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Effect of *Bauhinia holophylla* treatment in Streptozotocin-induced diabetic rats

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ABSTRACT

Bauhinia holophylla, commonly known as "cow's hoof", is widely used in Brazilian folk medicine for the diabetes treatment. Therefore, the aim of this study was at evaluating the aqueous extract effect of *Bauhinia holophylla* leaves treatment on the streptozotocin-induced diabetic rats. Diabetes was induced by Streptozotocin (40 mg/Kg) in female Wistar rats. Oral administration of aqueous extract of *Bauhinia holophylla* leaves was given to non-diabetic and diabetic rats at a dose of 400 mg/kg during 21 days. On day 17 of treatment, the Oral Glucose Tolerance Test was performed to determine the area under the curve. At the end of the treatment, the animals were anesthetized and blood was collected for serum biochemical parameters analysis. After treatment with *Bauhinia holophylla* extract, non-diabetic and diabetic rats presented no glycemic changes. On the other hand, the plant treatment decreased body weight and increased ALT and AST activities. In conclusion, the treatment with aqueous extract of *B. holophylla* leaves given to diabetic rats presented no hypoglycemic effect in nondiabetic animals and no antidiabetic effect in diabetic animals with the doses studied. In addition, the diabetic animals treated with the *B. holophylla* extract showed inconvenient effects and its indiscriminate consumption requires particular carefulness.

Key words: *Bauhinia holophylla*, diabetes, lipid profile, medicinal plants, rats.

INTRODUCTION

Diabetes mellitus is the name given to a group of disorders with different etiologies. It is characterized by disarrangements in carbohydrates, proteins and fat metabolism caused by complete or partial insufficiency of insulin secretion and/or insulin action (ADA 2016, Reece et al. 2004).

Several drugs are used to control diabetes, however, perfect glucose control is rarely achieved (Cooppan 2005). Moreover, plants have been used as an alternative therapy for the diabetes treatment. Many plants present hypoglycemic activity, which were demonstrated experimentally in animals and humans, but some still require further investigation (Volpato et al. 2002, Damasceno and Volpato 2008). Although several plants were tested for diabetes treatment, many of them were not evaluated, including species of the genus *Bauhinia*, popularly known as "cow's hoof" (Lorenzi and Matos 2002). These species typically present a wide distribution

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in tropical regions such as Asia, Africa and South America, so they have adapted well to the Brazilian climate (Donato 1995). Also, the biological properties of different preparations of *Bauhinia spp*. were investigated in *in vitro* and *in vivo* studies and their results encouraged the hypothesis of a hypoglycemic activity (Cechinel Filho 2009). Some attempts were made to attribute the antidiabetic effects of the leaf extracts to the kaempferitrin, the major flavonoid derivative (Pinheiro et al. 2006). Several studies showed the hypoglycemic effect of *Bauhinia forficata* (Silva et al. 2002, Pepato et al. 2004, Lino et al. 2004, Frankish et al. 2010), then other species of the same genus may have similar effect by the phylogenetic and chemistry proximity. Chemotaxonomy in conjunction with phylogenetic analysis may provide novel insights into species delimitation and chemical ecology (Kim et al. 2016).

Bauhinia holophylla (Leguminosae) is one of the species of *Bauhinia* used as antidiabetic. There are no studies in the literature to evaluate the hypoglycemic potential effect of *B. holophylla*. Our hypothesis is that this medicinal plant may have a beneficial result on hyperglycemic status, preventing the diabetic repercussions. Therefore, the aim of this study was at evaluating the aqueous extract effect of *B. holophylla* leaves treatment on the streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

EXTRACTION OF PLANT MATERIALS

The *Bauhinia holophylla* leaves were collected in Barra do Garças, Mato Grosso State, Brazil, between April and May 2013, in the morning period. The plant was identified and authenticated from the Botanic Department of Federal University of Mato Grosso (UFMT), where a voucher specimen (UFMT 05718) was left. Their leaves were dried at 50°C for a period of 24 h in an aerated stove, ground and a powder was prepared, similarly to the folk-medicine preparation method. *B. holophylla* aqueous extract was prepared by boiling 1 liter (L) of water for 5 minutes (min) and adding 70 grams (g) of the powder. The extract was agitated and covered until it reached room temperature. The residue was removed by filtration and the extract was then suitably concentrated in a rotary evaporator. A sample was separated for determination of the solid concentration, and the extract was divided into aliquots stored at -20°C until further use.

