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ORIGINAL ARTICLE

Chronic treatment with hydroalcoholic extract of *Plathymenia reticulata* promotes islet hyperplasia and improves glycemic control in diabetic rats

Tratamento crônico com extrato hidroalcoólico de *Plathymenia reticulata* promove hiperplasia de ilhotas e controle glicêmico em ratos diabéticos

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ABSTRACT

Objective: To investigate the anti-hyperglycemic effects of *Plathymenia reticulata* hydroalcoholic extract and related changes in body weight, lipid profile and the pancreas. **Methods:** Diabetes was induced in 75 adult male Wistar rats via oral gavage of 65mg/Kg of streptozotocin. Rats were allocated to one of 8 groups, as follows: diabetic and control rats treated with water, diabetic and control rats treated with 100mg/kg or 200mg/kg of plant extract, and diabetic and control rats treated with glyburide. Treatment consisted of oral gavage for 30 days. Blood glucose levels and body weight were measured weekly. Animals were sacrificed and lipid profile and pancreatic tissue samples analyzed. Statistical analysis consisted of ANOVA, post-hoc Tukey-Kramer, paired Student's *t* and χ^2 tests; the level of significance was set at 5%. **Results:** Extract gavage at 100mg/kg led to a decrease in blood glucose levels in diabetic rats in the second, third (198.71±65.27 *versus* 428.00±15.25) and fourth weeks (253.29±47.37 *versus* 443.22±42.72), body weight loss (13.22±5.70 *versus* 109.60±9.95) and lower cholesterol levels (58.75±3.13 *versus* 80.11±4.01) in control rats. Extract gavage at 200mg/Kg led to a decrease in glucose levels on the fourth week in diabetic rats, body weight loss in the second, third and fourth weeks in control rats, and lower cholesterol levels in diabetic and control rats. Islet hyperplasia (p=0.005) and pancreatic duct dilation (p=0.047) were observed in diabetic and control rats. **Conclusion:** *Plathymenia* extract reduced blood glucose levels in diabetic rats, and body weight in control rats, and promoted pancreatic islet hyperplasia in diabetic and control rats.

Keywords: *Plathymenia reticulada*; *Diabetes mellitus*; Streptozocin; Islets of langerhans; Plant extracts; *Fabaceae*; Pancreas

RESUMO

Objetivo: Avaliar o efeito anti-hiperglicêmico do extrato hidroalcoólico de *Plathymenia reticulata*, alterações no peso, lipídeos e efeito sobre o pâncreas. **Métodos:** O diabetes foi induzido pela administração de estreptozotocina 65mg/kg, em 75 ratos Wistar adultos machos, divididos em 8 grupos diferentes: ratos diabéticos e controle + água, ratos diabéticos e controle + 100mg/kg ou 200mg/kg de extrato, ratos diabéticos e controle + gliburida. O tratamento foi realizado por gavagem (oral) por 30 dias. Níveis de glicose e peso foram verificados semanalmente. Os

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animais foram sacrificados, e amostras de lipídeos e do pâncreas foram analisadas. A análise estatística incluiu ANOVA, *post-hoc* Tukey-Kramer, teste *t* de Student pareado e teste do χ^2, com nível de significância de 5%. **Resultados:** O extrato 100mg/kg promoveu redução nos níveis de glicose sanguínea em ratos diabéticos na segunda, terceira (198,71±65,27 *versus* 428,00±15,25) e quarta semanas (253,29±47,37 *versus* 443,22±42,72), perda de peso (13,22±5,70 *versus* 109,60±9,95) e diminuição do colesterol (58,75±3,13 *versus* 80,11±4,01) em ratos controle. Com extrato de 200mg/kg, houve redução dos níveis de glicose na quarta semana, nos ratos diabéticos; de peso na segunda, terceira e quarta semanas, nos ratos controle; e de colesterol nos animais diabéticos e controle. Ocorreram hiperplasia de ilhotas (p=0,005) e dilatação dos ductos pancreáticos (p=0,047) em ratos diabéticos e controles. **Conclusão:** O extrato de *Plathymenia* reduziu os níveis de glicose em ratos diabéticos e de peso em ratos controle, além de ter promovido hiperplasia de ilhotas pancreáticas em diabéticos e controles.

