Journal of Intercultural Ethnopharmacology
www.jicep.com
DOI: 10.5455/jice.20141028022407

Original Research

ScopeMed

Acute and subchronic antihyperglycemic activities of *Bowdichia virgilioides* roots in non-diabetic and diabetic rats

Ana Carolina Mazei Silva[1], Maísa Pavani dos Santos[1], Suélem Aparecida de França[1], Virginia Claudia da Silva[1], Luiz Everson da Silva[2], Uir Santana de Figueiredo[1], Evandro Luiz Dall'Oglio[1], Paulo Teixeira de Sousa Júnior[1], Carbene França Lopes[1], Amanda Martins Baviera[3], Nair Honda Kawashita[1]

[1]Department of Chemistry, Federal University of Mato Grosso, Cuiabá, Mato Grosso, Brazil, [2]Department of Science Education, Federal University of Paraná, Coastal Sector, Matinhos, Paraná, Brazil, [3]Department of Clinical Analysis, School of Pharmaceutical Sciences, São Paulo State University, Araraquara, São Paulo, Brazil

Address for correspondence:
Nair Honda Kawashita, Department of Chemistry, Federal University of Mato Grosso, Cuiabá, Mato Grosso, Brazil.
Tel.: +556536158765, Fax: +556536158798/99, E-mail: nairhonda@terra.com.br

Received: October 15, 2014
Accepted: October 28, 2014
Published: November 28, 2014

ABSTRACT

Aim: The present study was undertaken to evaluate the acute and subchronic antihyperglycemic effects of methanolic extract of *Bowdichia virgilioides* root bark of *B. virgilioides* in streptozotocin (STZ)-induced diabetic rats. **Materials and Methods:** The extract (100, 250 or 500 mg/kg) was orally administered to male Wistar diabetic (STZ, 42 mg/kg i.v.) and non-diabetic rats into two main protocols: (i) subchronic experiments, where animals were treated for 21 days with *B. virgilioides* extract and the following parameters were evaluated: Body weight, fluid and food intake (determined daily), urinary glucose and urea (every 3 days) and glycemia (every 5 days). At the end of the experimental period, skeletal muscles (extensor digitorum longus [EDL] and soleus), retroperitoneal and epididymal white adipose tissues were collected and weighed; liver samples were used for the determination of the lipid and glycogen contents; (ii) acute experiments, which evaluated the alterations on fasting and post-prandial glycemia and on glucose tolerance using the oral glucose tolerance test (OGTT). **Results:** In subchronic experiments, the treatment with *B. virgilioides* extract did not change any parameter evaluated in diabetic and non-diabetic animals. On fasting and post-prandial glycemia, the extract treatment did not promote changes in the glycemia values in diabetic or non-diabetic animals. In OGTT, the treatment with 500 mg/kg *B. virgilioides* extract reduced the hyperglycemia peak after a glucose overload, when compared with non-treated diabetic animals, resulting in a lower area under curve. **Conclusion:** The results of our work indicate that *B. virgilioides* root extract promotes an acute antihyperglycemic effect in STZ-diabetic rats; this effect probably occurs through an inhibition of the intestinal glucose absorption. The continuity of the research is necessary to elucidate these possibilities.

KEY WORDS: Antihyperglycemic effect, antihyperglycemic plants, *Bowdichia virgilioides*, fabaceae, medicinal plants

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia in the postprandial and/or fasting states. In addition, other disturbances such as dyslipidemia, obesity and oxidative stress are commonly involved in the development of the diabetic complications that may lead to a premature mortality. These alterations are mainly caused by insulin deficiency and/or insulin resistance. Based on studies in several countries, the World Health Organization and the American Diabetes Association have estimated that there will be 285 million people worldwide with diabetes in 2010 and projected to rise to 439 million in 2030 [1], indicating a situation of "epidemic diabetes." This rise can be attributed to several

factors, such as population growth, urbanization, changing lifestyle (sedentary daily life and increased consumption of energy-rich diets) and an increased prevalence of obesity among population as it represents a main risk factor for incidence of diabetes [2-4].

The available therapeutic approach to diabetes mellitus attempts to maintain the glycemia values close to normality and is based on diet, oral hypoglycemic drugs and insulin administration, used independently or in combination [5,6]. Despite the availability of insulin and multiple classes of hypoglycemic agents, several adverse effects are observed with the use of these drugs, such as hypoglycemia, liver dysfunction, lactic acidosis and others. In addition, the excessive cost of the diabetes treatment is another disadvantage. These negative aspects in the conventional diabetes therapy stimulate the use of alternative and/or complementary medicines, for example, the treatment with herbal preparations and/or constituents. In traditional practices, plant-based medicinal products are known since ancient times and have been used to control diabetes around the world.

World ethnobotanical information about medicinal plants reports almost 800 plants used in the control of diabetes mellitus [7-9]. More recently, in addition to the pharmacological diabetic animal models and classical methodologies used to the selection of plants with antidiabetic effect, the ethnopharmacological studies have been applied a diversity of novel approaches and refined assays to elucidate the mechanism of action that explains their hypoglycemic activity. Among these, it can be highlighted: studies in cell cultures [10,11], investigation of changes in components of the insulin intracellular signaling [12,13] and the use of genetically diabetic animal models [14,15]. These studies will generate essential information useful to the advances in the development of novel antidiabetic terapy.

