal Zanetti.

Preparation of extract

The leaves of *B. forficata* were dried and finely milled in a knife mill to a mean particle size of 0.3 mm. The comminuted leaves were extracted, as previously described by Souza et al. (2007) in 70 % w/w aqueous alcohol in a plant: solvent proportion of 1:5 w/w, in a water-jacketed reactor maintained at 50°C with constant stirring at 200 rpm for 1 h. The mixture was then vacuum-filtered and the filtrate was concentrated in a rotary evaporator (under 600 mm Hg vacuum at 50°C) to about 10 wt% solid contents. Colloidal silicon dioxide (Tixosil-333®, Rhodia, Brazil) was added to the concentrated extract before drying to improve the performance of the drying bed (6% w/w of extract weight or 60% w/w of extract solids content). The drying operation was performed in a conical-cylindrical spouted bed with a cylindrical column (150 mm of internal diameter and height 400 mm) connected to a conical base with internal angle of 40° and spouting gas orifice of 33 mm diameter. All parts were made of stainless steel. Teflon® beads of concave-cylindrical shape (mean diameter 5.45 mm, shape factor 0.96, specific surface 5.27 cm^2/g and density 2.16 g/cm^3) were used as inert material and were previously loaded to the dryer, in order to maintain the static bed height at 14 cm. The extract mixture was sprayed at the top of the column from a double fluid atomizer with internal mixing (0.8 mm hole) using a peristaltic pump and an air compressor. The feed rate of concentrated extract was fixed at 12 g/min, and the spouting gas (air at 150°C) flowed upwards at 1.8 times the minimum rate (≅ 0.0437 kg/s). Under this operating condition (selected on preliminary assays), the dryer presented a good performance, showing high production rate and small product accumulation, preventing the bed from collapsing. The concentrated extract composition dried on the surface of the inert bodies yielded a fine powder.

Animals and their treatment

Seventy male Wistar rats were fed a normal laboratory chow diet containing (wt/wt) 16% protein, 66% carbohydrate and 8% fat and were housed under a 12:12 h light and dark cycle at 22 - 25°C. The experimental protocols for the treatment and care of rats was carried out following the rules of the Brazilian National Ethics commissions (COBEA, 1991) and were approved by the Research Ethics Committee of the Araraquara School of Pharmaceutical Sciences (UNESP University) - CEP/FCF/Car – under protocol no. 21/2006.

Induction of diabetes

The animals (n = 70), weighing 158 ± 4 g were allowed to adapt to metabolic cages for 3 days, after which they were fasted for 14 - 16 h. Streptozotocin (STZ) was dissolved in 0.01 M citrate buffer (pH 4.5) and injected into the jugular vein of the fasted rats at 50 mg/kg b.w. All rats were then returned to their cages and given free access to food.

Preparation of suspensions and solutions

Fine suspension of plant extract at lower concentration

1.25 g of extract powder from the spouted bed was mixed with 10

mL water and 1 mL of the suspension was administered by gavage per rat, at 8 and 18 h each day. This dose was equivalent to the mean dose given in the form of a decoction in the previous study (Pepato et al., 2002).

Fine suspension of plant extract at higher concentration

2.5 g of extract powder was suspended in 10 mL water and the suspension used to treat rats as described above.

Low concentration Tixosil colloidal suspension

0.4688 g Tixosil was mixed with 10 mL water at the time of treatment and 1 mL per rat was administered by gavage at 8 and 18 h.

High concentration Tixosil

0.9376 g in 10 mL water was used to treat rats as described above.

Insulin suspension

2.5 U of Humulin NPH U-100 insulin (Lilly, Indianapolis, USA) was injected subcutaneously (s.c.) in 0.3 mL/rat at 8 and 18 h in the group treated with insulin (positive controls).

