Anti-diabetic activity of *Bauhinia forficata* decoction in streptozotocin-diabetic rats

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**Abstract**

The effects of using *Bauhinia forficata* leaf decoction (150 g leaf/l water; 35.2 ± 7.8 ml/100 g body weight mean daily dose) as a drinking-water substitute for about 1 month on streptozotocin-diabetes (STZ-diabetes) in male Wistar rats were investigated. The physico-metabolic parameters measured were: body weight, food and liquid intake, urinary volume, hepatic glycogen, serum triglycerides and cholesterol, plasma glucose, urinary glucose and urea, and the weight of epididymal and retroperitoneal adipose tissue and soleus and extensor digitorum longus muscles. The STZ-diabetic rats treated with decoction showed a significant reduction in serum and urinary glucose and urinary urea as compared to the STZ-diabetic control, no difference being seen between decoction-treated and -untreated non-diabetic rats. The other physico-metabolic factors showed no changes in treated STZ-diabetic rats. The improvement in carbohydrate metabolism seen in the rats treated with *Bauhinia forficata* decoction does not appear to be linked to the inhibition of glycogenolysis or the stimulation of glycogenesis nor does it appear to act in a way similar to insulin or the sulfonylureas, although it may act by the inhibition of neoglycogenesis in a manner similar to that of the biguanides.

**Keywords:** Anti-diabetic activity; *Bauhinia forficata*; Anti-hyperglycemic effect; Streptozotocin-diabetic rats

1. **Introduction**

The genus *Bauhinia* belongs to the family Caesalpiniaceae (formally Leguminosae) (Viana et al., 1999; Panda and Kar, 1999) and has some species that are used as traditional folk medicines in the treatment of diabetes. Studies carried out with *Bauhinia manca*, *Bauhinia divaricata*, *Bauhinia purpurea* and *Bauhinia variegata* have demonstrated hypoglycemic activity in laboratory animals (Vasconcelos, 2000; Ivorra et al., 1989).

*Bauhinia forficata* is the *Bauhinia* species most used as an anti-diabetic herbal remedy in Brazil, where it is known as *Pata de Vaca* (cows hoof). This species is an arboreal plant of Asiatic origin which adapts well to the Brazilian climate, reaching 12 m in height (Miyake et al., 1986; Donato, 1995).

The first reports of *Bauhinia forficata* hypoglycemic activity in diabetic patients were made by Juliani (1929) and Juliani (1931). The fact that few reports of the effects of this plant occur in the literature, and that those which do give contradictory results (Caricati-Neto et al., 1985; Russo et al., 1990) or unsuccessful results (Costa, 1945), makes it important that more detailed investigations using good experimental models are carried out on the effects of oral treatment with this plant. In this paper we investigate the effects of oral treatment with a decoction of *Bauhinia forficata* on characteristic metabolic parameters of streptozotocin-diabetic and non-diabetic rats.
2. Materials and methods

2.1. Decoction preparation

Material from a Bauhinia forficata tree in the Medicinal Plants Garden of the School of Pharmacy, Araraquara, São Paulo State, Brazil was identified, authenticated and deposited in the Herbarium of the Department of Industrial Pharmacy, Federal University of Santa Maria, Rio Grande do Sul, Brazil as accession No. 119, by Dr Gilberto Dolejal Zanetti. Leaves were collected from this tree between April and May (the end of Autumn in the Southern Hemisphere) and a decoction prepared by the method normally used in the collection of fresh leaves. The leaves were boiled 150 g of fresh leaves in 1 l of water for 5 min, allowing the decoction to stand for 30 min and filtering it through a simple paper filter. The final yield was 87% by volume, the decoction being prepared every 2 or 3 days and kept in brown-glass bottles at 4 °C.

2.2. Animals and their treatment

All 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:dark cycle at 22–25 °C. The experimental protocols and the treatment and care of the rats had the prior approval of the ethics committee.

A group of male Wistar rats were adapted to metabolic cages for 2 or 3 days. The animals were then anesthetized with ethyl ether and 40 mg/kg body weight streptozotocin (STZ) dissolved in 0.01 M citrate buffer, pH 4.5, was injected into the jugular vein. The rats were fasted for 14–16 h and their mean weight was 160 ± 2 g. Another group of rats weighing 144 ± 2 g were treated in the same way except that they received STZ at the dose 50 mg/kg body weight because young rats proved to be more resistant than older ones to the diabetogenic action of STZ (Pepato et al., 1996). All rats were returned to their metabolic cages where they had free access to food and water.