Bowdichia virgilioides belongs to Fabaceae family and is a plant commonly distributed in the Amazonian lowlands of Brazil, Bolivia and up north to Central America, typically in savanna fields; it is known by the popular names: *Sucupira-do-cerrado, sucupira-do-campo, angelim-amargoso, coração-de-negro*. *B. virgilioides* preparations have also been used in popular medicine for the treatment of leishmaniasis and malaria [16,17]. Almeida *et al*. [18] have demonstrated that essential oil from *B. virgilioides* leaves presented antimicrobial activity against several pathogenic microorganisms. Although the use of *B. virgilioides* to treat diabetes was previously cited by Oliveira and Saito [19], describing its popular preparation to the complementary treatment of this disease, no other studies have been carried out to confirm the beneficial effect of this plant species in biochemical or physiological parameters altered in diabetes.

Chemical studies with *B. virgilioides* have revealed the presence of alkaloids, terpenoids and benzofuran derivatives in the stem bark [20-23], volatile constituents, essential oils, flavonoids and isoflavonoids in roots [24-27], tannins in inner bark and leaves [28] and geraniol, caryophyllene and anthocyanin in fruits [29,30]. Some of these constituents have been related with the antidiabetic activity of other plant species, for example, flavonoids [31,32], terpenoids [33,34], anthocyanins [35] and gallotannins [36].

In this way, the present study was undertaken to evaluate the acute and subchronic antihyperglycemic effects of methanolic extract of *B. virgilioides* root bark of *B. virgilioides* in streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Materials

Root bark of *B. virgilioides* was collected in the Poconé region (S15 47 159; W56 21 345; S 16 18 112; W 056 33 952), Mato Grosso state, Brazil in February of 2006. The plant was identified, and a voucher specimen (number 24,600) was stored at the Central Herbarium of the Federal University of Mato Grosso (UFMT).

Preparation of the *B. virgilioides* Methanolic Extract (BvMeOH)

The air-dried and ground *B. virgilioides* roots (4.85 kg) were macerated (7 days) and extracted, sequentially, with hexane (1 × 3 L/kg) and methanol (4 × 3 L/kg), respectively. The BvMeOH (350 g; 7.2%) was obtained after filtration and removal of the solvent under reduced pressure. The powdered BvMeOH was stored at 4°C and dissolved to the desired concentration prior to use.

Animals

Male Swiss-Webster mice (25-30 g) and Wistar rats (180-210 g) were housed in a room under standard laboratory conditions (12:12 hour light-dark cycle, 24±1°C) and were given free access to water and normal lab chow diet (Purina® Labina). All experiments took place between 08:00 and 10:00 a.m. The animals were managed according to the Brazilian College of Animal Experimentation and approved by the Committee for Ethics in Animal Experimental of the UFMT (no: 23108.043016/10-6).

Acute Toxicity Test

Groups of male mice received orally by gavage a single administration of BvMeOH in different concentrations: 100, 250, 500, 1000, 2000, 3000, 4000 or 5000 mg/kg. The control group received vehicle (1% Tween 80). The animals were individually observed during 0, 5, 10, 15 and 30 minutes; 1, 2, 4 and 8 h and during 1 week (one time a day) after BvMeOH or vehicle administration. The behavior alterations observed were registered in a table adapted from Malone's study [37].

Induction of Diabetes

STZ, 42 mg/kg; Sigma Aldrich, St. Louis, MO, USA dissolved in 0.01 mol/L citrate buffer (pH 4.5) was administered by a single intravenous injection in previously 16 hours fasted rats. Five days

after STZ administration, animals with post-prandial glycemia between 350 and 500 mg/mL were used in the experiments. Non-diabetic control (NC) animals received a citrate buffer injection.

Subchronic Experiments

Five days after STZ or citrate buffer injection, the animals were divided into the following groups: DC group – diabetic control (DC) rats; DT100 group – diabetic rats treated with 100 mg/kg of BvMeOH; DT250 group – diabetic rats treated with 250 mg/kg of BvMeOH; DT500 group – diabetic rats treated with 500 mg/kg of BvMeOH; DG group – diabetic rats treated with glibenclamide; DI group – diabetic rats treated with insulin; NC group – NC rats; NT100 group – non-diabetic rats treated with 100 mg/kg of BvMeOH; NT250 group – non-diabetic rats treated with 250 mg/kg of the BvMeOH and NT500 group – non-diabetic rats treated with 500 mg/kg of the BvMeOH. The extract was dissolved in 1% Tween 80. The diabetic and non-diabetic groups (except DI), received the BvMeOH and/or Tween 80 (controls) orally by gavage, once a day, during 22 days. DG group received 2mg/kg of glibenclamide at 9:00 a.m. and 6:00 p.m., from day 5 to day 21. DI received 3 U/rat of Insulin NPH – Lilly (s.c.), at 9:00 a.m. and 6:00 p.m., from day 5 to day 21.