EXPERIMENTAL ANIMALS

Female Wistar rats (200-230 g), approximately 90 days old, were obtained from the UFMT Vivarium and were maintained under standard laboratory conditions (22±3°C, 12-h light/dark cycle), with pelleted food (Purina rat chow, Purina®, São Paulo, SP, Brazil) and tap water *ad libitum*. The procedures and animal handling were performed in accordance with the guidelines provided by the Brazilian College of Animal Experimentation in agreement with the International Guiding Principles for Biomedical Research Involving Animals promulgated by the Society for the Study of Reproduction and were authorized by the Ethical Committee for Animal Research of the UFMT, Brazil (protocol number 23108.001989/13-0).

After two weeks of acclimatization, the diabetes was induced in rats with Streptozotocin (STZ, Sigma Chemical Company®, St. Louis, Millstone). STZ was intravenously (i.v.) administered in a dose of 40 mg/Kg dissolved in citrate buffer (0.1 M, pH 6.5). Control rats received i.v. citrate buffer. Blood glucose concentrations were measured by One Touch Ultra glucometer (Johnson & Johnson®) seven days after diabetes induction, and glucose concentrations exceeded 300 mg/dL confirmed the diabetic state (Damasceno et al. 2012).

EXPERIMENTAL GROUPS

After diabetic state was confirmed, the rats were placed in four experimental groups (n=11 animals/

group): Control - rats treated with vehicle (water); Treated Control - rats treated with *B. holophylla* extract; Diabetic - diabetic rats treated with vehicle; and Treated Diabetic - diabetic rats treated with *B. holophylla* extract. The treatment dose of 400 mg/Kg/day of the *B. holophylla* extract was orally given by gavage during 21 days. Body weight, water and food intake and blood glucose were also evaluated weekly in the morning period.

BIOCHEMICAL PROFILE ANALYSIS

Oral glucose tolerance test (OGTT) was performed at day 17 of treatment. After an overnight fasting of 12 hours, a glucose solution (200g/L) was administered into the stomach of the rats through a gastric catheter, at a final dose of 2 g/Kg body weight. The treated groups also received the plant extract 15 min. before glucose administration. Blood glucose concentrations were measured at 0 (previous administration of glucose solution/plant extract – fasting glucose), 30, 60 and 120 min (Mello et al. 2001, Sinzato et al. 2012). Glucose responses during the OGTT were evaluated by estimation of the total area under the curve (AUC), using the trapezoidal method (Tai 1994).

At day 21 of treatment, the rats were anesthetized by sodium pentobarbital (Tiopentax® - 50 mg/Kg) and blood samples were collected by decapitation for biochemical determinations. The blood samples were collected and put into anticoagulant-free test tubes, maintained in ice for 30 min and then centrifuged at 1300×g during 10 min at 4◦C. The supernatant was collected as serum and stored at -80◦C for further determination of biochemical parameters.

Serum concentrations of total protein (TP) was determined colorimetric method, and total cholesterol (CHO), triglycerides (TG), high-density lipoprotein (HDL-c) concentrations; and also alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were estimated by enzymatic method (Young 2000) by Winner® assay kits. The values were expressed in milligrams per deciliter (mg/dL). Very-low-density lipoprotein (VLDL) serum level estimated value was calculated through the triglyceride concentrations (Knopfholz et al. 2014).

STATISTICAL EVALUATION

Analysis of variance (ANOVA) followed by Tukey's Multiple Comparison test was used to compare mean values. Differences were considered statistically significant when $p < 0.05$.

RESULTS

As shown in Figure 1, blood glucose levels presented near 100 mg/dL in control groups. In the diabetic groups, glucose levels were higher than 300 mg/dL. The treatment with *B. holophylla* aqueous extract did not interfere significantly with the glycaemia of control or diabetic groups compared with their corresponding groups.

The animals of control and treated groups showed no difference in body weight, water and food intake during the study. The body weight in diabetic groups was statistically lower compared to control on days 14 and 20. In addition, the plant treatment caused a reduction in the body weight compared to the diabetic group (non-treated) on days 14 and 20. The rats from diabetic groups presented higher food intake (around 30 g/day) in relation to control groups (about 16 g/day) in all experimental period. In addition, the average water intake was increased in diabetic groups as compared to non-diabetic groups (Table I).

