Antidiabetic Activity of *Bauhinia forficata* Extracts in Alloxan-Diabetic Rats

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The antidiabetic activity of aqueous, ethanolic and hexanic extracts of *Bauhinia forficata* was investigated in a model of alloxan-induced diabetes in rats. The biochemical parameters studied were: plasma glucose, serum triglycerides, cholesterol, high density lipoprotein (HDL), and low density lipoprotein (LDL). Extracts were administered daily for 7 d at doses of 200 and 400 mg/kg, *p.o.*, 48 h after alloxan injection (60 mg/kg, i.v.). The alloxan-diabetic rats showed significant reductions in plasma glucose, triglycerides, total cholesterol and HDL-cholesterol after treatment with the extracts and glibenclamide (used as standard) as compared to the diabetic controls. Levels of LDL were not altered. In conclusion, our results showed that the plant extracts when administered by gavage may reduce glucose, triglycerides, total cholesterol and HDL-cholesterol levels. These results suggest the validity of the clinical use of *B. forficata* in the treatment of diabetes mellitus type II.

Key words antidiabetic activity; aqueous; etanolic; hexanic; glucose; triglyceride

Diabetes mellitus is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins, and an increased risk of complications from vascular disease.[1)]

More than 400 species of plants have been reported to display hypoglycemic effects, but only a few of them have been investigated.[2)] *Bauhinia forficata* is the *Bauhinia* species most used as an antidiabetic herbal remedy in Brazil, where it is known as "Pata de Vaca" (cow's hoof).[3)] This species is an arboreal plant of Asiatic origin which adapted well to the Brazilian climate, reaching 12 m in height.[1)]

There are only a few reports on the effects of this plant in the literature and some of them presenting contradictory or unsuccessful results. This fact emphasizes the importance of more detailed investigations using good experimental models in order to clarify the effects of oral treatment with this plant.[4)] In preliminary work we showed that the crude aqueous extract from *B. forficata* has a hypoglycemic effect.[5)] In this paper we investigate further the effects of oral daily treatment for 7 d with extracts of *Bauhinia forficata* on glucose and lipid levels of alloxan-diabetic rats.

MATERIALS AND METHODS

Plant The leaves of *Bauhinia forficata* were collected near the city of Pacoti, state of Ceará, Brazil. The material was brought to the Federal University of Ceará for preparation of the extracts at the Organic and Inorganic Chemistry Department. The exsiccatae of the plant is deposited in the Prisco Bezerra Herbarium of the Federal University of Ceará under the number 17856.

Aqueous Extract *B. forficata* fresh leaves were ground and submitted to heating under pressure, with distilled water as solvent. After extraction this material was kept at 4°C, and later centrifuged and the supernatant submitted to lyophilization.

Hexane Extract *B. forficata* leaves were dehydrated at room temperature, ground and submitted to extraction with hexane. The extract was concentrated at reduced pressure and kept in a refrigerator until use.

Ethanol Extract *B. forficata* leaves previously extracted with hexane were submitted to extraction with ethanol. The extract was concentrated at reduced pressure and used for pharmacological assays.

Drugs Alloxan was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and Glibenclamide from Teuto, Brasileiro LTDA, (GO, Brasil). All drugs were dissolved in distilled water for oral administration.

Animals Male Wistar rats (180—250 g) were housed in standard environmental conditions (22±1 °C, humidity 60±5%, 12 h light : 12 h dark cycle) with free access to a standard commercial diet and water *ad libitum*. Experiments were performed according to the Guide for the Care and Use of Laboratory Animals, from the US Department of Health and Human Services, Institute of Laboratory Animal Resources, Washington DC, 1985.

Study of the Aqueous, Ethanol and Hexanic Extracts in Diabetic Rats Rats fasted for at least 16 h received alloxan (ALX) 60 mg/kg through the penile vein. The diabetic state was assessed by blood glucose levels 48 h later and usually around 40% death was registered at this time. Animals which presented glucose levels lower than 200 mg/dl glucose levels, were rejected. The rats were divided into 10 groups of 5—8 animals each. Group I and II: Normal control (NC) and Cremophor control (CC) received distilled water and Cremophor 3% (vehicle) 10 ml/kg, *p.o.*, respectively. Groups III, IV, V, VI, VII, VIII and IX received ALX and 48 h later were treated with distilled water (diabetic control) (DC), aqueous (AE), ethanol (EE) and hexane (HE) extracts respectively, at doses of 200 and 400 mg/kg, *p.o.*; and Group X was treated with Glibenclamide (GLI) 5 mg/kg, *p.o.* as standard. Blood samples were collected just prior two and seven days after treatment. Cremophor was utilized for solubilization of the hexanic extract.