Descritores: *Plathymenia reticulata*; *Diabetes mellitus*; Estreptozocina; Ilhotas pancreáticas; Extratos vegetais; *Fabaceae*; Pâncreas

INTRODUCTION

Diabetes mellitus, one of the most common chronic diseases, affects more than 245 million people worldwide and is the fourth or fifth leading cause of death in developing countries. The number of cases amounted to 135 million in 1995, and 415 million in 2015 globally and is estimated to reach 642 million in 2040, with two thirds of affected individuals living in developing countries.[1] Brazil saw a 61,8%-increase in diabetes cases over the course of 10 years.[2]

Increased survival of diabetic individuals led to higher odds of chronic disease-related complications resulting from time of exposure to hyperglycemia. These complications can be highly debilitating to affected individuals, with reduced life expectancy and quality of life, not to mention the cost burden on the health system.[3]

Several plant extracts have been shown to lower blood glucose levels in animals and the great diversity of chemical compound classes suggests a variety of underlying modes of action. Despite therapeutic potential in some cases, in others hypoglycemia may result from toxicity, particularly hepatotoxicity.[4,5]

Plathymenia reticulata Benth, of the *Leguminosae* family, is a typical plant of the *Cerrado,* rich in phenolic compounds such as tannins and flavonoids. Hydrolysable tannins have the ability to inhibit the development of insects, fungi and bacteria.[6] Flavonoids are widely distributed in nature and have important biological effects, including antimicrobial and cardiovascular activity.[7]

The plant known by the common name *vinhático*, a tree belonging to the genus *Plathymenia Benth*, has been studied and two species described: *P. reticulata Benth* (*vinhático* of the field) and *Plathymenia foliolosa Benth* (*vinhático* of the forest).[8] A third species, *Plathymenia modest Burk,* has been reported and grows in Argentina, according to Rizzini.[9] According to Corrêa et al.,[10] the scientific name of *P. reticulata Benth* is "*Chrysoxylon vinhatico casar*". However, Antezana[11] refers to *P. reticulata Benth* as *P. foliolosa Benth*.

The chemical characteristics *of P. reticulata* have been associated with anti-inflammatory activity[6] and two cassane diterpenes have been described,[12] as well as antimicrobial activity. The fraction defined as the condensed tannin-rich fraction (CTPr) is a good source of natural inhibitors of the local inflammation induced by components of a snake venom.[13] Inhibition of 84.7% of staphylococci by hydroalcoholic *P. reticulata* extract at 0.625mg/mL has been reported.[14] Despite the use of this plant extract to treat diabetes in popular medicine, its hypoglycemic potential has not been scientifically proven.

OBJECTIVE

To evaluate the anti-hyperglycemic effects of hydroalcoholic *Plathymenia* extract and related changes in body weight, lipid profile and the pancreas.

METHODS

This experimental study was approved by the Ethics Committee for Animal Experimentation (CEEA 002/2011). Seventy-five young adult male Wistar rats weighing between 180 and 220g obtained from the *Universidade de Uberaba* vivarium were used. Animals were housed in temperature controlled environment (22° to 25°C) and received animal feed and water *ad libitum*. Animals were submitted to a 10-day adaptation period, then randomly allocated to different experimental groups.

Induction of diabetes

Animals were submitted to a 24-hour fasting period, then treated with intraperitoneal injection of 65mg/kg of aqueous streptozotocin solution, which was previously prepared in 10mmol/L sodium citrate buffer (pH 4.5).[15,16] Blood glucose level monitoring was started 7 days after diabetes induction; tests were performed on blood samples collected from the caudal vein using Accu-Chek Performa. Diabetes was defined as body weight loss associated with fasting blood glucose levels higher than 200mg/dL.