2.2.1. Decoction administration

2.2.1.1. Diabetic group. Body weight, serum glucose levels, urinary glucose excretion and food intake were measured 3 days after STZ injection (40 mg/kg) and used as parameters to obtain matching pairs of rats with diabetes of a similar level of severity. One rat of each pair was randomly assigned to the experimental group which was to receive Bauhinia forficata decoction in place of drinking-water (the STZ Bauhinia forficata-treated [STZBFT] group), while the other rat of each pair was assigned to the control group which received drinking-water (the STZ control [STZCONT] group). Both groups received water for the first five days after STZ injection, after which the water in the cages of the STZBFT group was replaced by Bauhinia forficata decoction while the rats in the STZCONT group continued to have access to plain water. Body weight, food and liquid intake, urine excretion, plasma glucose (blood collected from the tip of the tail) and urinary glucose and urea were measured about every 7 days up to 36 days after STZ injection (31 days of treatment). We chose chronic treatment because previous experiments in our laboratory had shown that acute treatment with Bauhinia forficata decoction produced no alteration in the level of glycemia. After 31 days of chronic treatment with Bauhinia forficata decoction (mean daily dose 35.2 ± 7.8 ml/100 g body weight), the rats were sacrificed by decapitation and samples of the free running blood collected for the determination of the plasma level of glucose, serum cholesterol and triglycerides. The epididymal fat-pad and the retroperitoneal adipose tissue lying over the psoas, the soleus and extensor digitorum longus (EDL) muscles were removed and weighed and the liver removed for glycogen determination.

2.2.1.2. Non-diabetic group. After an adaptation period in metabolic cages the rats were paired according to body weight (124 ± 1.5 g) and randomly assigned to either the non-diabetic Bauhinia forficata-treated (NDBFT) group which received Bauhinia forficata decoction in place of drinking-water (mean daily dose 18.6 ± 0.66 ml/100 g body weight) or the non-diabetic control (NDCONT) group which had access to plain water. The experiment continued for 34 days during which time similar physico-metabolic determinations were made as for the STZ-diabetic rats.

2.2.2. Insulin administration

Eight days after of STZ (50 mg/kg) injection rats in the diabetic insulin group (DI) were treated twice a day (9 a.m. and 6 p.m.) by subcutaneous injection of 3 units of NPH insulin (Biohulin NU-100, Biobras, Montes Claros, MG, Brazil) for 38 days, after which the same metabolic parameters were evaluated. The diabetic control group (DCONT) received the same volume of 0.9% NaCl solution, administered identically. These insulin-treated rats served as a further control for the experimental model.

2.3. Chemical and statistical analyses

Urinary glucose was measured by the o-toluidine method of Duboswski (1962) and urea by the urease method (Bollet et al., 1961; Bergemeyer, 1985). Hepatic glycogen was extracted with 30% KOH and precipitated with alcohol (Carrol et al., 1956) and the quantity recovered determined (in triplicate) by the
colorimetric anthrone method of Collowick and Kaplan (1957), using an Hitachi U 3000 or Micronal B 382 spectrophotometer. Serum glucose, cholesterol and triglycerides were determined in a Bayer Technicon RA-100 autoanalyzer. All reagents were purchased from Merk or Sigma and were of at least analytical grade purity.

The animal data was assessed by Analysis of Variance and the Student’s t-test at a significance level of \( P < 0.05 \).

3. Results

Before administration of Bauhinia forficata decoction both groups of STZ-treated rats presented practically the same degree of physico-metabolic symptoms as seen in the diabetic state.

Figs. 1–3 show data for plasma glucose, urinary glucose and urinary urea levels respectively for the non-diabetic (NDBFT and NDCONT) and diabetic (STZBFT and STZCONT) groups of rats. It can be seen that after 31 days of treatment the diabetic group treated with decoction showed a significant reduction in plasma glucose \( (P < 0.05) \) (Fig. 1) and urinary glucose \( (P < 0.01) \) (Fig. 2) and urinary urea \( (P < 0.05) \) levels (Fig. 3) in relation to the STZCONT. The beneficial effect of Bauhinia forficata decoction on plasma glucose level appeared on about the 18th day of treatment (23 days after STZ injection). Although Fig. 3 shows that, as compared to the non-diabetic group untreated (NDCONT), the non-diabetic group treated with decoction (NDBFT) showed an increase in the level of urinary urea on day 13 of Bauhinia forficata treatment and an increase in plasma glucose on day 16 of treatment (Fig. 1). Comparison of both non-diabetic groups (NDBFT and NDCONT) during the whole 34 days of treatment showed no overall significant differences between these two groups regarding glycemia and urinary urea.