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Determination of Plasma Glucose and Lipid Concentrations Blood from the retro-orbital plexus was collected and centrifuged at 3000 rpm for 10 min, and the glucose level was determined by the glucose oxidase–peroxidase enzymatic method (Lab Test Set for Glucose, Sistemas Diagnosticos Ltda., Brazil); cholesterol, high density lipoprotein (HDL) cholesterol, triglyceride levels and low density lipoprotein (LDL) were determined by the Enzimático–Trinder method.

Statistical Analyses All results are presented as mean±S.E.M. Data was analyzed by ANOVA followed by Student–Neuman–Keuls as the *post hoc* test. Results were considered significant at $p<0.05$.

RESULTS

The treatment for seven days with AE, EE and HE (200, 400 mg/kg) and GLI (5 mg/kg), caused a significant reduction of the hyperglycemia as compared to DC. Thus AE reduced hyperglycemia by 60 and 64%, and EE by 42 and 55% at doses of 200 and 400 mg/kg, *p.o.*, respectively, when compared with DC. The HE 400 and GLI 5 mg/kg reduced the hyperglycemia by 45 and 54%, respectively (Fig. 1).

Data on lipid concentrations are presented in Fig. 2 and Table 1. There were significant alterations in levels of serum lipids in the diabetic treated rats as compared with the untreated diabetic group. The AE 200 reduced cholesterol levels in 28 (Table 1) and triglyceride levels in 78% (Fig. 2), but did not alter serum HDL concentration (Table 1). The AE 400 reduced triglyceride levels in 91% (Fig. 2) and total cholesterol and HDL levels in 50 and 35%, respectively (Table 1). The EE (200, 400 mg/kg, *p.o.*) reduced total cholesterol in 43 and 50%, HDL in 27 and 30% (Table 1) and triglyceride levels in 81 and 83% (Fig. 2), respectively. The HE (200, 400) reduced the level of total cholesterol in 40 and 49%, and HDL in 68 and 52% (Table 1), respectively. Triglycerides were reduced only with HE 400 mg/kg, *p.o.* (82%) (Fig. 2). Similarly, GLI reduced cholesterol in 38%, HDL in 30% (Table 1) and triglyceride concentrations in 79% (Fig. 2).

DISCUSSION

The main characteristics of diabetes mellitus are polydipsia, polyuria and polyphagia, weight loss, muscle weakness and hyperglycemia.[6] This work evaluated biochemical parameters such as: serum triglycerides, cholesterol, LDL, HDL, and plasma glucose in experimental diabetes caused by alloxan in rats.

The unique capacity of alloxan to selectively destroy the pancreatic beta cells was first described by Dunn *et al.*, 1943.[7] These investigators examined the nephrotoxicity of uric acid derivatives in the rabbit and found accidentally that alloxan caused the destruction of the majority of pancreatic beta cells. Subsequently, alloxan administration has been found to lead to long-lasting diabetes in many animal species. The site at which alloxan interacts with the cell membrane is uncertain.[4]

Previous studies demonstrated that the alcoholic extract from the bark of *B. forficata* had a hypoglycemic effect.[8] Reports demonstrated that the alcoholic extract from its leaves did not reduce glucose concentrations in diabetes induced by

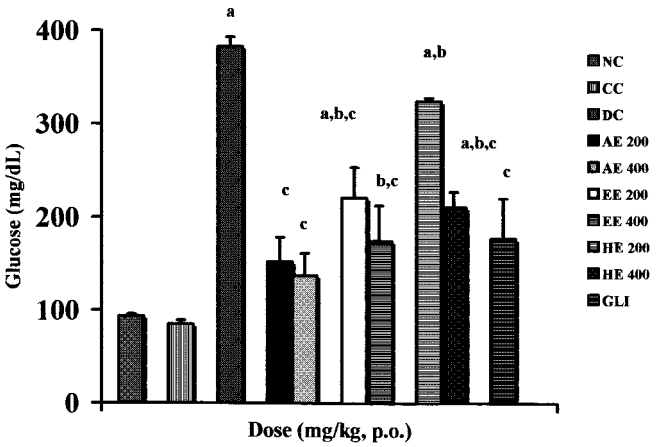

Fig. 1. The Effect of Aqueous Extract (AE), Ethanol (EE) and Hexanic Extracts (HE) of *Bauhinia forficata* on Levels of Plasma Glucose in ALX-Diabetic Rats

All values represent mean±S.E.M. (5—60). a,b,c) $p<0.05$ *vs.* normal control (NC), cremophor control (CC) and diabetic control (DC). (ANOVA and Student–Newman–Keuls as the *post hoc* test).