Plant study

Collection and botanical identification

Exsiccate samples used for botanical identification and secondary stem inner bark samples used for pharmacological tests were obtained from a *P. reticulata Benth* specimen (family *Leguminosae*, subfamily *Mimosoideae*) collected in September (bloom time) 2006, at the following geographical location: 15° 47' 16.65 " S and 48° 33' 26.96 " (Google Earth, 2010), of Edilândia, Municipality of Cocalzinho - GO/Brasilia, Distrito Federal. The voucher plant specimen was prepared according to standard herborization methods[17,18] and stored in the Biology Department Herbarium of *Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto* of the *Universidade de São Paulo* (USP), Ribeirão Preto (SP), registration number 13120 SPFR, collector name and number M. Groppo 2073.(b)

Plant extract preparation

Plant extracts were obtained from previously selected and fragmented inner bark samples using alcohol solution (70ºC, room temperature). The extract was dried in steam drying then resuspended in distilled water at 100 and 200mg of plant/kg of animal/mL concentration for pharmacological test purposes.

Pharmacognostic control of plant material and plant extract

Tests were performed according to the Brazilian Pharmacopoeia (4th edition)[19] and Simões et al.[20] Pharmacognostic control consisted of the following tests: ash content, acid ash content, moisture content, total volatile and ethanol extractable substances, lipid and resin content, extract density and pH determination, plant and plant extract dry matter content, physicochemical and colorimetric confirmation of chemical groups (saponins, phenols, tannins and flavonoids) according to references provided.

Treatment of animals

Animals in this study were allocated to one of eight groups, as follows: diabetic and non-diabetic animals treated with oral gavage of 100mg/kg (D100 and C100) or 200mg/kg (D200 and C200) of hydroalcoholic plant extract, or 600mcg/kg of glyburide (DG and CG), or not treated (CD and CC – oral gavage of water). Plant extract or water (controls) were given for 30 days.

Blood glucose and body weight measurement

Blood glucose was measured weekly from blood samples collected from the caudal vein using Accu-Chek Performa, following overnight fasting of at least 12 hours. Body weight was measured and recorded prior to the above described procedure.

Animal sacrifice

On the thirtieth and last day of treatment, animals (63) were subjected to a 12-hour fasting period, then anesthetized with intraperitoneal sodium thiopental (50mg/kg). Abdominal and thoracic organs were removed *en block* and blood collected via cardiac or inferior vena cava puncture using a 10mL syringe and 25×8 gauge needle. Blood samples were transferred to dry tubes and spun down at 4,500rpm for 15 minutes. The serum was then transferred to a second tube for determination of high-density lipoprotein-cholesterol (HDL-c), total cholesterol and triglyceride levels using commercial kits (LABTEST). Tests were performed at the biochemistry section of the Clinical Analysis Laboratory of the *Universidade de Uberaba*, Uberaba (MG).

Pathological examination

Thoracic and abdominal organ samples were preserved in 10% formalin and sent to the Animal Pathology Laboratory of *Hospital Veterinário de Uberaba*, State of Minas Gerais, Brazil, for slide preparation. Samples were cut to obtain fragments of pancreas measuring approximately $1cm^3$. Fragments were fixed in 10% formalin and sent to the Histology Laboratory of *Universidade de Uberaba*. Samples used for slide preparation were dehydrated in increasing alcohol concentrations (up to 100%), then bleached in xylene solution and embedded in paraffin. Sections (6μm) were cut using a microtome. Samples were rehydrated, stained with hematoxylin-eosin (HE), dehydrated, cleared, covered with resin and protected by a coverslip.

Statistical analysis

Data were entered into Statistical Package for Social Science (SPSS), version 14.0 data base. Results were expressed as mean±standard error of the mean (SEM). The analyses among the groups were performed using analysis of variance (ANOVA) of a track and the Tukey-Kramer multiple comparisons test (post-hoc). Intragroup analyses were performed using the paired Student's *t* test. Pathological findings were submitted to the χ^2 or the Fischer test as necessary. The level of significance was set at alpha of 5%.

RESULTS

There was a significant decrease in blood glucose levels in diabetic animals treated with 100mg/kg hydroalcoholic

extract (D100) compared to non-treated diabetic animals (DC) in the second (DC with 374.78±32.77mg/dL *versus* D100 with 246.22±45.33mg/dL; p=0.039), third (DC with 428.00±15.25mg/dL *versus* D100 with 198.71±65.27mg/dL; p=0.005) and fourth (DC with 443.22±42.72mg/dL *versus* D100 with 253.29±47.37mg/dL; p=0.021) weeks of treatment. Treatment with 200mg/kg of hydroalcoholic extract (D200) also led to a significant reduction of blood glucose levels in diabetic animals (DC with 443.22±42.72mg/dL *versus* D200 with 201.00±66.97mg/dL; p=0.013), but only after four weeks of treatment. The average change in blood glucose levels during the experiment (*i.e.*, the difference between final and baseline glycemia) was significantly lower in the D200 compared to the control group (-243.50±92.66mg/dL and 87.77±64.01mg/dL respectively; p=0.012) (Table 1).