The mean level of plasma glucose and urinary urea recorded during of the experiment (31 days for the STZ-diabetic groups and 34 days for the non-diabetic groups), which was significantly higher in the STZ-diabetic groups than in the non-diabetic groups, irrespective of whether or not the rats were treated with Bauhinia forficata decoction. The difference in plasma glucose and urinary urea between the non-diabetic group not treated (NDCONT) and the diabetic group not treated (STZCONT), was 321 mg/dl for glucose and 223 mg/24 h 100 g body weight for urinary urea. Similarly, the difference in these values between the non-diabetic group treated with decoction (NDBFT)
and the STZ-diabetic group treated with decoction (STZBFT) was 234 mg/dl for glucose and 98 mg/24 h 100 g body weight for urinary urea. It should be noted that the differences are higher in the groups which did not receive decoction, supporting the beneficial effects *Bauhinia forficata* decoction presented above.

Table 1 shows that treatment with *Bauhinia forficata* decoction did not produce any alterations in body weight, water or food intake, urinary volume, serum lipids or hepatic glycogen either in non-diabetic or STZ-diabetic rats. Comparing the data for the STZBFT and STZCONT groups with that of their matched controls, NDBFT and NDCONT groups, it can be seen that there are significant differences in body weight gain (ΔW), food and liquid intake and urinary volume. Although there was no significant difference regarding hepatic glycogen between the two *Bauhinia forficata* untreated groups (NDCONT and STZCONT) there was a significant difference between the two *Bauhinia forficata* treated groups (NDBFT and STZBFT).

Table 2 shows the metabolic parameters of STZ-diabetic rats before and after treatment with insulin (DI) or NaCl solution (DCONT). It can be seen that, as expected, there was a significant improvement in the metabolic status of the rats after treatment with insulin, indicating that the experimental model adopted was adequate.

The lipid-related data in Table 1 shows that there was no significant alteration in the level of serum lipids over a diabetic period of about 1 month, although the significant differences in epididymal fat-pad tissue and retroperitoneal adipose tissue between the two groups of STZ-diabetic rats (STZBFT and STZCONT) as compared with the two groups of non-diabetic rats (NDBFT and NDCONT) shown in Table 3 indicates fat mobilization. Treatment with *Bauhinia forficata* decoction did not significantly affect the weight of either adipose or muscle tissue.

### 4. Discussion

By reducing plasma and urinary glucose and urinary urea (*Figs. 1–3*), oral treatment of STZ-diabetic rats with *Bauhinia forficata*, carried out by replacing their drinking-water with *Bauhinia forficata* decoction, produced a beneficial effect on the STZ-induced diabetes of the rats.

As early as 1941 Juliani working with pancreatectomized or with adrenaline administration in dogs and rabbits, had noticed that the administering of tablets containing the active components of *Bauhinia forficata* produced prophylactic anti-hyperglycemic effect in relation to diabetogenic states and had beneficial effects on hyperglycemia in transitory diabetes (Juliani, 1941). Vasconcelos et al. (2000) observed a hypoglycemic effect when a leaf extract of *Bauhinia forficata* was administered to hyperglycemic rats previously injected with scorpion venom.

Previously published work (Almeida and Agra, 1986; Vasconcelos et al., 2000) corroborates our results which show that *Bauhinia forficata* has no effect on glycemia in non-diabetic rats. Furthermore, the data given in Table 1 show that there was no significant difference in liquid intake between the two groups (NDCONT and NDBFT) of non-diabetic rats (indicating that the decoction was palatable for the rats).

It is well known that sulfonylureas produce hypoglycemia in non-diabetic animals because of the ability of these compounds to stimulate β pancreatic cells to liberate more insulin, although sulfonylureas do not reduce glycemia in alloxan-diabetic animals (Goth, 1978). However, exogenous insulin administration reduces glycemia in non-diabetic, STZ-diabetic and alloxan-diabetic animals (Gilman et al., 1981). In our study, treatment of STZ-diabetic rats either with insulin or with *Bauhinia forficata* induced a fall in the level of
glycemia and urinary glucose and urea (Table 2, Figs. 1–3).