Body weight, fluid and food intake were determined daily, urinary glucose and urea every 3 days and glycemia every 5 days. Blood samples for plasma glucose determination were collected from the tip of the tail. At the end of the experimental period, rats were anesthetized and sacrificed by decapitation, and samples of the free running blood were collected for glucose measurement. skeletal muscles (EDL and soleus), retroperitoneal and epididymal white adipose tissues were collected and weighed. Hepatic lipid and glycogen contents were determined. Urinary glucose was determined using the dinitrosalicylic acid (Sigma Aldrich, St. Louis, MO, USA) reaction [38], urinary urea by the urease reaction (Labtest® kit) and plasma glucose was measured by the glucose oxidase method (Labtest® kit). Hepatic glycogen was determined after acidic hydrolysis and titration for monomeric glucose that was quantified with anthrone reagent [39]. The liver lipid content was determined by gravimetric method after extraction as described by Folch *et al*. [40].

Oral Glucose Tolerance Test (OGTT)

The OGTT was performed in overnight fasted NC, DC and normal and diabetic 100, 250 and 500 mg/kg extract-treated animals (NT100, NT250, NT500, DT100, DT250, DT500). The control groups received 1% Tween 80. All animals received an oral load of glucose (2.5 g/kg). The BvMeOH or vehicle was administered at same time as glucose. Plasma glucose was measured in blood withdrawn from the tip of the tail, before load ($t = 0$) and after 15, 30, 45, 60, 75 and 90 min.

Fasting and Post-prandial Glycemia

To evaluate the effect of BvMeOH on fasting and post-prandial glycemia, the blood glucose level of each rat was determined at the beginning of the experiment. In the fasting glycemia determination, blood samples were collected after an overnight fasting (14 h). The same groups and proceedings were done after feeding of animals to evaluate the extract effects on post-prandial glycemia. The different doses of BvMeOH administered to normal and diabetic rats were the same as previously described.

Statistical Analysis

Data were expressed as mean ± standard error of mean. Statistical analysis was performed using the Statistic Software package (Statsoft, Tulsa, OK, USA). Bartlett's test for the homogeneity of variances was initially used to determine whether the data complied with the assumptions for parametric analysis of variance (ANOVA). The one-way ANOVA was employed to analyze the data between treated groups and their respective control groups (diabetic or non-diabetic). In the subchronic experiment the summed data of each parameter during statistical evaluation were compared. Glycemia areas under curves (AUC) were compared. Differences were considered significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$.

RESULTS

Acute Toxicity Study

Normal mice treated with BvMeOH in doses ranging from 100 to 500 mg/kg did not present any behavior alteration. A dose of 1000 mg/kg reduced the motility of animals 15 min after its administration. At higher doses (2000, 3000, 4000 and 5000 mg/kg), it was initially observed palpebral ptosis and subsequent somnolence that persisted until 1 h after the treatment. No deaths were observed in this assay. Based on these results the doses of 100, 250 and 500 mg/kg were chosen for the antihyperglycemic assays.

Subchronic Treatment

The subchronic treatment with BvMeOH for 21 days did not reduce the high post-prandial glycemia of diabetic rats. The other biochemical parameters evaluated (liver glycogen and lipid content, urinary glucose and urinary urea) did not also differ between diabetic groups treated or not with BvMeOH [Tables 1 and 3]. The extract did not alter the lower body weight gain of diabetic, as well as the food and liquid intake, the urinary volume [Table 2] and the weights of skeletal muscles and white adipose tissues [Table 3]. The treatment with insulin and glibenclamide improved all the parameters described above. In the same way, the BvMeOH treatment did not change any parameter in normal, non-diabetic animals (data not shown).

Acute Treatment

The BvMeOH did not alter the fasting and post-prandial glycemia values in normal and diabetic groups [Figures 1c-1f]. In OGTT, the treatment with 500 mg/kg BvMeOH reduced the hyperglycemia peak after a glucose overload when compared

Table 1: Effects of *B. virgilioides* root bark extract on plasma glucose, urinary glucose and urinary urea of non-diabetic, DC, DT100, DT250, DT500, DI, and DG groups in subchronic experiment during 21 days

Groups	1	6	11	16	21
Plasma glucose (mg/dL)					
NC	153±6	146±5	145±5	146±5	157±5
DC	482±40	555±54	519±47	489±16	585±31
DT100	468±12	437±62	394±53	458±46	614±63
DT250	461±20	382±54	404±53	429±51	476±75
DT500	511±15	597±26	564±31	520±48	475±60
DI (3 U/rat)	461±90	470±92	201±33*	208±40#	214±49#
DG (2 g/kg)	415±67	338±59	321±55*	326±59*	299±49#
Urinary glucose (mg/24 h)					
DC	8.14±0.90	9.29±1.06	8.48±1.25	9.53±1.77	10.45±0.60
DT100	8.00±1.07	8.14±1.42	9.31±1.38	8.20±0.96	9.52±0.90
DT250	8.56±0.93	9.54±1.31	9.68±0.86	10.16±0.74	10.14±1.00
DT500	9.00±1.20	10.73±0.76	7.26±0.79	6.10±1.09	6.81±1.00
DI (3 U/rat)	9.33±0.68	9.22±1.52	0.05±0.03##	0.16±0.08##	0.05±0.03##
DG (2 g/kg)	4.81±1.47	4.51±1.61	3.97±1.40#	4.92±1.67#	4.55±1.50#
Urinary urea (g/24 h)					
NC	0.43±0.03	0.42±0.05	0.59±0.04	0.63±0.06	0.74±0.03
DC	1.27±0.15	1.48±0.17	1.56±0.13	1.44±0.17	1.64±0.15
DT100	1.29±0.08	1.40±0.17	1.67±0.13	1.47±0.13	1.94±0.34
DT250	1.16±0.09	1.28±0.16	1.47±0.10	1.57±0.07	1.69±0.27
DT500	1.57±0.23	1.57±0.16	1.13±0.10	0.98±0.12	0.94±0.14
DI (3 U/rat)	1.43±0.02	1.33±0.06	1.03±0.07	1.02±0.11	0.86±0.14*
DG (2 g/kg)	0.97±0.18	1.09±0.19	1.17±0.15	1.33±0.16	1.14±0.16