Treatment and sampling

Four days after the STZ treatment, blood samples were collected from the tip of the tail in Eppendorf tubes, with and without liquaemin sodium (Roche, Indianapolis, IN, USA). The samples were used to assays the plasma glucose and the serum enzyme markers of hepatic toxicity, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). From the original 70 diabetic rats, 60 were chosen to form 6 treatment groups of 10 animals, the groups being matched as closely as possible in plasma glucose profile. The 60 rats were all diabetic (D) and the 6 groups were treated twice a day as follows: group DW was given 1 mL water by gavage; DBfT< was given 1 mL *B. forficata* suspension at the lower concentration with Tixosil, by gavage ; DBfT> was given 1 mL *B. forficata* suspension at the higher concentration, with Tixosil by gavage; DT< received 1 mL Tixosil at the lower concentration by gavage; DT> received 1 mL Tixosil at the higher concentration, by gavage and DI received 2.5 U insulin, injected subcutaneously (s.c.); n = 10 for all treatment groups; the 10 rats that could not be matched closely were not used. All treatments started on the 4th day after the STZ injection and lasted for 35 days. During this period, body weight, daily intake of food and liquid, excretion of urine, urinary glucose, urea and proteins were measured every 7 days, while plasma glucose and ALP were measured every 15 days. Physiological and biochemical variables were determined on day 39 after the STZ injection and day 35 after the start of treatments by gavage; then the rats were sacrificed 2 h after treatments (8:00 – 10:00 am) by decapitation. Samples of the free-running blood were collected and the serum AST, ALT and ALP activities, glucose, cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides were determined. Serum, plasma and/or urine were stored at -20°C whenever they could not be analyzed on the day of collection. The epididymal fat pad and retroperitoneal adipose tissue lying over the psoas and the *soleus* and *extensor digitorum longus* (EDL) muscles were removed and weighed.

Chemical analysis

Plasma glucose, serum cholesterol, HDL-cholesterol and triglycerides and the transaminases (AST and ALT) were determined with a Labmax 240 automatic clinical analyzer (Hirose eletronic system Co Ltd, Nasu-Gun, Tochigi, Japan) with reagents kits from Labtest Diagnostics (Lagoa Santa, MG, Brazil). Urinary glucose was measured by the *o*-toluidine method (Dubowski, 1962), urea by the urease method with a Labtest reagent kit (Bolleter et al., 1961; Bergemeyer, 1985) and urinary protein by a modified Bradford method (Bradford, 1976), with a diagnostic kit from Doles Reagents (Goiânia, GO, Brazil). ALP was assayed by colorimetric methods (IFCC, 1983) with reagents from the Labtest kit. Readings were taken with a Power Wave XS2-Biotek spectrophotometer (Biotek Instruments, USA). Unless otherwise indicated, all reagents were purchased from Merck or Sigma and were of analytical grade.

Statistical analysis

Data are expressed as mean ± SEM. They were analyzed by one-way and two-way analysis of variance (ANOVA) followed by the Fischer-LSD test with the computer package SigmaStat 2.03. Significance was accepted when $p < 0.05$.

RESULTS AND DISCUSSION

The insulin-treated group of rats (DI) showed significant improvements. Their body weight was greater while their intake of food and water and rate of urination were reduced; same is applicable to the other biochemical marker variables, relative to group DW (Table 1). Thus, in view of the reproduction of the symptoms caused by the absence of insulin, the STZ-induced diabetic model was perfectly suitable. This assertion is reinforced by the observed reversibility of all these symptoms by the injection of exogenous insulin (Mori et al., 2003; Sacks, 2006). Neither the spouted bed drying aid, Tixosil, nor the leaf extract-coated Tixosil product obtained by spouted bed drying at either of the two concentrations tested showed any beneficial effect on the physiological changes classically associated with diabetes. Thus, the physiological variables measured in all these groups had values significantly higher than in DI (excepting body weight, which was lower than in DI) and not significantly different from those in DW. Curiously, in both groups treated with the spouted-bed dried *B. forficata* extract, DBfT< and DBfT>, the diabetic symptoms were worse than in DT> (this observation is discussed later). The latter group was statistically different from DW in urine volume and from DT< in water intake. The group treated with the higher concentration of plant extract (DBfT>) showed lower body weight than group DW and a higher urine volume than DT<. These results refer to the intergroup comparisons of average values during the experiment. The intergroup and intra-group temporal comparisons (between values at single times) gave the same general results.

Regarding the biochemical assays, group DI again showed the expected behavior, with improved values of

Table 1. Mean metabolic variables of diabetic rats during 35-day treatment with *Bauhinia forficata* leaf extract dried in a spouted bed (BfT).