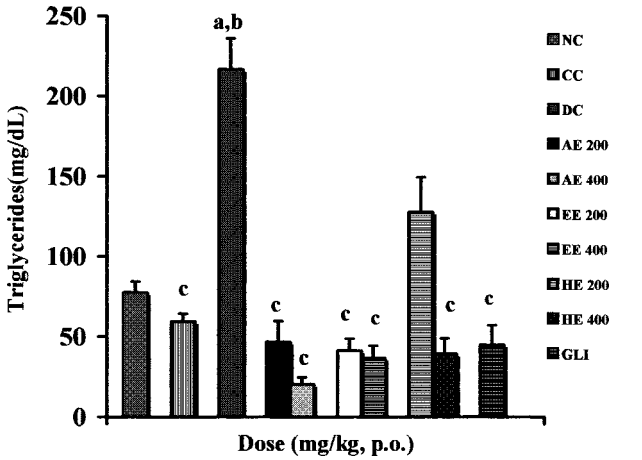

Fig. 2. The Effect of Aqueous (AE), Ethanol (EE) and Hexanic Extracts (HE) of *Bauhinia forficata* on Levels of Plasma Triglycerides in ALX-Diabetic Rats

All values represent mean±S.E.M. (5—60). a,b,c) $p<0.05$ in relation to normal control (NC), cremophor control (CC) and diabetic control (DC), respectively (ANOVA and *post hoc* Student–Newman–Keuls test).

Table 1. Plasma Lipid Profiles in Controls and Alloxan-Induced Diabetic Rats without and after Treatment with *Bauhinia forficata* and Glibenclamide

	Total cholesterol	LDL-cholesterol	HDL-cholesterol
NC	74.3±1.81 (24)	36.5±1.78 (24)	22.3±0.55 (24)
CC	61.8±3.55 (11)$^{c)}$	31.3±2.47 (14)	18.2±1.43 (08)
DC	82.2±2.33 (60)$^{a)}$	34.7±2.06 (60)	19.4±0.52 (60)
AE 200	53.2±5.19 (08)$^{a,c)}$	31.1±3.34 (07)	16.8±1.04 (08)$^{a)}$
400	40.8±5.86 (07)$^{a,c)}$	24.1±3.76 (07)	12.7±0.60 (06)$^{a,c)}$
EE 200	46.9±5.43 (11)$^{a,c)}$	22.4±3.57 (09)	14.1±1.96 (10)$^{a,c)}$
400	41.0±3.40 (13)$^{a,c)}$	20.8±2.00 (10)	13.6±2.28 (10)$^{a,c)}$
HE 200	49.2±4.35 (05)$^{a,c)}$	17.5±4.44 (05)	6.2±0.46 (06)$^{a,b,c)}$
400	41.8±6.08 (09)$^{a,c)}$	22.1±5.31 (06)	10.1±2.06 (06)$^{a,b,c)}$
GLI 5	50.0±8.78 (08)$^{a,c)}$	30.4±6.89 (07)	13.5±1.27 (08)$^{a,c)}$

Values are expressed as mg/dl and are the mean of the number of parenthesized animals. *a,b,c*) $p<0.05$ in relation to normal control (NC), cremophor control (CC) and diabetic control (DC), respectively (ANOVA and *post hoc* Student–Newman–Keuls test).

streptozotocin in rats.[9] In the present work we showed that the alcoholic extract reduced the serum glucose levels in diabetic rats but values did not return to those of normal controls.

All forms of diabetes mellitus are due to a decrease in the circulating concentration of insulin (insulin deficiency) and a decrease in the response of peripheral tissues to insulin (insulin resistance). These abnormalities lead to alterations in the metabolism of carbohydrates, lipids, ketones, and amino acids; the central feature of the syndrome is hyperglycemia. Insulin also enhances the transcription of lipoprotein lipase in the capillary endothelium. Thus, in the untreated or undertreated diabetic patient, hyperlipidemia often occurs.[5] Recent reports demonstrated that oral administration with doses of 400, 600 and 800 mg/kg of the *n*-butanol fraction of *B. forficata* reduced the hyperglycemia in alloxan-induced diabetic rats.[10]