B. virgilioides: Bowdichia virgilioides, NC: Non-diabetic control, DC: Diabetic control, DT100: Diabetic treated with 100 mg/kg extract, DT250: Diabetic treated with 250 mg/kg extract, DT500: Diabetic treated with 500 mg/kg extract, DI: Diabetic treated with insulin, DG: Diabetic treated with glibenclamide, SEM: Standard error of the mean, ANOVA: Analysis of variance. The values are expressed as mean±SEM of 5-8 animals. *$P<0.05$; #$P<0.01$; ##$P<0.001$ versus DC (ANOVA one-way)

Table 2: Effects of *B. virgilioides* root bark extract on body weight, food and liquid intake and urinary volume of non-diabetic, DC, DT100, DT250, DT500, DI, and DG groups in subchronic experiment during 21 days

Groups	Body weight (g)		Food intake (g)	Liquid intake (mL)	Urinary volume (mL)
	Initial	Final			
NC	198±3	318±8*	513±10*	741±17*	128±12*
DC	194±9	206±15	791±27	3194±280	2350±261
DT100	207±4	243±6	755±30	3029±332	2207±276
DT250	203±3	258±6	778±31	3203±247	2335±218
DT500	199±6	202±11	730±37	3266±198	2340±183
DI (3 U/rat)	191±7	285±17#	567±9##	1538±67#	1023±318##
DG (2 g/kg)	213±3	295±12#	630±44#	1674±332#	1670±129#

The values are expressed as mean±SEM of 5-8 animals. *$P<0.05$, #$P<0.01$, ##$P<0.001$ versus DC (ANOVA one-way). *B. virgilioides: Bowdichia virgilioides*, NC: Non-diabetic control, DC: Diabetic control, DT100: Diabetic treated with 100 mg/kg extract, DT250: Diabetic treated with 250 mg/kg extract, DT500: Diabetic treated with 500 mg/kg extract, DI: Diabetic treated with insulin, DG: Diabetic treated with glibenclamide, SEM: Standard error of the mean, ANOVA: Analysis of variance

with DC rats [Figure 1a]. Therefore, the AUC of DT500 group was significantly lower than of DC and DT250 groups, although it was higher than the AUC of NC group. The AUC of normal rats treated with BvMeOH, at any tested dose, was not different in comparison with normal, non-treated rats [Figure 1b].

DISCUSSION

The results obtained in this study showed that, although *B. virgilioides* extract did not change any parameter evaluated in diabetic rats after the subchronic experiment, the extract showed an acute antihyperglycemic effect (500 mg/kg), reducing the hyperglycemia peak when a glucose load was administered to diabetic animals.

Diabetes experimental model with STZ-induced animals is widely used for screening of compounds including natural products with different antidiabetic activities. After STZ administration, the drug is taken up by pancreatic beta cells through glucose transporter type 2 (GLUT2), promoting irreversible destruction of these cells by two main mechanisms: DNA alkylation, which promotes its fragmentation, and exposition of beta cells to reactive oxygen species damage. The final result is degranulation and/or reduction of insulin secretion [41,42]. Although insulin treatment has become one of the most important therapeutic agents to ameliorate symptoms of type I diabetes, there are continued efforts to find insulin substitutes, mainly from plant sources. Traditional herbal remedies prescription instead of conventional treatment can be justified by some of its advantages, for example, their effectiveness, no or few side-effects and relatively low cost. Therefore, several studies have focused on the comprehension of the ethnopharmacological, phytochemical and clinical importance of medicinal plants for diabetes treatment.