Variations observed within diabetic animal groups revealed reduction in blood glucose levels compared to baseline in the first week of treatment in animals receiving 100mg/kg (t=3.254; p=0.010). In animals treated with 200mg/kg, blood glucose levels decreased in the second compared to the first (t=2.609; p=0.048) and in the fourth compared to the third (t=2.815; p=0.067) week of treatment. Blood glucose levels did not decrease in animals treated with glyburide or water (controls) (Figure 1).

Data presented in table 2 show that treatment of non-diabetic rats with 100mg/kg hydroalcoholic extract (C100) led to a significant decrease in blood glucose levels in these animals compared to controls in the first (CC with 93.60±2.68mg/dL *versus* C100 with 81.20±1.94mg/dL; p=0.006), second (CC with 102.60±2.31mg/dL *versus* C100 with 80.80±4.84mg/dL; p=0.014) and fourth (CC with 93.10±3.61mg/dL *versus* C100 with 74.33±1.31mg/dL; p=0.001) week of treatment. Treatment with 200mg/kg of hydroalcoholic extract (C200) led to a significant reduction in blood glucose levels in the 4th week (CC with 93.10±3.61mg/dL *versus* C200 with 74.83±8.41mg/dL; p=0.055).

Variations observed within non-diabetic animal groups revealed reduction in blood glucose levels in animals treated with 100mg/kg of extract in the first week compared to baseline (t=2.594; p=0.029) and in the fourth compared to the third week (t=3.690; p=0.006) (Figure 2).

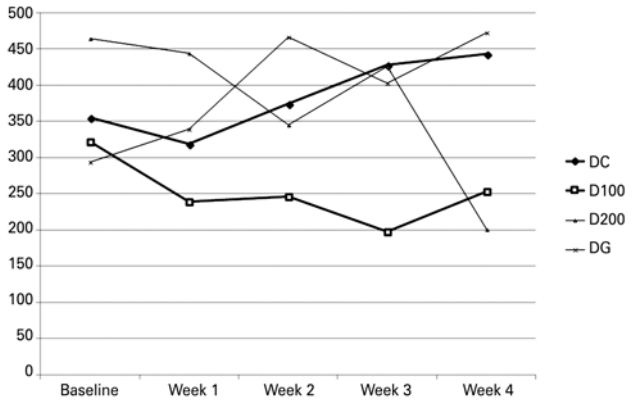
Effects on body weight gain were also evaluated. Treatment with 100mg/kg (D100) or 200mg/kg (D200) of hydroalcoholic extract did not promote significant weight gain in diabetic animals, except in the second week in those receiving 200mg/kg of extract (DC with 213.78±11.42 *versus* D200 with 165.17±3.04; p=0.037). There was no significant body weight variation (final minus initial body weight) in diabetic animals (Table 1).

Table 1. Analysis of blood glucose levels, body weight, and lipids in diabetic animals treated with 100 (D100) or 200mg/kg (D200) of hydroalcoholic extract or glyburide (DG) for 4 weeks, compared to controls (CD)