Mean metabolic variable	DW	DI	DT<	DT>	DBfT<	DBfT>
Body weight (bw) (g)	212.77 ± 6.04	249.98 ± 6.04[a]	202.72 ± 6.54[b]	219.88 ± 6.79[c]	201.43 ± 6.46[f,g]	194.03 ± 7.16[h,l,k]
Urine volume (mL/d. per 100g bw)	52.84 ± 3.60	20.20 ± 3.60[a]	50.88 ± 3.90[b]	41.40 ± 4.05[c,d]	58.01 ± 3.86[f,g]	62.55 ± 4.27[h,j,k]
Water intake (mL/d. per 100g bw)	65.21 ± 4.58	34.79 ± 4.58[a]	74.74 ± 4.96[b]	55.90 ± 5.15[c.e]	71.09 ± 4.90[f,g]	75.84 ± 5.44[h,k]
Food intake (g/d. per 100g bw)	15.78 ± 0.58	11.92 ± 0.58[a]	15.68 ± 0.62[b]	14.28 ± 0.65[c]	16.80 ± 0.68[f,g]	16.80 ± 0.68[h,k]
Blood glucose (mg/dL)	514 ± 28	231 ± 28[a]	551 ± 31[b]	535 ± 31[c]	589 ± 30[f]	619 ± 33[h,i]
Glucosuria (g/d. per 100g bw)	3.99 ± 0.28	1.68 ± 0.28[a]	3.95 ± 0.30[b]	2.97 ± 0.31[c,d,e]	4.40 ± 0.30[f,g]	4.35 ± 0.33[h,k]
Urinary urea (mg/d. per 100g bw)	0.61 ± 0.03	0.32 ± 0.03[a]	0.55 ± 0.04[b]	0.52 ± 0.04[c]	0.64 ± 0.04[f,g]	0.65 ± 0.04[h.k]
Proteinuria (mg/d. per 100g bw)	10.83 ± 0.79	6.53 ± 0.80[a]	10.31 ± 0.85	8.52 ± 0.90	11.02 ± 0.85[f,g]	12.25 ± 0.95[h.k]

Diabetic rats treated with: DW: Water; DI: insulin; DT<: Tixosil at lower concentration (0.0469 g/mL); DT>: Tixosil at higher concentration (0.0938 g/mL); DBfT<: Bf T at lower concentration (0.125 g/mL); DBfT> : BfT at higher concentration (0.25 g/mL). Results expressed as mean ± standard error of 5 measurements performed during the period of treatment (35 d) (n=50), except blood glucose which is mean of 3 measurements (35 d) (n=30). Intergroup comparisons by two-way ANOVA plus Fischer-LSD test ($p<0.05$): a: DI vs. DW; b: DT< vs. DI; c: DT> vs. DI; d: DT> vs. DW; e: DT> vs. DT<; f: DBfT< vs. DI; g: DBfT< vs. DT>; h: DBfT> vs. DI; i: DBfT> vs. DW; j: DBfT> vs. DT<; k: DBfT> vs. DT>.

all the measured variables, relative to DW, which in turn reproduced the diabetic condition very well. The reduced excretion of protein in the DI is also a sign of improvement of the diabetes, since it indicates recovery of kidney function which can be seriously damaged in this syndrome (Mogensen, 1995; Babu and Srinivasan, 1999).

All the treated rat groups other than DI behaved like the water-treated group (DW) in nearly all the biochemical assays, showing significantly higher values than rats in DI. Except in terms of plasma glucose, the rats treated with *B. forficata* extract (DBfT< and DBfT>) gave results significantly different from DT>, while DBfT> had a higher plasma glucose level than DW. Meanwhile, urine glucose was apparently better in DT> than in DA or DT<. As in the case of the physiological variables, the intergroup and intragroup single-time results confirmed this analysis of biochemical results.

To articulate the results, it seems that the apparent effects on diabetes of the drying aid, Tixosil, can be interpreted as random effects seen at a few data points and thus without physiological meaning. In other words, Tixosil had no consistent effect on experimental diabetes, either at the low or the high concentration. Furthermore, it may be concluded that the treatment with spouted-bed processed *B. forficata* extract also had no beneficial effect on the biochemical markers for diabetes assessed here, either at the lower or higher dose.