Studies with herbal remedies have shown that several mechanisms are involved, isolated or combined, in the promotion of its antidiabetic effect: stimulation of pancreatic cells insulin release, reduction of liver glucose production, enhancement of glucose uptake by peripheral tissues and inhibition of intestinal glucose absorption. In this way, it was

Table 3: Effects of *B. virgilioides* root bark extract on retroperitoneal, epididymal and perirenal adipose tissue weight, liver and soleus and EDL muscles weight and glycogen and lipid liver content of NC, DC, DT100, DT250, DT500, DI, and DG groups in subchronic experiment during 21 days

Groups	Retroperitoneal weight (g)	Epididymal weight (g)	Perirenal weight (g)	Liver weight (g)	Soleus weight (g)	EDL weight (g)	Liver glycogen content (mg/g)	Liver lipid content (mg/g)
NC	2.42±0.42	2.71±0.25	0.70±0.12	11.95±0.82	0.28±0.01	0.26±0.02	25.17±3.45	48.55±0.80
DC	0.51±0.21	0.91±0.18	0.10±0.04	11.58±0.94	0.23±0.02	0.19±0.02	8.82±2.77	41.24±3.02
DT100	0.42±0.18	0.99±0.19	0.09±0.03	12.85±0.76	0.22±0.02	0.20±0.02	5.92±3.29	44.68±1.49
DT250	0.48±0.09	1.16±0.09	0.13±0.04	12.68±0.36	0.22±0.01	0.20±0.01	7.47±3.66	45.96±1.80
DT500	0.46±0.23	0.92±0.16	0.09±0.03	10.85±1.03	0.18±0.01	0.17±0.01	8.32±2.61	38.87±1.90
DI (3 U/rat)	2.23±0.42$^{\#\#}$	2.54±0.68$^{\#\#}$	0.45±0.11$^{\#}$	12.85±0.73	0.24±0.01	0.25±0.01	20.12±3.02$^{\#\#}$	45.50±1.10
DG (2 g/kg)	1.22±0.41$^{\#}$	1.80±0.27$^{\#}$	0.23±0.06*	13.37±0.40	0.25±0.01	0.25±0.01	19.64±5.15$^{\#\#}$	40.03±7.03

EDL: Extensor digitorum longus, *B. virgilioides: Bowdichia virgilioides*, NC: Non-diabetic control, DC: Diabetic control, DT100: Diabetic treated with 100 mg/kg extract, DT250: Diabetic treated with 250 mg/kg extract, DT500: Diabetic treated with 500 mg/kg extract, DI: Diabetic treated with insulin, DG: Diabetic treated with glibenclamide, SEM: Standard error of the mean, ANOVA: Analysis of variance. The values are expressed as mean±SEM of 5-8 animals. *$P<0.05$, $^{\#}P<0.01$, $^{\#\#}P<0.001$ versus DC (ANOVA one-way)

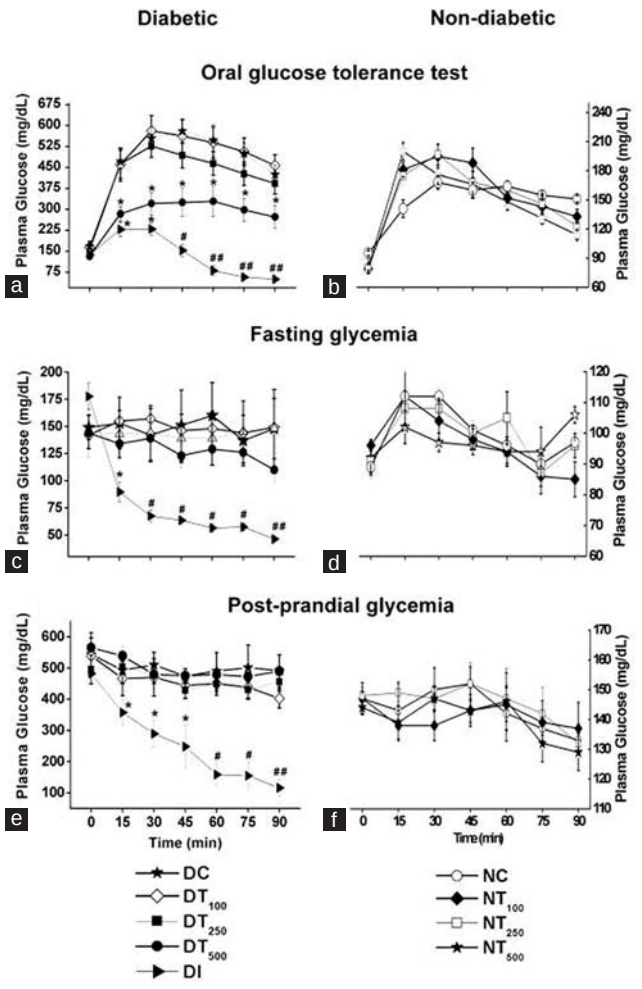

Figure 1: Effects of *Bowdichia virgilioides* root bark extract on oral glucose tolerance (a and b), fasting glycemia (c and d), and post-prandial glycemia (e and f) of diabetic control (DC) and non-diabetic control, diabetic and non-diabetic treated with 100 mg/kg extract, diabetic and non-diabetic treated with 250 mg/kg extract diabetic and non-diabetic treated with 500 mg/kg extract, diabetic treated with insulin. Values are expressed as mean ± standard error of the mean of 5-8 animals. *$P < 0.05$, $^{\#}P < 0.01$, $^{\#\#}P < 0.001$ versus DC (one-way analysis of variance)