	CD	D100	D200	DG	p value
Blood glucose					
Baseline	355.44±25.83 (n=9)	322.90±14.09 (n=10)	464.00±22.49* (n=8)	294.56±24.07 (n=9)	0.009
1st week	319.00±43.85 (n=9)	239.50±29.27 (n=10)	444.38±23.73 (n=8)	339.29±43.29 (n=7)	0.362
2nd week	374.78±32.77 (n=9)	246.22±45.33† (n=9)	345.83±18.11 (n=6)	466.14±17.02 (n=7)	0.039
3rd week	428.00±15.25 (n=9)	198.71±65.27† (n=9)	426.00±9.73 (n=5)	403.71±60.56 (n=7)	0.005
4th week	443.22±42.72 (n=9)	253.29±47.37† (n=9)	201.00±66.97* (n=4)	472.67±32.31 (n=6)	0.021; 0.013
Blood glucose variation	87.77±64.01 (n=9)	- 73.28±45.13 (n=9)	- 243.50±92.66* (n=4)	199.16±52.84 (n=6)	0.012
Body weight					
Baseline	188.44± 7.59 (n=9)	210.60±8.11 (n=10)	185.00±2.84 (n=8)	191.11±4.96 (n=9)	0.086
1st week	193.88±8.19 (n=9)	198.00±9.45 (n=10)	167.63±5.30 (n=8)	198.63±10.42 (n=8)	0.189
2nd week	213.78±11.42 (n=9)	191.56±11.61 (n=9)	165.17±3.04† (n=6)	210.14±14.19 (n=7)	0.037
3rd week	205.44±15.28 (n=9)	182.88±12.43 (n=8)	164.00±3.67 (n=5)	211.50±18.15 (n=6)	0.156
4th week	207.56±14.71 (n=9)	196.14±14.51 (n=7)	165.40±3.84 (n=5)	197.83±19.80 (n=6)	0.322
Body weight variation	19.11±11.63 (n=9)	-10.28±7.71 (n=7)	-19.40±6.73 (n=5)	9.66±13.93 (n=6)	0.080
Lipids					
Total cholesterol	84.22±7.26 (n=9)	62.28±2.93† (n=7)	52.40±5.83* (n=5)	87.40±5.76 (n=5)	0.053; 0.008
HDL-c	44.33±4.70 (n=9)	19.86±3.33* (n=7)	21.60±4.24* (n=5)	40.33±5.23 (n=5)	0.002; 0.011
LDL-c	32.78±4.01 (n=9)	33.57±4.18 (n=7)	23.80±1.83 (n=5)	38.40±5.57 (n=5)	0.219
Triglycerides	34.89±6.31 (n=9)	46.43±4.29 (n=7)	34.00±4.92 (n=5)	50.60±22.62 (n=5)	0.599

Results expressed as mean and standard error of the mean (analysis of variance, *post-hoc* Tukey). * p<0.01; † p< 0.05.

In contrast, natural weight gain was significantly lower in non-diabetic animals treated with 200mg/kg of hydroalcoholic extract (C200) in the second (CC with 273.20±9.21g *versus* C200 with 225.17±9.42g; p=0.018), third (CC with 292.60±10.36g *versus* C200 with 242.83±7.23g; p=0.003) and fourth (CC with 310.80±11.00g *versus* C200 with 241.60±12.60g; p<0.001) week of treatment. Total body weight gain did not differ significantly between groups treated with 100 or 200mg/kg of extract (CC with 109.60±9.95g *versus* C100 with 13.22±5.70g *versus* C200 with 29.00±9.08g; p<0.001) (Table 2).



Intragroup analysis: paired Student's t test. * p<0.05; † p<0.01.

Figure 1. Blood glucose levels in diabetic animals treated with hydroalcoholic *Plathymenia reticulata* extract at doses of 100mg/kg (D100) or 200mg/kg (D200), glybenclamide (DG) or water (DC)

Treatment of diabetic animals with 100mg/kg of extract significantly reduced total (DC with 84.22±7.26mg/dL *versus* D100 with 62.28±2.93mg/dL; p=0.053) and HDL (DC with 44.33±4.70mg/dL *versus* D100 with 19.86±3.33mg/dL; p=0.002) cholesterol levels (Table 1). Total (CC with 80.11±4.01mg/dL *versus* C100 58.75±3.13mg/dL; p=0.01) and HDL (CC with 37.60±3.75mg/dL *versus* C100 with 20.86±1.33mg/dL; p=0.005) cholesterol levels decreased in non-diabetic animals (Table 2).