In Table 2, it can be seen that serum levels of cholesterol and HDL-cholesterol tended to be slightly lower in group DI, but the statistical treatment of the data did not indicate any difference in these values among any of the groups. On the other hand, serum triglycerides, was significantly lower in group DI than in DW. In addition, group DBfT< showed significantly higher triglycerides than DI, DT< and DT>.

In sum, it is apparent that the pharmaceutical form of the *B. forficata* leaf extract dried by the spouted bed system had no effect on the serum lipids analyzed here and even the insulin treatment was not effective in improving cholesterol or HDL-cholesterol levels, although it did improve the serum content of triglycerides. In our laboratory, earlier experiments based on the same model of experimental diabetes gave similar results, even though exogenous insulin is normally expected to raise the HDL-cholesterol fraction and diminish the total cholesterol content in the serum of diabetic animals (Mesotten et al., 2004).

The epididymal fat pads (Table 2) were lighter in groups DT>, DBfT< and DBfT> than in DI while groups DI had a mean pad weight more than double that of the diabetic control (DW). Similar results were seen for the retroperitoneal adipose tissue (Table 2), which was significantly lighter in all the groups cited above, as well as in DT<. These results, together with those for the serum triglycerides, indicated once again that this model of experi-mental diabetes give a satisfactory response in the positive control group (insulin-treated). The extract-treated groups did not show any increase in fat deposits in the dissected tissues, corroborating the results of serum lipid assays that pointed to the lack of improvement of

Table 2. Serum lipid levels and masses of adipose tissue and muscles on day 35 of treatment with *Bauhinia forficata* leaf extract dried in a spouted bed (BfT).

Lipid	DW	DI	DT<	DT>	DBfT<	DBfT>
Serum lipids (mg/dL)						
Cholesterol	66 ± 5	56 ± 3	81 ± 20	62 ± 4	77 ± 9	74 ± 11
HDL-Cholesterol	49 ± 2	43 ± 2	51 ± 3	47 ± 2	49 ± 3	50 ± 2
Triglycerides	331 ± 75	126 ± 20[a]	320 ± 119	500 ± 123	259 ± 81[d,f,g]	207 ± 61
Tissue masses (g/100g bw)						
Epididymal	0.8703 ± 0.2906	2.0663 ± 0.2189[a]	1.2172 ± 0.5550	1.1252 ± 0.1535[c]	1.1129 ± 0.3029[d]	0.6960 ± 0.1739[e]
Retroperitoneal	1.3558 ± 0.2283	2.2281 ± 0.3064[a]	1.1541 ± 0.7353[b]	0.6469 ± 0.2348[c]	0.7362 ± 0.3137[d]	0.5355 ± 0.2758[e]
Soleus	0.1196 ± 0.0092	0.1364 ± 0.0055	0.1049 ± 0.0174	0.1263 ± 0.0071	0.1070 ± 0.014	0.0800 ± 0.0204[e,h,i]
EDL	0.1093 ± 0.0128	0.1430 ± 0.0061[a]	0.0974 ± 0.0191[b]	0.1261 ± 0.0107	0.0958 ± 0.0139[d]	0.0603 ± 0.0187[e, h]

Diabetic rats treated with: DW: Water; DI: insulin; DT<: Tixosil at lower concentration (0.0469 g/mL); DT>: Tixosil at higher concentration (0.0938 g/mL); DBfT<: BfT at lower concentration (0.125 g/mL); DBfT>: BfT at higher concentration (0.25 g/mL). Results expressed as mean ± standard error (n=10). Intergroup comparisons by two-way ANOVA plus Fischer-LSD test ($p<0.05$) for the same period: a: DI vs. DW; b: DT< vs. DI; c: DT> vs. DI; d: DBfT< vs. DI; e: DBfT> vs. DI; f: DBfT< vs. DT<; g : DBfT< vs. DT>; h : DBfT> vs. DW; i : DBfT> vs. DT>.

lipid metabolism by either dose of the *B. forficata* extract.