recently demonstrated that the root bark of *Paeonia suffruticosa* presented an *in vitro* antidiabetic effect through inhibition of glucose uptake by intestinal brush border membrane vesicles and enhancement of the glucose uptake by culture fibroblasts and adipocytes cells [43]. This same study also demonstrated that peonol, an active compound isolated from non-polar fraction of *Paeonia suffruticosa* root bark, had an *in vivo* beneficial effect, with amelioration of oral glucose tolerance in diabetic rats, corroborating the study from Jung *et al.* [44] that demonstrated the antihyperglycemic effect of this plant. Many other studies showed significant improvement in the glucose tolerance of STZ-diabetic rats treated with different plant extracts, for example *Plantago ovata* husks [45], *Sclerocarya birrea* stem bark [46] and *Ipomoea aquatica* leaf stem [47]. According to these studies, the antihyperglycemic effect could be achieved, at least in part, by inhibition of intestinal glucose absorption. In this same way, the present work showed that the administration of *B. virgilioides* root extract in association with a glucose loading markedly reduced the hyperglycemia peak in diabetic rats. The absence of effects on fasting and post-prandial glycemia values and in subchronic treatment suggested that the plant extract probably exerts its effect via inhibition of the glucose intestinal absorption, and might not have effect on glucose uptake by peripheral tissues. However, changes in the glucose tolerance were not observed in normal rats treated with *B. virgilioides* extract.

Medicinal plants acting at intestinal level to promote the antihyperglycemic effect may inhibit glucose absorption at two distinct targets: (i) classical carbohydrate absorption mediated by the Na+/glucose co-transporter, and (ii) facilitative transport through GLUT2 present in the apical membrane [48]. It has been shown that insulin reduces the GLUT2 quantity in both apical and basolateral enterocyte membranes, promoting a rapid traffic of this glucose transporter away from the plasma membrane and preventing GLUT2 insertion into the apical membrane, independently of the glucose amount in the luminal intestine [49]. Furthermore, apical membrane GLUT2 is dramatically increased in an experimental diabetes model characterized by insulinopenia [50], and changes in GLUT2 quantity in the apical enterocyte membrane of

diabetic rats treated with antihyperglycemic plant extracts cannot be ruled out. In agreement with this present work, the glycemia reduction observed in diabetic rats treated with *B. virgilioides* extract could be explained, at least in part, by a decrease of the large GLUT2 number in the apical enterocyte membrane. The same effect was not observed after the treatment of normal rats, which possibly have the regulation of this glucose transporter by insulin under regular conditions. Similar results were found by Ndong *et al*. [51], which examined the effects of *Moringa oleifera* extract on the glucose tolerance in normal Wistar rats and Goto-Kakizaki diabetic rats. *Moringa oleifera* oral administration ameliorated the glycemia in both rats, but the beneficial effect promoted by plant treatment was more evident in Goto-Kakizaki diabetic rats than in normal Wistar rats. Further studies are needed to clarify the different responses between normal and diabetic rats in the antihyperglycemic effect of *B. virgilioides* extract, mainly in the GLUT2 regulation at enterocyte membrane. Finally, another mechanism by which this extract could be promoting a decrease in the glucose intestinal absorption is through enhanced intestinal motility, a mechanism that could be more investigated.

The chemical characterization of *B. virgilioides* extract indicated the presence of alkaloids, flavonoids and terpenoids (unpublished data). Since flavonoids and terpenoids are associated with antidiabetic activity of several plant species, it could be speculated that the antihyperglycemic effect of *B. virgilioides* extract is related to these compounds. Complementary studies are necessary to clarify this hypothesis.

The results of our work indicate that *B. virgilioides* root extract promotes an acute antihyperglycemic effect in STZ-diabetic rats; this effect probably occurs through an inhibition of the intestinal glucose absorption. The continuity of the research is necessary to elucidate these possibilities. Although several studies have demonstrated the antidiabetic properties of medicinal plants, the use of phytochemicals in diabetes treatment has not been validated with scientific criteria that support their substitution for the conventional therapy. This indicates that, in addition to studies showing the plant antihyperglycemic activity, other approaches are necessary to explore more profoundly these findings, which will help to consolidate the use of medicinal plants in the treatment of diabetes mellitus.

ACKNOWLEDGMENTS

We thank the technicians Air Francisco Costa, Marlene Mariano and Libério Neto for their help and Msc. Harri Lourenzi of the Plantarum Institute for identification and authentication of *Bowdichia virgilioides*. We are also grateful for the support given by Fundação de Amparo à Pesquisa do Estado de Mato Grosso (Cuiabá, Brazil), Coordenação de Aperfeiçoamento de Pessoal Superior (Brasília, Brazil), Conselho Nacional de Pesquisa Científica (CNPq) and Centro de Pesquisa do Pantanal (CPP) for financial support.