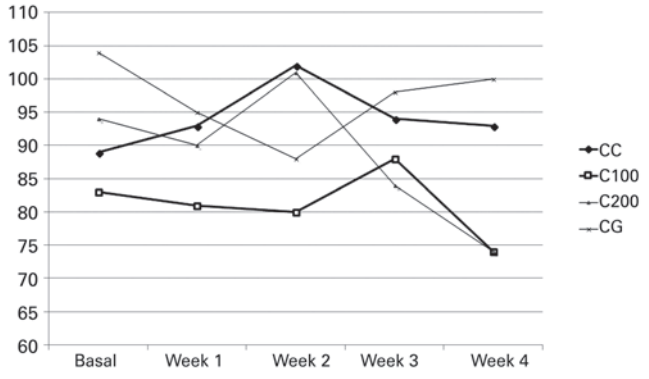
The treatment with 200mg/kg of extract led to a significant decrease in total cholesterol levels in both diabetic (DC with 84.22±7.26mg/dL *versus* D200 with 52.40±5.83mg/dL; p=0.008) and non-diabetic (CC with 80.11±4.01mg/dL n=9 *versus* C200 with 64.83±2.84mg/dL; p=0.037) rats, and to lower HDL-c levels (DC with 44.33±4.70mg/dL *versus* D200 with 21.60±4.24mg/dL; p=0.011) in diabetic rats (Table 2).

Histopathological analysis of the pancreas revealed lack of significant differences with respect to hemorrhage, hydropic degeneration, hyperemia and presence of pancreatic cysts. Significant islet hyperplasia (χ^2=20.384; p=0.005) (Figure 3) and pancreatic duct dilation (χ^2 test of 14.232; p=0.047) were observed in D200 and C100 groups (Table 3).

Table 2. Analysis blood glucose levels, body weight, and lipids in non-diabetic animals treated with 100 (D100) or 200mg/kg (D200) of hydroalcoholic extract or glyburide (GC) for 4 weeks, compared to controls (CC)

	CC	C100	C200	GC	p value
Blood glucose					
Baseline	89.40±3.32 (n=10)	83.80±1.81 (n=10)	94.67±3.42 (n=9)	104.38±4.16* (n=8)	<0.001
1st week	93.60±2.68 (n=10)	81.20±1.94† (n=10)	90.00±5.47 (n=9)	95.88±2.61 (n=8)	0.006
2nd week	102.60±2.31 (n=10)	80.80±4.84† (n=10)	101.14±8.41 (n=6)	88.86±8.46 (n=8)	0.014
3rd week	94.40±4.03 (n=10)	88.56±3.39 (n=9)	84.57±2.88 (n=6)	98.25±5.40 (n=8)	0.149
4th week	93.10±3.61 (n=10)	74.33±1.31† (n=9)	74.83±8.41†(n=6)	100.50±4.23 (n = 8)	0.001; 0.055
Blood glucose variation	3.7±6.35 (n=10)	- 8.89±1.89 (n=9)	- 17.00±9.92 (n=6)	- 3.87±6.18 (n=8)	0.154
Body weight					
Baseline	201.20±7.25 (n=10)	317.20±5.76* (n=10)	207.25±5.52 (n=9)	195.00 ±3.45 (n=8)	<0.001
1st week	232.60±11.07 (n=10)	316.30±8.39* (n=10)	195.25±18.86 (n=9)	201.00±5.82 (n=8)	<0.001
2nd week	273.20±9.21 (n=10)	293.60±12.69 (n=10)	225.17±9.42† (n=6)	241.88±5.04 (n=8)	0.018
3rd week	292.60±10.36 (n=10)	318.11±11.21 (n=9)	242.83±7.23† (n=6)	270.88±3.60 (n=8)	0.003
4th week	310.80±11.00 (n=10)	330.11±7.65 (n=9)	241.60±12.60† (n=6)	287.13±4.54 (n=8)	<0.001
Body weight variation	109.60±9.95 (n=10)	13.22±5.70* (n=9)	29.00±9.08* (n=6)	92.12±3.32 (n=8)	<0.001; <0.001
Lipids					
Total cholesterol	80.11±4.01 (n=9)	58.75±3.13* (n=8)	64.83±2.84† (n=6)	83.43±4.02 (n=7)	0.001; 0.037
HDL-c	37.60±3.75 (n=9)	20.86±1.33* (n=8)	31.33±3.66 (n=6)	50.17±2.94† (n=7)	0.004; 0.053
LDL-c	27.33±4.65 (n=9)	30.25±2.18 (n=8)	23.00±3.59 (n=6)	24.83±4.78 (n=7)	0.623
Triglycerides	75.89±10.00 (n=9)	52.62±3.92 (n=8)	51.33±3.65 (n=6)	45.00±4.01† (n=7)	0.013

Results express as mean and standard error of the mean (analysis of variance, *post-hoc*: Tukey). *p<0.01; † p<0.05.