Figure 2a shows the glycemic curve of the oral glucose tolerance test of control groups and Figure 2b of diabetic groups. The rats in both diabetic groups presented statistically higher blood glucose levels in relation to those of control animals throughout the test. The non-treated groups showed the glycemic curve with a similar pattern with increasing glucose levels at 30 minutes compared to time 0, and returning to the basal level at the time 120 in both groups (control and diabetic). The blood glucose levels of diabetic animals treated with the plant presented no significant statistically difference between all timepoints measured (Figure 2b).

The control groups showed an area under the curve around 14,000 mg/dL/120 minutes. The rats

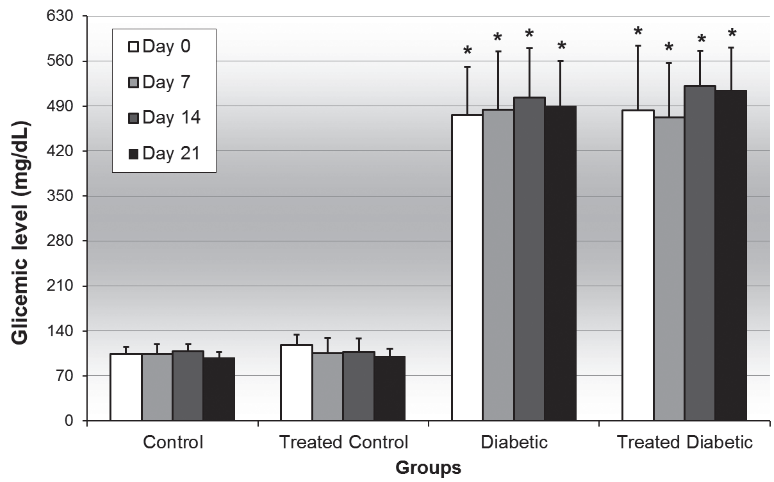

Figure 1 - Blood glucose levels on days 0, 7, 14 and 21 of the control and diabetic rats treated or not with a *Bauhinia holophylla* aqueous extract (400 mg/Kg) during 21 days of experiment. Data shown as mean± standard deviation. * p<0.05 compared control group (ANOVA followed Tukey's multiple comparison test).

TABLE I
Body weight, water intake and food intake on days 0, 7, 14 and 21 of the control and diabetic rats treated or not with a *Bauhinia holophylla* aqueous extract (400 mg/Kg) during 21 days of experiment.

	Groups			
	Control	Treated Control	Diabetic	Treated Diabetic
Body weight (g)				
Day 0	249.7 ± 22.1	251.0 ± 21.7	243.6 ± 19.3	232.8 ± 17.7
Day 7	255.0 ± 18.5	245.0 ± 20.3	232.5 ± 17.9	213.4 ± 26.5*
Day 14	253.9 ± 15.9	243.0 ± 22.6	230.6 ± 19.6*	210.4 ± 24.6*#
Day 20	255.8 ± 16.1	241.5 ± 19.0	230.9 ± 22.4*	208.1 ± 20.8*#
Water intake (mL)				
Day 0	39.1 ± 8.0	36.5 ± 5.3	114.6 ± 49.2*	99.1 ± 53.4*
Day 7	35.5 ± 6.1	36.0 ± 6.7	117.9 ± 55.0*	115.7 ± 55.3*
Day 14	34.6 ± 5.7	35.8 ± 6.5	138.9 ± 44.1*	124.1 ± 45.9*
Day 20	35.5 ± 4.7	35.2 ± 6.4	146.4 ± 45.5*	125.5 ± 47.5*
Food intake (g)				
Day 0	17.6 ± 3.9	16.1 ± 2.8	23.6 ± 7.2	24.6 ± 8.7
Day 7	17.3 ± 3.3	17.6 ± 3.1	26.9 ± 8.3*	25.1 ± 4.8*
Day 14	17.1 ± 2.2	16.7 ± 2.5	32.1 ± 7.4*	30.2 ± 8.9*
Day 20	16.6 ± 2.0	16.9 ± 3.6	35.4 ± 8.5*	28.9 ± 8.1*

Data shown as mean± standard deviation (SD). * p<0.05 compared to control group (ANOVA followed Tukey's multiple comparison test). # p<0.05 compared to diabetic group (ANOVA followed Tukey's multiple comparison test).