REFERENCES

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010;87:4-14.
2. Caballero B, Rubinstein S. Environmental factors affecting nutritional status in urban areas of developing countries. Arch Latinoam Nutr 1997;47:3-8.
3. Seidell JC. Obesity, insulin resistance and diabetes – A worldwide epidemic. Br J Nutr 2000;83 Suppl 1:S5-8.
4. Misra A, Khurana L. Obesity and the metabolic syndrome in developing countries. J Clin Endocrinol Metab 2008;93:S9-30.
5. Warren RE. The stepwise approach to the management of type 2 diabetes. Diabetes Res Clin Pract 2004;65 Suppl 1:S3-8.
6. Cohen A, Horton ES. Progress in the treatment of type 2 diabetes: New pharmacologic approaches to improve glycemic control. Curr Med Res Opin 2007;23:905-17.
7. Ivorra MD, Payá M, Villar A. A review of natural products and plants as potential antidiabetic drugs. J Ethnopharmacol 1989;27:243-75.
8. Alarcon-Aguilara FJ, Roman-Ramos R, Perez-Gutierrez S, Aguilar-Contreras A, Contreras-Weber CC, Flores-Saenz JL. Study of the anti-hyperglycemic effect of plants used as antidiabetics. J Ethnopharmacol 1998;61:101-10.
9. Yeh GY, Eisenberg DM, Kaptchuk TJ, Phillips RS. Systematic review of herbs and dietary supplements for glycemic control in diabetes. Diabetes Care 2003;26:1277-94.
10. Alonso-Castro AJ, Salazar-Olivo LA. The anti-diabetic properties of *Guazuma ulmifolia* Lam are mediated by the stimulation of glucose uptake in normal and diabetic adipocytes without inducing adipogenesis. J Ethnopharmacol 2008;118:252-6.
11. Tamrakar AK, Kumar R, Sharma R, Balapure AK, Lakshmi V, Srivastava AK. Stimulatory effect of *Ceriops tagal* on hexose uptake in L6 muscle cells in culture. Nat Prod Res 2008;22:592-9.
12. Wang ZQ, Ribnicky D, Zhang XH, Raskin I, Yu Y, Cefalu WT. Bioactives of *Artemisia dracunculus* L enhance cellular insulin signaling in primary human skeletal muscle culture. Metabolism 2008;57:S58-64.
13. Baviloni PD, dos Santos MP, Aiko GM, Reis SR, Latorraca MQ, da Silva VC, *et al*. Mechanism of anti-hyperglycemic action of *Vatairea macrocarpa* (Leguminosae): Investigation in peripheral tissues. J Ethnopharmacol 2010;131:135-9.
14. Shinde J, Taldone T, Barletta M, Kunaparaju N, Hu B, Kumar S, *et al*. Alpha-glucosidase inhibitory activity of *Syzygium cumini* (Linn.) Skeels seed kernel *in vitro* and in *Goto-Kakizaki* (GK) rats. Carbohydr Res 2008;343:1278-81.
15. Vianna R, Brault A, Martineau LC, Couture R, Arnason JT, Haddad PS. *in vivo* anti-diabetic activity of the ethanolic crude extract of *Sorbus decora* C.K. Schneid. (Rosacea): A medicinal plant used by Canadian james bay cree nations to treat symptoms related to diabetes. Evid Based Complement Alternat Med 2011;2011:237941.
16. Bourdy G, DeWalt SJ, Chávez de Michel LR, Roca A, Deharo E, Muñoz V, *et al*. Medicinal plants uses of the Tacana, an Amazonian Bolivian ethnic group. J Ethnopharmacol 2000;70:87-109.
17. Deharo E, Bourdy G, Quenevo C, Muñoz V, Ruiz G, Sauvain M. A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part V. Evaluation of the antimalarial activity of plants used by the Tacana Indians. J Ethnopharmacol 2001;77:91-8.
18. Almeida JR, Silva-Filho RN, Nunes XP, Dias CS, Pereira FO, Lima EO. Antimicrobial activity of the essential oil of *Bowdichia virgilioides*, Kunt. Braz J Pharmacol 2000;16:638-41.
19. Oliveira F, Saito ML. Some Brazilian plants employed in diabetes treatment. Braz J Pharmacol 1989;2/4:170-96.
20. Torrenegra R, Bauereiss P, Achenbach H. Homoormosanine-type alkaloids from *Bowdichia virgilioides*. Phytochem 1989;28:2219-21.
21. Marinho LC, Cunha MT, Thomas G, Barbosa-Filho JM. Constituents of *Bowdichia virgilioides*. Fitoterapia 1994;65:475-6.
22. Melo FN, Navarro VR, Silva MS, Da-Cunha EV, Barbosa-Filho JM, Braz-Filho R. Bowdenol, a new 2,3-dihydrobenzofuran constituent from *Bowdichia virgilioides*. Nat Prod Lett 2001;15:261-6.
23. Barbosa-Filho JM, Da Silva Almeida JR, De Oliveira Costa VC, Da-Cunha EV, Da Silva MS, Braz-Filho R. Bowdichine, a new diaza-adamantane alkaloid from *Bowdichia virgilioides*. J Asian Nat Prod Res 2004;6:11-7.
24. Arriaga MC, Machado MI, Gomes GA, Craveiro AA. Volatile constituents from roots of *Bowdichia virgilioides*, Kunt. J Essent Oil Res 1998;10:205-6.