Intragroup analysis: paired Student's t test. * $p<0.05$; † $p<0.01$.

Figure 2. Blood glucose levels in non-diabetic animals treated with hydroalcoholic *Plathymenia reticulata* extract at doses of 100mg/kg (C100) or 200mg/kg (C200), glybenclamide (CG) or water (CC)

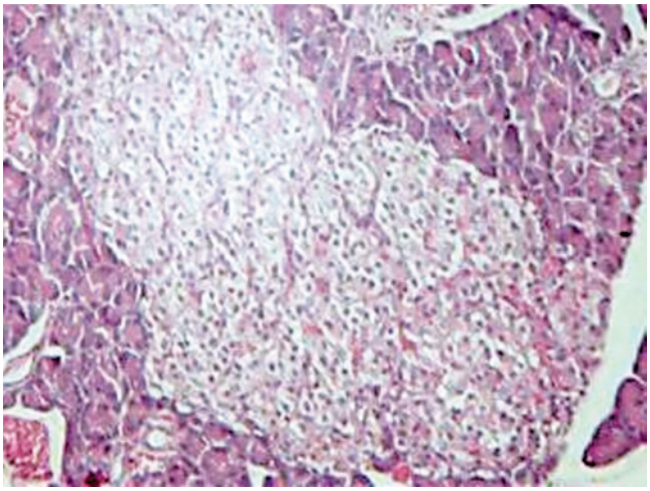


Figure 3. Hyperplastic islets of Langerhans in diabetic animals treated with *Plathymenia*

Table 3. Percentage of pathological changes found in the pancreas of diabetic animals treated with hydroalcoholic *Plathymenia* extract and controls

Pancreas	CC	DC	C100	C200	D100	D200	GD	GC	p value*
No changes	28.6	28.6	71.4	28.6	42.9	50.0	42.9	28.6	0.755
Islet hemorrhage	0	0	0	0	0	0	0	0	
Pancreatic hemorrhage	0	0	0	0	0	0	0	0	
Hydropic degeneration	0	42.9	0	14.3	0	0	28.6	14.3	0.13
Hyperemia	0	0	0	0	14.3	33.3	0	0	0.08
Pancreatic cysts	14.3	0	0	57.1	57.1	33.3	47.9	14.3	0.06
Islet hyperplasia	42.9	0	28.6	0	0	33.3	0	71.4	0.005
Pancreatic duct dilation	0	28.6	0	0	0	0	0	0	0.047

* χ^2 test.

DISCUSSION

P. reticulata is a protein- and enzyme-rich plant growing in the Brazilian *Cerrado*.[21] The antimicrobial activity of the hydroalcoholic extract from this plant against *Gram*-positive bacteria (*Streptococcus mutans* and *Staphylococcus sp.*) has been demonstrated *in vitro*.[14] Crude hydroalcoholic extracts (hot and cold) have been shown to have anti-inflammatory activity, decreasing leukocyte migration in carrageenan-induced peritonitis in mice. Crude hydroalcoholic extracts also induced anti-edematous activity against carrageenan-induced paw edema, showed antiproliferative activity against lung, ovary and melanoma tumor cells, and inhibited nociception in acetic acid-induced pain tests, with a similar profile to indomethacin.[7]

Antifungal activity of the alcoholic extract (made from Brazilian *cachaça*) has been recently reported.[22] Also, the hexane, dichloromethane, ethyl acetic fractions of ethanolic *P. reticulata* extract were able to inhibit *Bothrops jararacussu* poison toxicity, possibly via a tannin-mediated effect.[23]

Anti-hyperglycemic effects of *P. reticulata* extract have not been reported to date. However, this study introduces a prospective novel treatment for diabetes mellitus, with potential pancreatic protection, and a new disease prevention strategy, supported by the ability of the extract to promote weight loss and islet hyperplasia in non-diabetic animals.