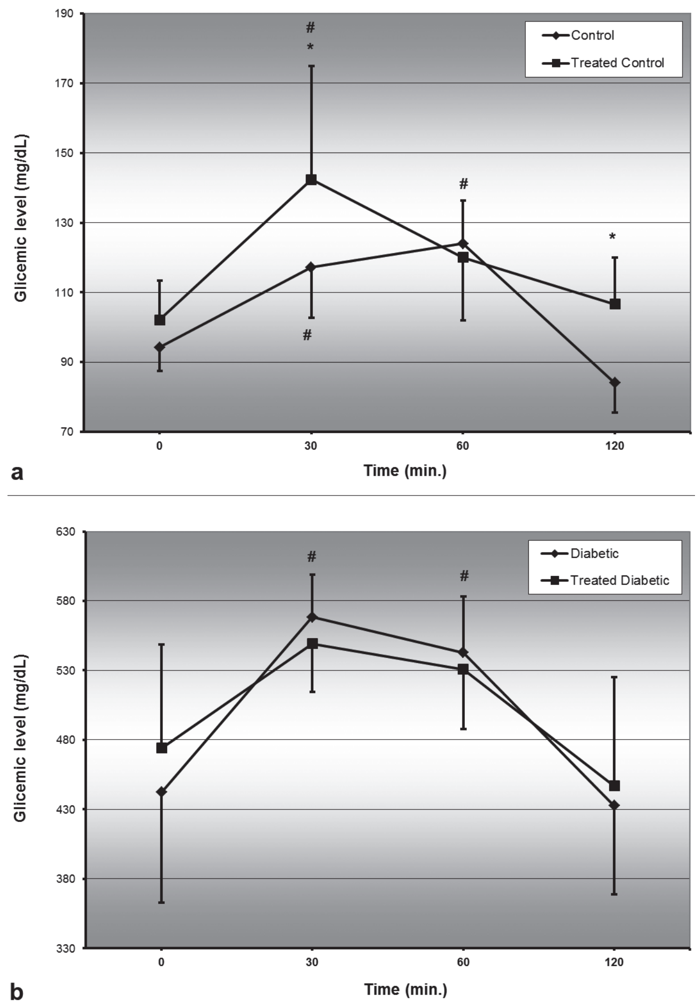

Figure 2 - Oral glucose tolerance test on the day 17 of treatment of control (**a**) and diabetic (**b**) rats treated or not with a *Bauhinia holophylla* aqueous extract (400 mg/Kg) during 21 days of experiment. Data shown as mean± standard deviation (SD). * $p<0.05$ compared to control group (ANOVA followed Tukey´ s multiple comparison test). [#] $p<0.05$ compared to Time 0 (ANOVA followed Tukey's multiple comparison test).

from diabetic groups showed an area under the curve higher compared to control groups, reaching approximately 60,000 mg/dL/120 minutes. The *B. holophylla* extract treatment did not change data about area under the curve as compared to the untreated diabetic group (Figure 3).

As shown in Table II, the treated control group presented HDL-cholesterol values decreased as compared to those of control group. The diabetic group presented higher levels of serum total proteins in relation to those of control group. Both diabetic groups showed higher levels of triglycerides, total cholesterol, VLDL-cholesterol concentrations, and also ALT and AST activities compared to those of control group. The treated diabetic group showed decreased HDL-cholesterol levels as compared to those of the control and diabetic groups. In addition, the plant treatment caused increased ALT and AST activities in diabetic animals compared to diabetic group.