Since Bauhinia forficata did not reduce glycemia in non-diabetic animals it is possible that its mechanism of action is different to that both of the sulfonylureas and insulin. These data support the work of Russo et al. (1990), who found no significant differences in the level of serum insulin between subjects of control groups and type II diabetic and non-diabetic groups who were administered Bauhinia forficata chronically or acutely.

It has also been reported (Mirsky, 1956) that diabetic animals present higher insulinase activities than non-

![Graph](image)

**Table 1**

<table>
<thead>
<tr>
<th>Metabolic parameters</th>
<th>Non-diabetic groups</th>
<th>STZ-diabetic groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated* (NDCONT)</td>
<td>Treated for 34^b^ days (NDBFT)</td>
</tr>
<tr>
<td>Body weight (g)(\text{c})</td>
<td>233.6 ± 3.7</td>
<td>232.1 ± 3.5</td>
</tr>
<tr>
<td>Change in body weight ((\Delta \text{w}))(\text{c})</td>
<td>10.9 ± 3.4</td>
<td>108.0 ± 3.3</td>
</tr>
<tr>
<td>Food intake (g/24 h)(\text{c,d})</td>
<td>11.9 ± 0.3</td>
<td>11.6 ± 0.2</td>
</tr>
<tr>
<td>Liquid intake (ml/24 h)(\text{c,d})</td>
<td>18.4 ± 0.3</td>
<td>18.6 ± 0.3</td>
</tr>
<tr>
<td>Urinary volume (ml/24 h)(\text{c,d})</td>
<td>4.1 ± 0.3</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>78.2 ± 10.0</td>
<td>61.3 ± 7.2</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>65.2 ± 3.4</td>
<td>60.0 ± 2.3</td>
</tr>
<tr>
<td>Hepatic glycogen (mg%)</td>
<td>3.9 ± 0.1</td>
<td>4.1 ± 0.4</td>
</tr>
</tbody>
</table>

\(\Delta \text{w} = \) Difference in body weight (measured about every 7 days post STZ administration) as compared to body weight on the day of STZ injection; Significant at *\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\) for comparisons between non-diabetic and STZ-diabetic groups. All values represent means ± standard error of the mean (\(n = 10\)).

\(\text{a}\) Drinking-water in place of Bauhinia forficata decoction.

\(\text{b}\) Bauhinia forficata decoction in place of drinking-water.

\(\text{c}\) Mean ± standard error of the mean of 5 determinations made during the treatment period.

\(\text{d}\) Per 100 g of body weight.
Table 2
Metabolic parameters of STZ-diabetic rats before and after treatment with insulin

<table>
<thead>
<tr>
<th>Metabolic parameters</th>
<th>Before insulin treatment</th>
<th>After 22 days insulin treatment</th>
<th>After 38 days insulin treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DCONT</td>
<td>DI</td>
<td>DCONT</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>158±4</td>
<td>161±4</td>
<td>192±12**</td>
</tr>
<tr>
<td>Fluid intake (ml/24 h)</td>
<td>67±5</td>
<td>66±3</td>
<td>58±6</td>
</tr>
<tr>
<td>Food intake (g/24 h)</td>
<td>20.4±1.23</td>
<td>18.5±0.7</td>
<td>26.8±3.0</td>
</tr>
<tr>
<td>Urinary volume (ml/24 h)</td>
<td>39±4</td>
<td>42±3</td>
<td>44±5</td>
</tr>
<tr>
<td>Urinary glucose (g/24 h)</td>
<td>1.89±0.18</td>
<td>1.77±0.23</td>
<td>1.8±0.20</td>
</tr>
<tr>
<td>Urinary urea (mg/24 h)</td>
<td>421±35</td>
<td>412±22</td>
<td>378±36</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>504±22</td>
<td>512±38</td>
<td>577±22**</td>
</tr>
</tbody>
</table>

Treatment was started 8 days after STZ injection. Except for body weight and plasma glucose, all values are reported per 100 g body weight. DI, diabetic insulin group, DCONT, diabetic control group. All values represent means±standard error of the mean (n = 10). *P < 0.001, diabetic treated group compared to the same group before insulin treatment; **P < 0.01, ***P < 0.001, DCONT compared to the same group before insulin treatment (paired Student t-test).

diabetic animals, while Achrekar et al. (1991) found reduced insulin degradation after the administration of the pulp and seeds of Eugenia jambolana (‘Jambolan’) because of insulinase inhibition, an effect similar to the chemical tolbutamide which is also an insulinase inhibitor (Mirsky and Diengoff, 1957). Taken together these reports may explain why Bauhinia forficata decoction showed hypoglycemic action only in diabetic rats, and the investigation of the effect of Bauhinia forficata decoction on insulinase activity may well confirm the hypothesis that this plant contains chemicals with anti-insulinase activity that may well be useful in maintaining the levels of any residual insulin which may be present in diabetics.