Although the *soleus* muscle showed a slight tendency to be heavier (on average) in the insulin-treated group (Table 2), the mean weight in DI was not in fact significantly different from that in DW. DBfT>, however, had a mean value significantly lower than that of DT>, DW and DI. In contrast, the mean weight of EDL muscle in DI was significantly higher than that in DW and in all other groups except DT>. It was noteworthy that group DBfT> suffered a significant loss of EDL muscle mass, relative to DW. The fact that the effects of diabetes are more easily detected in EDL than in the *soleus* muscle, as is the effect of treatment with insulin in reversing the loss of EDL muscle mass, can be explained by previously published results (Pepato et al., 1996). In the diabetic state, the EDL muscle exhibits greater proteolytic activity than the *soleus* arising probably from the action of catabolic glucocorticoid hormones whose levels are raised in response to insulin deficiency. Such protein digestion appears to affect the EDL muscle more strongly because it is less physiologically active and contractile than the *soleus*. Furthermore, insulin exerts an additive effect on tissue engaged in contractile activity and on glucose transport that is greater in the *soleus* than in the EDL, since the oxidative muscle fibers of the former possess a larger number of GLUT 4 carriers than the glycolytic fibers of the latter (Henriksen, 1990).

The form of *B. forficata* extract prepared with the spouted bed dryer showed a complete lack of action on protein metabolism, both in the above results regarding the EDL muscle and in the urinary urea levels observed during the treatment.

Considering the results obtained here for all the variables used as signs of diabetes, we can report that the treatment of the rats with this leaf extract, dried in the spouted bed, was inefficacious against diabetes at either concentration (DBfT< or DBfT>). Nevertheless, other studies have proved that a decoction of *B. forficata* leaves, at the same low concentration (DBT<), has the capacity to reduce the level of glucose in the serum and urine, and that of urinary urea, though no effect was observed on the other variables analyzed (Pepato et al., 2002).

Several hypotheses could be raised to try and explain these contradictory results, including possible inadequacies in the model of diabetes. However, control tests in both the present and the previous study (Pepato et al., 2002) demonstrated that this model was reliable and appropriate.

The quantity of active principles in plants can be influenced by their genetic constitution, light, temperature, soil type, water and other factors (Lopez, 2006). However, in both the previous (decoction) and present (spouted bed) preparations of the extract, care was taken to collect the *B. forficata* leaves at the same point in the year (month of May) and from the same tree.

The severity of diabetes mellitus can also be a determining factor in the action of a herbal drug. In the present case, this may be disregarded as the STZ induced severe diabetes (Lercoi et al., 2003) both in the present experiment with the spouted bed and in the experiment in which the