Pepato *et al.*[11] demonstrated that chronic administration (31 d of treatment) of *B. forficata* decoction to the diabetic group caused a significant reduction in plasma glucose. The beneficial effect of this decoction on plasma glucose level appeared around the 18th day of treatment. The present study confirmed the ability of *B. forficata* to reverse the hyperglycemia of alloxan treated rats in sub-acute treatment. Evidence was presented to show that, in addition to its hypoglycemic activity, *B. forficata*, also possesses lipid lowering properties in diabetic rats. A previous paper, however demonstrated that there was no significant alteration in the level of serum lipids after 1 month treatment with *B. forficata* decoction in streptozotocin-induced diabetic rats.[12]

The reduction of cholesterol in the prevention of coronary arterial disease decreases the incidence of heart attacks.[13] Several researchers, among them Yugarani (1992)[14] have demonstrated that flavonoids act as reducers of lipid activities in animals.[15] It has been reported that *B. forficata* has terpenoids, esteroids and flavonoids.[16—18] Similarly, our results showed that extracts from *B. forficata* reduced serum lipid concentration and that this effect may occur due to the presence of flavonoids[19] in the plant extracts. The results show that the oral administration of *B. forficata* extracts had a beneficial effect on the diabetic state reducing the hyperglycemia as well as hyperlipidemia. Studies with flavonoids are underway to further elucidate their mechanism of action.

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REFERENCES

1) Davis S. N., Granner D. K., "Insulin, Oral Hypoglycemic Agents, and the Pharmacology of the Endocrine Pancreas," 9th ed., Chap. 60, ed. by Hardman J. G., Limbird L. E., Molinoff P. B., Ruddon R. W., Gilman A. G., McGraw-Hill, New York, 1996, pp. 1487—1518.
2) Silva K. L., Biavatti M. W, Leite S. N., Yunes R. A., Delle M. F., Cechinel V., *J. Biosciences*, **55**, 478—480 (2000).
3) Filho J. M., Almeida R. N., Galvão K. A. A., Morais R. M., In Livro de Resumos. X Simpósio de Plantas Medicinais do Brasil, São Paulo-Sp. 1988, p. 47.
4) Luz M. M. S., Santos C. A. M., Sato M. E. O., Arruda A. M. S., In Livro de Resumos. XIV Simpósio de Plantas Medicinais do Brasil, Florianopolis, 1996, p. 84.
5) Howell S. L., Taylor K. W., *J. Endocrinol*, **37**, 421 (1967).
6) Bragança L. A. R., "Plantas Medicinais Antidiabeticas," UFF, Niterói, 1996, p. 283.
7) Dunn J. S., Mclechie N. G. B., *Lancet*, **2**, 384 (1943).
8) Almeida R. N., "I Simposio de Plantas Medicinais do Brasil," São Paulo, 1984, p. 9.
9) Damasceno D. C., Volpato G. T., Rudge M. V. C., Traballi A. L. M., Silva C. F., Oliveira M., Calderon I. M. P., *Diabetes Clinica*, **6**, 435—439 (2002).
10) Silva F. R. M. B., Szpoganicz B., Pizzolatti M. G., Willrich M. A. V. Sousa E., *J. Ethnopharmacology*, **83**, 33—37 (2002).
11) Pepato M. T., Keller E. H., Baviera A. M., Kettelhut I. C., Vendramini R. C., Brunetti I. L., *J. Ethnopharmacology*, **81**, 191—197 (2002).
12) Oliveira E. P., Martins L. P. G., Uyemma S. A., Pedrazzi A. H. P., *Rev. Bras. Anal. Clin.*, **29**, 62—68 (1997).
13) Miura T., Kubo M., Itoh Y., Iwamoto N., Kato M., Park S. R., Ukawa Y., Kita Y., Suzuki I., *Biol. Pharm. Bull.*, **25**, 1234—1237 (2002).
14) Yugaran T., Tan B. K. H., Das N. Y., *Lipids*, **27**, 181—186 (1992).
15) Tomita T., Lacy P., Matschinsky F. M., McdanieL M. L., *Diabetes*, **23**, 517 (1974).
16) Silva K. L., Filho C., *Química nova.*, **25**, 449—454 (2002).
17) Silva K. L., Leite S., Biavatti M. W., Cechinel F. V., In Livro de Resumos. XV Simpósio de Plantas Medicinais do Brasil, São Paulo-Sp., 1998, p. 198.
18) Donato A. M., *Bradea*, **6**, 357—371 (1986).
19) Costa A. F., "Farmacognosia," 2nd ed., Vol. 1—2, Fundação Calouste Gulbenkian, Lisboa, 1977.