The fact that in our study there was no effect on hepatic glycogen levels in both the diabetic and non-diabetic groups (Table 1) suggests that the drop in glycemia in diabetic animals does not involve a reduction in glycogenolysis and/or increase in glycogenesis, similar results having been seen in rabbits treated with Jambolan seeds (Kedar and Chakrabarti, 1983). This, along with the reduction in glycemia seen in the STZ-diabetic rats treated with Bauhinia forficata decoction (Fig. 1), suggests that the decoction increased the sensitivity of cells to any residual insulin present in the rats, in other words, in moderate diabetes decoction increased insulin efficiency.

The results obtained for hepatic glycogen and glycemia along with the reduction in urinary urea seen in the STZ-diabetic rats treated with decoction suggests that the mechanism of action of Bauhinia forficata is related to a reduction in counterregulatory hormones and/or the inhibition of gluconeogenesis in a manner similar to that caused by the biguanides (Akhrar and Iqbal, 1991). The fact that there was no alteration in urinary urea in the non-diabetic group (Fig. 3) may have been related to their lower intake of decoction (Table 1).

The decreased rates of glucose (Fig. 2) and urea excretion (Fig. 3) could not be accounted for by reduced carbohydrate and protein intake because there was no alteration in food intake in animals treated with decoction (Table 1).

The maintenance of muscle weight seen in the non-diabetic group is consistent with the results for urinary urea. In the STZ-diabetic group there was a drop in urinary urea but, again, no alteration in muscle weight (Table 3), although it is possible that this parameter is insufficiently sensitive in this case. The hypothesis that

Table 3
Weight of epididymal and retroperitoneal adipose tissue, and soleus and EDL muscles in non-diabetic and diabetic rats treated and untreated with Bauhinia forficata decoction

<table>
<thead>
<tr>
<th>Weight of tissue/100 g body weight</th>
<th>Non-diabetic groups</th>
<th>STZ-diabetic groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreateda (NDCONT)</td>
<td>Treated for 34b days (NDBFT)</td>
</tr>
<tr>
<td>Epididymal</td>
<td>0.728±0.015</td>
<td>0.739±0.048</td>
</tr>
<tr>
<td>Retroperitoneal</td>
<td>0.527±0.045</td>
<td>0.515±0.036</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.086±0.002</td>
<td>0.083±0.003</td>
</tr>
<tr>
<td>EDL</td>
<td>0.094±0.003</td>
<td>0.094±0.003</td>
</tr>
</tbody>
</table>

Significant at *P < 0.05, **P < 0.01 or ***P < 0.001 for comparisons between non-diabetic and STZ-diabetic groups. All values represent means±standard error of the mean (n = 10).

a Drinking-water in place of Bauhinia forficata decoction.
b Bauhinia forficata decoction in place of drinking-water.
the decoction could inhibit enzymes involved in the urea cycle should be considered.

The improvement in urinary urea excretion in STZ-diabetic rats treated with decoction, was not sufficient to reach the levels observed in the non-diabetic rats, and there being no alteration in urine levels in the non-diabetic rats. Similar results were obtained with diabetic rabbits treated with *Eugenia jambolana* (Kedar and Chakrabarti, 1983) and non-diabetic rats treated with *Allium cepa* (onion) (Babu and Srinivasan, 1997).

As expected, we observed a sharp reduction in epididymal and retroperitoneal adipose tissue in the STZ-diabetic rats as compared to the non-diabetic rats (Table 3). The maintenance of serum triglyceride levels (Table 1) in STZ-diabetic rats treated with *Bauhinia forficata* decoction is in accord with the results for epididymal and retroperitoneal adipose tissue (Table 3).

Pharmacological, biochemical, histological and chemical studies are needed to elucidate the exact mechanism of action of *Bauhinia forficata* leaf decoction and to isolate any active compounds. Such investigations should also be carried out regarding type 2 diabetes.

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