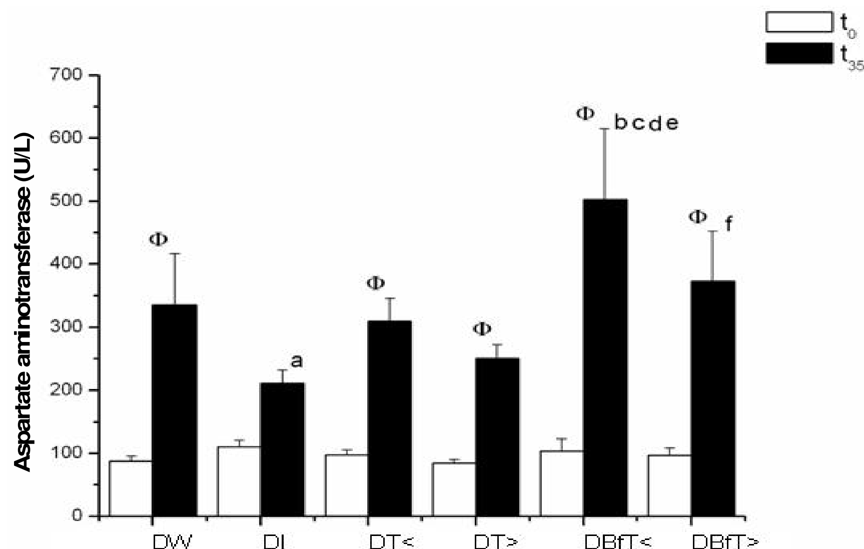

Figure 1. Serum activity of aspartate aminotransferase (U/L) in diabetic rats before the start (t_0) and after 35 days (t_{35}) of treatment with *Bauhinia forficata* leaf extract dried in a spouted bed (BfT). Values represent mean ± SE. Diabetic rats treated with: DW: Water; DI: insulin; DT<: Tixosil at lower concentration(0.0469 g/mL); DT >:Tixosil at higher concentration (0.0938 g/mL); DBfT < BfT at lower concentration (0.125 g/mL); DBfT>: BfT at higher concentration(0.25 g/mL). Intergroup comparisons for the same period by two-way ANOVA plus Fischer-LSD test ($p < 0.05$): a: DI vs. DW; b: DBfT < vs. DI; c: DBfT < vs. DW; d: DBfT< vs. DT <; e: DBfT < vs. DT >; f: DBfT > vs. DI.Φ: Intragroup comparisons (two-way ANOVA plus Fischer-LSD test, $p < 0.05$) between t_{35} and t_0 .

decoction was administered (Pepato et al., 2002). A crucial factor affecting the success of plant-based therapy is the time of treatment. This was in fact very similar in the two experiments, treatment being started 5 days after STZ and concluded 31 days later in the trial with leaf decoction, against 4 days after STZ and 35 days later in the present study.

The high concentration of the extract attained in the spouted bed process could also be a cause of the loss of beneficial effect of the *B. forficata* preparation as cases are known where such, increases the content of a herbal remedy instead of increasing its power leading to a loss of effect or even promote adverse side-effects (Prince et al.,1998). To investigate this possibility, we carried out the treatment with the spouted-bed *B. forficata* preparation, not only at the high concentration (group DBfT>) but also at a concentration in which the plant material was equal to that in the decoction used in the earlier study (DBfT<). These findings demonstrated that the high dose of the new form was not responsible for its lack of effect on the disease.

Another possible explanation of the failure of this treatment is some harmful effect of the drying aid, Tixosil. However, according to the results of the tests with Tixosil alone (DT> and DT<), this substance had neither deleterious nor beneficial effects on the diabetes. Moreover, colloidal silica is widely used as an excipient in pharmaceutical formulae and presents no risk to health when ingested in small quantities. There is still the possibility that some interaction between the silica and the active compounds from the plant might result in their conversion to other, inactive compounds.

Another hypothesis that could explain these results is that the spouted bed drying process may have altered the plant constituents; actually, analysis of the flavonoids present showed that about 20% were degraded (data not shown). Previous work on herbal drugs and on this drying method (Heigl and Franz, 2003; Souza and Oliveira, 2005) suggest that the relatively high temperature of the spouting air (150°C) and the presence of colloidal silica might hydrolyze or modify flavonoids or other compounds in the extract, leading to a loss of hypoglycemic activity. This suggestion is reinforced by the loss of effect of the original decoction dried in the spouted bed and used at the original (lower) concentration (group DBfT<).

There are numerous divergences among published data on the antidiabetic activity of *B. forficata*. One factor that needs to be taken into account is the variety of solvents and diverse methods of preparation of the extract (e.g. in water, aqueous alcohol, ethanol, hydrophobic solvents). Such discrepancies are also found in respect to other plants employed in popular medicine for the treatment of diabetes, such as the jambolan tree (*Eugenia jambolana*) in which Grover et al. (2000) detected hypoglycemic activity, while same was not observed in a study carried out in our laboratory (Pepato et al., 2001).

Regarding hepatic toxicity, Figure 1 shows the results for the marker enzyme AST, where it is seen that just before