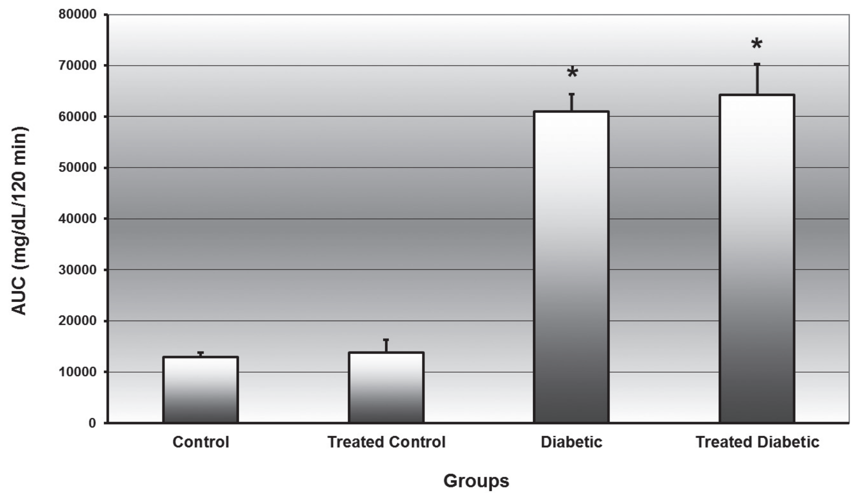

Figure 3 - Area under the curve (AUC) in the day 17 of treatment of control and diabetic rats treated or not with a *Bauhinia holophylla* aqueous extract (400 mg/Kg) during 21 days of experiment. Data shown as mean± standard deviation. * p<0.05 compared control group (ANOVA followed Tukey's multiple comparison test).

TABLE II
Biochemical parameters of control and diabetic rats treated or not with a *Bauhinia holophylla* aqueous extract (400 mg/Kg) during 21 days of experiment.

	Groups			
	Control	Treated Control	Diabetic	Treated Diabetic
Total protein (g/dL)	4.3 ± 0.4	4.3 ± 0.2	5.0 ± 0.3*	4,7 ± 0,7
Triglycerides (mg/dL)	91.8 ± 27.3	120.0 ± 36.3	593.3 ± 358.6*	797.7 ±104.3*
Cholesterol (mg/dL)	81.1 ± 6.7	77.5 ± 13.7	113.0 ± 20.4*	107.7 ± 13.9*
HDL (mg/dL)	60.4 ± 8.2	26.9 ± 2.1*	61.9 ± 10.2	40.2 ± 5.7*#
VLDL (mg/dL)	18.3 ± 5.5	24.0 ± 7.3	118.7 ± 71.7*	159.5 ± 20.9*
ALT (U/l)	78.2 ±12.1	54.5 ± 13.9	186.7 ± 93.2*	390.0 ± 52.0*#
AST (U/l)	196.6 ± 29.6	180.0 ± 42.6	327.6 ± 146.3*	981.0 ± 151.3*#

Data shown as mean± standard deviation (SD). * p<0.05 compared to control group (ANOVA followed Tukey's multiple comparison test) # p<0.05 compared to diabetic group (ANOVA followed Tukey's multiple comparison test).

DISCUSSION

The estrogen affects the glucose and lipoprotein metabolism. Studies demonstrated that uncontrolled hyperglycemia, hypertension and dyslipidemia is more common in women than male with diabetes (Göbl et al. 2010, Mascarenhas-Melo et al. 2013). This fact explains our choice for females to evaluate the hypoglycaemic effect of *B. holophylla*. These rats entered in the experimental design with 90 days old. In the first day of treatment, the rats were with 120 days old (adulthood). Sengupta (2013) reported that the females rats become sexually mature at 6 weeks (40 days old), and adulthood period begins after the eighth week of post-natal life (~ 60 days old), confirming that the female rats used in this study were also adult.

In our laboratory, previous studies showed no alteration on blood glucose level of rats treated with 200 mg/Kg of the *B. forficata* aqueous extract, which is a traditional dose of this medicinal herbal used by the population (Volpato et al. 1999). Pepato et al. (2002) also showed that acute treatment with *B. forficata* decoction produced no alteration in glycemic levels under to 31 days. Contradictorily, several authors demonstrated hypoglycemic effect of *Bauhinia* species extract or fraction. In addition, some species even has showed insulin mimetic properties and hypoglycemic action, for example *B. variegata* (Silva et al. 2002, Pepato et al. 2004, Lino et al. 2004, Frankish et al. 2010). Rozza et al. (2015) verified that *Bauhinia holophylla* hydroalcoholic extract (150 mg/Kg) enhanced glutathione peroxidase (GSH-Px) and glutathione reductase (GR) activities and the reduced glutathione (GSH) level, suggesting an antioxidant activity from *B. holophylla*. In our study, *B. holophylla* treatment did not interfere in blood glucose levels of the diabetic and it causes no hypoglycemia in non-diabetic rats. This might be justified due to administered dose, extraction method, short treatment period, and the sensitivity difference among the plants used and the animals tested (Campos et al. 2009), which would prevent the effective action by the plant extract. Another variable to consider is the type of diabetes studied. Streptozotocin is known to reproduce the severe diabetes (glycemia above 300 mg/dL), and the plant extract could be more efficient treating individuals with a moderate diabetes (glycemia between 120 and 300 mg/dL). Previous studies testing plant extracts confirmed that animals with severe diabetes also presented no antidiabetic effect (Volpato et al. 2007, 2008, 2011).