25. Velozo LS, Silva BP, Silva BE, Parente JP. Constituents from the roots of *Bowdichia virgilioides*. Fitoterapia 1999;70:532-535.
26. Arriaga AM, Gomes GA, Braz-Filho R. Constituents of *Bowdichia virgilioides*. Fitoterapia 2000;71:211-2.
27. Juck DB, De Rezende LC, David JP, De Queiroz LP, David JM. Two new isoflavonoids from *Bowdichia virgilioides*. Nat Prod Res 2006;20:27-30.
28. Thomazzi SM, Silva CB, Silveira DC, Vasconcellos CL, Lira AF, Cambui EV, *et al*. Antinociceptive and anti-inflammatory activities of *Bowdichia virgilioides* (sucupira). J Ethnopharmacol 2010;127:451-6.
29. Mell CD. Interesting sources of natural dyestuffs. Text Colorist 1929;51:453-5.
30. Jorge-Neto J. Pharmacognostic study of essential oil of sucupira, *Bowdichia virgilioides*. Ver Fac Farm Odontol Araraquara 1970;4:203-4.
31. Marzouk MS, Soliman FM, Shehata IA, Rabee M, Fawzy GA. Flavonoids and biological activities of Jussiaea repens. Nat Prod Res 2007;21:436-43.
32. Sharma B, Balomajumder C, Roy P. Hypoglycemic and hypolipidemic effects of flavonoid rich extract from *Eugenia jambolana* seeds on streptozotocin induced diabetic rats. Food Chem Toxicol 2008;46:2376-83.
33. Tan MJ, Ye JM, Turner N, Hohnen-Behrens C, Ke CQ, Tang CP, *et al*. Antidiabetic activities of triterpenoids isolated from bitter melon associated with activation of the AMPK pathway. Chem Biol 2008;15:263-73.
34. Lü H, Chen J, Li WL, Ren BR, Wu JL, Kang HY, *et al*. Hypoglycemic and hypolipidemic effects of the total triterpene acid fraction from Folium Eriobotryae. J Ethnopharmacol 2009;122:486-91.
35. Grace MH, Ribnicky DM, Kuhn P, Poulev A, Logendra S, Yousef GG, *et al*. Hypoglycemic activity of a novel anthocyanin-rich formulation from lowbush blueberry, *Vaccinium angustifolium* Aiton. Phytomedicine 2009;16:406-15.
36. Klein G, Kim J, Himmeldirk K, Cao Y, Chen X. Antidiabetes and Anti-obesity Activity of Lagerstroemia speciosa. Evid Based Complement Alternat Med 2007;4:401-7.
37. Malone MH, Robichaud RC. A hippocratic screen for pure or crude drug materials. Lloydia 1962;25:320-332.
38. Summerson WH, Hank PB, Oser BL. Practical Physiological Chemistry, 12th ed. New York: Blakiston Co.; 1947.
39. Carroll NV, Longley RW, Roe JH. The determination of glycogen in liver and muscle by use of anthrone reagent. J Biol Chem 1956;220:583-93.
40. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957;226:497-509.
41. Szkudelski T. The mechanism of alloxan and streptozotocin action in beta cells of the rat pancreas. Physiol Res 2001;50:536-46.
42. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia 2008;51:216-26.
43. Lau CH, Chan CM, Chan YW, Lau KM, Lau TW, Lam FC, *et al*. Pharmacological investigations of the anti-diabetic effect of cortex moutan and its active component paeonol. Phytomedicine 2007;14:778-84.
44. Jung CH, Zhou S, Ding GX, Kim JH, Hong MH, Shin YC, *et al*. Antihyperglycemic activity of herb extracts on streptozotocin-induced diabetic rats. Biosci Biotechnol Biochem 2006;70:2556-9.
45. Hannan JM, Ali L, Khaleque J, Akhter M, Flatt PR, Abdel-Wahab YH. Aqueous extracts of husks of Plantago ovata reduce hyperglycaemia in type 1 and type 2 diabetes by inhibition of intestinal glucose absorption. Br J Nutr 2006;96:131-7.
46. Dimo T, Rakotonirina SV, Tan PV, Azay J, Dongo E, Kamtchouing P, *et al*. Effect of *Sclerocarya birrea* (Anacardiaceae) stem bark methylene chloride/methanol extract on streptozotocin-diabetic rats. J Ethnopharmacol 2007;110:434-8.
47. Sokeng SD, Rokeya B, Hannan JM, Junaida K, Zitech P, Ali L, *et al*. Inhibitory effect of Ipomoea aquatica extracts on glucose absorption using a perfused rat intestinal preparation. Fitoterapia 2007;78:526-9.
48. Kellett GL, Brot-Laroche E, Mace OJ, Leturque A. Sugar absorption in the intestine: The role of GLUT2. Annu Rev Nutr 2008;28:35-54.
49. Tobin V, Le Gall M, Fioramonti X, Stolarczyk E, Blazquez AG, Klein C, *et al*. Insulin internalizes GLUT2 in the enterocytes of healthy but not insulin-resistant mice. Diabetes 2008;57:555-62.
50. Burant CF, Flink S, DePaoli AM, Chen J, Lee WS, Hediger MA, *et al*. Small intestine hexose transport in experimental diabetes. Increased transporter mRNA and protein expression in enterocytes. J Clin Invest 1994;93:578-85.
51. Ndong M, Uehara M, Katsumata S, Suzuki K. Effects of oral administration of *Moringa oleifera* Lam on glucose tolerance in goto-kakizaki and wistar rats. J Clin Biochem Nutr 2007;40:229-33.

Source of Support: Nil, Conflict of Interest: None declared.