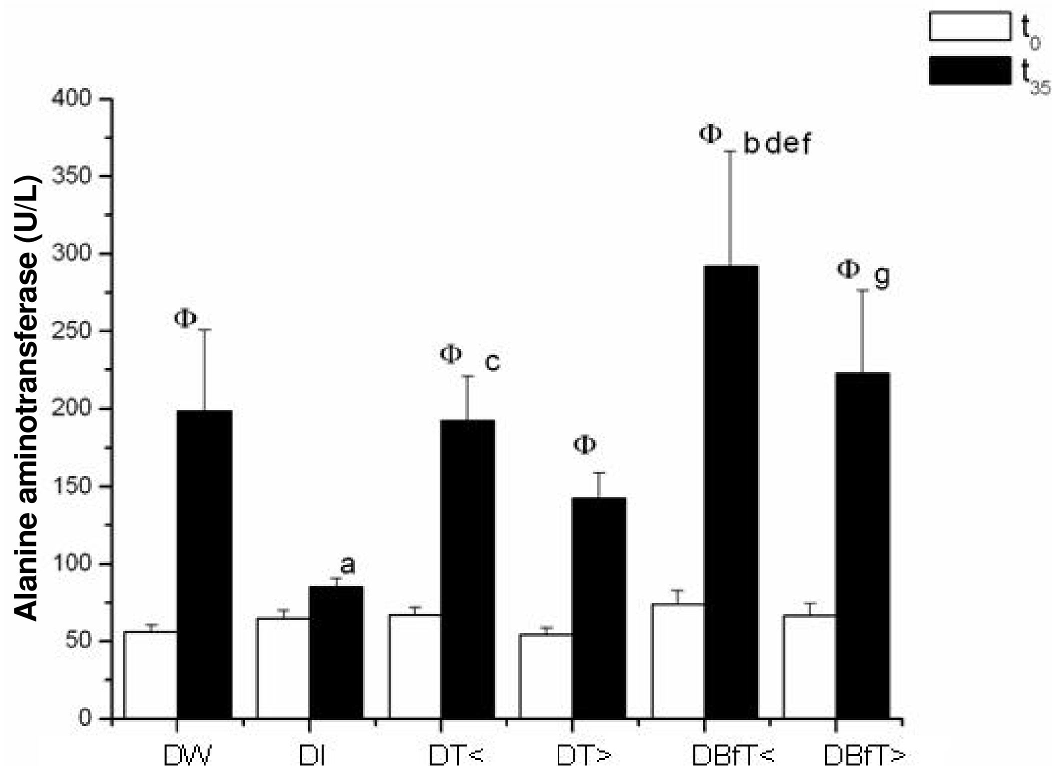

Figure 2. Serum activity of alanine aminotransferase (U/L) in diabetic rats before the start (t_0) and after 35 days (t_{35}) of treatment with *Bauhinia forficata* leaf extract dried in a spouted bed (BfT). Values represent mean ± SE. Diabetic rats treated with: DW: Water; DI: insulin; DT<: Tixosil at lower concentration(0.0469 g/mL); DT >:Tixosil at higher concentration (0.0938 g/mL); DBfT <: BfT at lower concentration (0.125 g/mL); DBfT>: BfT at higher concentration(0.25 g/mL). Intergroup comparisons for the same period by two-way ANOVA plus Fischer-LSD test ($p < 0.05$): a: DI vs. DW; b: DBfT< vs. DI; c: DT< vs. DI; d: DBfT< vs. DW; e: DBfT< vs. DT< ; f : DBfT< vs. DT> ; g : DBfT> vs. DI. Φ: Intragroup comparisons (two-way ANOVA plus Fischer-LSD test, $p < 0.05$) between t_{35} and t_0.

the treatment with extract (t_0), all groups show similar activities. After 35 days of treatment, there was a statistical difference between group DI and the groups DW, DBfT< , DBfT>, DI showing the least activity of all the groups. Group DBfT< exhibited the highest activity of all and this was significantly different from DW, DT< and DT>. It is also noteworthy that in the intragroup comparisons, the activity at the end of treatment (day 35) was significantly higher than at t_0 in all groups, except DI. Very similar results were obtained for the ALT activity (Figure 2), except that the difference at 35 days between DI and DT< was now significant.

The results for the mean serum ALP activity during the period of treatment are shown in Table 3. Note that the value for DI is significantly lower than those obtained for all other groups. Activities in groups DBfT< and DBfT> were also appreciably higher than in DT<, while that in DBfT< was significantly higher than that found in DW.

It is thus clear that the model of experimental diabetes used in this study was capable of reproducing the hepatic toxicity characteristic of diabetes mellitus, revealed by the increased serum activity of the enzymes AST, ALT and ALP in the diabetic rats treated with water (DW) (Mori et al., 2003). It was also demonstrated that treatment with insulin effectively reduced the liver damage; this was evident both in the intragroup comparisons, where insulin was seen to abolish the statistical difference between the activity at t_0, and on day 35, was present in all groups other than DI (data not shown for ALP), and in the intergroup comparisons between groups DI and DW, which show significant reductions in the serum activities of the marker enzymes by insulin on day 35 (data not show for ALP). This effect has been known since on earlier study of alloxan-diabetic rats (Awadallah and El-Dessoukey, 1977) and has been observed in diabetic patients (Arkkila et al., 2001).