The body weight loss represents one of the most common signs of diabetes. Despite the increased appetite, insulin deficiency reduces all anabolic processes and accelerates catabolic processes, contributing further to body weight loss, which is already occurring by glycosuria and polyuria (ADA 2014). This status was showed in this study, since treated animals from both diabetic groups showed body weight loss, especially in the last days of treatment. The *B. holophylla* treatment of diabetic animals led to a decreased body weight, possible due to a toxic effect of the plant in an impaired organism.

The exaggerated appetite, another physiological dysfunction caused by diabetes, is a symptom due to glucose loss in the urine at least in part, which deprives the body of a considerable part of the calories ingested with food (Kahn et al. 2005). Large quantities of glucose are eliminated through the urine, elevating the osmotic pressure and reducing water reabsorption in the renal tubules. Due to this increased diuresis, there is excessive water intake by subject via stimulation of the thirst center in hypothalamus (Powers and D'Alessio 2011). The increase in water and food consumption in diabetes was confirmed in our study, and *B. holophylla* treatment did not contribute to improve these disorders.

The blood glucose data generated from the OGTT shows that in non-diabetic rats the insulin uptake follows the expected physiological response, with blood glucose levels returning to its original state at the timepoint 120 min, characterizing a glycemic return (Campos et al. 2007). However, in non-diabetic rats treated with the plant, blood glucose level returned to the initial value already at the timepoint 60 min, which might indicate an increase in insulin release and action, resulting in a faster glucose uptake. The blood glucose level in the OGTT of diabetic rats showed that there was no reduction of the response time or the blood glucose levels of these rats. It is important to consider the diabetes type studied in this experiment. In the present study, the rats presented glycemia similar to uncontrolled human type 1 diabetes, since the Streptozotocin has a beta-cytotoxic action (Tancrede et al. 1983), leading to abnormal/lacking insulin secretion. Since there was no change in the area under the curve of Streptozotocin-induced diabetic rats, we suggest that the plant extract did not contribute to reduce the blood glucose levels.

In diabetic individuals, the lack of insulin causes inhibition of protein synthesis and increased degradation, which increases amino acid levels in the blood to be subsequently used for gluconeogenesis (Abu-Lebdeh and Nair 1996). Our study showed that *B. holophylla* treatment caused a reduction on the serum protein level.

In the present study, the lipid profile of diabetic rats was abnormal as expected for diabetic patients. The triglycerides, total cholesterol and VLDL-cholesterol levels were higher as compared to the nondiabetic rats due to the action of lipase, whose activity is exacerbated in individuals with reduced glucose utilization. According to Umrani and Goyal (2002), the streptozotocin-induced diabetic animals presented a decreased insulin levels, increased triglycerides, and total cholesterol levels, corroborating with our results. Therefore, the *B. holophylla* treatment also causes no changes in these biochemical parameters. Some studies showed a biological tendency of the HDL-cholesterol level to accompany the total cholesterol levels (Kiss et al. 2006, Turner et al. 2004). Our results showed reduced HDL-cholesterol levels in treated groups, which might indicate metabolic changes in the liver as a result of treatment, either due to reduction in its production or its function.

The ALT and AST enzymes are found mainly in the hepatocytes, and when high levels are detected, it is possible to confirm the diagnosis of liver damage (Pratt and Kaplan 2000). Our study presented high ALT and AST activities in diabetic groups. The *B. holophylla* treatment also caused increased activities of these enzymes, suggesting this plant led to a liver injury. This finding in association with the decreased body weight reinforce the possible toxic effect of the plant in an impaired organism by diabetes.

In conclusion, the treatment with aqueous extract of *B. holophylla* leaves given to diabetic rats presented no hypoglycemic effect in nondiabetic animals and no antidiabetic effect in diabetic animals with the doses studied. In addition, the diabetic animals treated with the *B. holophylla* extract caused inconvenient effects and its indiscriminate consumption requires particular carefulness.

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