The mode of action of the plant in this study translated into significant anti-hyperglycemic effects, leading to blood glucose level decrease in diabetic and non-diabetic rats; this decrease was significant in non-diabetic animals, albeit with no clinical manifestations (hypoglycemia). Pancreas-protective effects (*i.e.*, pancreatic islet hyperplasia) were also observed. Hypoglycemic effects may reflect the ability of the plant extract to promote beta cell neogenesis, as the presence of islet hyperplasia and pancreatic duct dilation suggests, or restore beta cell dedifferentiation, a process through which dedifferentiated cells revert to progenitor-like cells expressing Neurogenin 3, Oct 4, Nanog, and L-Myc. Some diabetes medications in the market are able to promote beta cell neogenesis or restore dedifferentiation.[24,25]

The hypoglycemic effect occurred early in animals treated with 100mg/kg (on the second week) and late in those treated with 200mg/kg, and may have been due to hormesis - a dose-response relationship phenomenon characterized by low-dose stimulation and high-dose inhibition. This concept introduces a changing perception that the fundamental nature of the dose response is neither linear nor threshold, but U-shaped,

changing the concept and conduct of toxicological and risk assessment.[26]

Beta cell dysfunction plays a key role in the onset and progression of type 2 diabetes. Among acquired factors, lipotoxicity and glucotoxicity may be of of particular relevance to cell damage. More recently, a predominant role of inflammation in beta cell dysfunction in type 2 diabetes has been proposed.[27] Hence, the plant studied may be indicated for both, type 1 and type 2 diabetes mellitus.

The role of mitochondrial dysfunction on beta cell injury (*i.e.*, beta cell apoptosis resulting from production of reactive oxygen species (ROS) in response to metabolic stress) has been reported in type 2 diabetes[28] and animals with type 2 diabetes.[29] Other recent reports based on autopsy specimens suggested amyloid deposits in pancreatic islets contribute to decreased beta cell mass in patients with type 2 diabetes.[30] Animal experiments in monkeys (nonhuman model primate) also showed correlations with alpha cell hyperplasia, leading to insulin deficiency, hyperglucagonemia and insulin resistance.[31]

Body weight loss in non-diabetic rats treated with 200mg/kg or 100mg/kg suggests additional effects on obesity control. Weight loss may have reflected reduced food intake or impaired food absorption in response to plant extract tannins.[23] Studies analyzing food and water intake in these animals are being conducted to unveil the mechanisms underlying anti-obesity effects.

Similar hormesis effect can observed in body weight variation in non-diabetic animals, which had lower weight gain when treated with the 100mg/kg, followed by treatment with 200mg/kg.[26] Initial body weight differences between animals lead to the use of body weight variation (initial weight minus final weight) as a parameter in this study.

Positive effect on total cholesterol levels were observed; however, HDL-c levels decreased. HDL-c carries cholesterol to the liver, where it is picked up by SR-B1 receptors, contributing to vascular bed protection against atherogenesis via mechanisms such as removal of oxidized low-density lipoprotein-cholesterol (LDL), inhibition of adhesion molecule and monocyte fixation to the endothelium and stimulation of nitric oxide release.[32]

Lower total cholesterol levels are beneficial for the population at large and for diabetic individuals in particular. However, HDL-c reduction may increase cardiovascular risks, an undesirable effect for diabetics.

The experimental model of diabetes induced by high streptozotocin doses is a useful tool for investigation of several aspects of type 1 diabetes.[33] In this study, oral gavage of hydroalcoholic *P. reticulata Benth* extract led to a significant drop in blood glucose levels and promoted islet hyperplasia in treated animals. These findings may be the first step towards development a novel oral drug for treatment of type 1 and possibly to type 2 diabetes.

CONCLUSION

Further studies are warranted to unveil the mechanisms underlying extract effects, investigate extract toxicity and single out the compound responsible for the effects described. This study may raise prospects of new therapies for type 1 or type 2 diabetes, based on hypoglycemic effects and beta cell preservation or, more importantly, lead to the identification of a compound capable of preventing the onset of type 2 diabetes in susceptible individuals via pancreatic protection and weight loss. Beneficial effects described may reflect the ability of the plant extract to promote beta cell neogenesis or restore beta cell dedifferentiation.

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