The treatment with spouted-bed dried extract of *B. forficata* was ineffective, at either concentration, in reversing the liver damage caused by induced diabetes since the activities of the three marker enzymes were significantly higher on day 35 of the treatment than just before it started (t_0), as well as being higher on day 35 in both extract-treated groups than in group DI (data not shown for ALP). Given that this metabolic syndrome is itself

Table 3. Mean serum activity of alkaline phosphatase (ALP) during 35-day treatment with *Bauhinia forficata* leaf extract dried in a spouted bed (BfT).

Enzyme	DW	DI	DT<	DT>	DBfT<	DBfT>
ALP (U/L)	149.02 ± 6.22	115.46 ± 6.21[a]	143.43 ± 6.63[b]	154.40 ± 6.88[c]	172.16 ± 6.58[d,e,f]	167.03 ± 7.19[g,h]

Diabetic rats treated with: DW: water; DI: insulin; DT<: Tixosil at lower concentration (0.0469 g/mL); DT>: Tixosil at higher concentration (0.0938 g/mL); DBfT<: BfT at lower concentration (0.125 g/mL); DBfT>: BfT at higher concentration (0.25 g/mL). Results expressed as mean ± standard error of 3 measurements performed during the period of treatment (35 d) (n=30). Intergroup comparisons by two-way ANOVA plus Fischer-LSD test (p<0.05) for the same period: a: DI vs. DW; b: DT< vs. DI; c: DT> vs. DI; d: DBfT< vs. DI; e: DBfT< vs. DW; f: DBfT< vs. DT<; g: DBfT> vs. DI; h: DBfT> vs. DT<.

capable of raising the serum activities of the toxicity marker enzymes, a rigorous analysis is needed when these activities are used as signs of hepatic toxicity caused by pharmaceutical preparations used to treat diabetes. In the present study, this analysis indicated that, in AST and ALT activities (Figures 1 and 2), group DBfT< was significantly higher than the rats treated with water (DW) or with Tixosil (DT< and DT>), while in ALP activity (Table 3), it was higher than DW or DT<, sug-gesting that the extract might cause toxicity. Although statistical differences were not evident between DBfT> and DW, for enzymes AST and ALT, it may be mentioned that no such differences were detected between DBfT> and DBfT<, impl-ying a possible indication of toxicity at the higher dose of *B. forficata*.

These results suggest that the pharmaceutical form of *B. forficata* prepared by spouted bed drying shows some hepatic toxicity. This contrasts with the toxicity analysis carried out during chronic treatment with the decoction of the same plant, in while the markers amylase (pancreas), creatine kinase (muscle), lactate dehydrogenase (muscle and liver), bilirubin (liver and gall-bladder) and angiotensin-converting enzyme (kidneys and renal microcirculation) did not reveal any toxicity (Pepato et al., 2004).

It is clear that the colloidal silicon dioxide (Tixosil) used in the spouted bed cannot, in itself, be the cause of the toxicity, since both groups treated with Tixosil alone showed similar enzyme activities to the water-treated control (Figures 1 and 2; Table 3). However, there is the remote possibility that the high temperature in the spouted bed modified the silica, leading to the formation of toxic derivatives, or that the silica interacted with the extract, forming new compounds that, in turn, provoked toxicity.

Finally, we must not exclude the possibility that, by testing other conditions of preparation of the leaf extract with the spouted bed, for example by varying the method of extraction (type of solvent, plant:solvent mass ratio, time and temperature) and of drying (temperature of spouting gas, type and parameters of solid particles, etc), better results could be obtained.

Conclusion

Daily oral treatment of diabetic rats over 35 days with hydroalcoholic leaf extract of *B. forficata*, dried in a spouted bed under the conditions des-cribed did not improve the physiological or metabolic variables that are typically altered in the diabetic state. Moreover, the treatment produced signs of hepatic toxicity, possibly due to secondary products of constituents of *B. forficata* and/or Tixosil, generated by interactions or the high bed temperature. For these reasons, this method of preparing the extract which involves drying in the spouted bed cannot be recommended, while the decoction prepared from *B. forficata* may lead to a usable form.

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