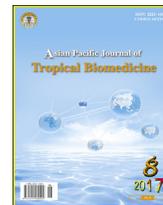




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Rourea cuspidata: Chemical composition and hypoglycemic activity



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ABSTRACT

Objective: To investigate the antidiabetic effect of *Rourea cuspidata* hydroalcoholic stem extract in normal and streptozotocin-induced diabetic rats.

Methods: In order to evaluate the chemical composition, different extracts from stem in ascending solvent order of polarity were prepared. The extracts were analyzed by high resolution mass spectrometry and 7 compounds were identified, including hyperin, an important and already reported active compound in the literature. Hyperin was also quantified by HPLC-UV in all the extracts. The hydroalcoholic stem extract (Ss5), which showed the highest concentration of hyperin, was administered to STZ-induced diabetes rats to evaluate the potential hypoglycemic activity. Total cholesterol, HDL, triglycerides, ALT and AST were also evaluated. In the present study, the effects of oral administration of hydroalcoholic stem extract (200 mg/kg b. wt.) for 28 days on the level of serum glucose, total cholesterol, HDL, triglycerides, aspartate amino transferase (AST) and alanine amino transferase (ALT) in normal and streptozotocin-induced diabetic rats were evaluated. Histopathological changes in diabetic rats' pancreas were also studied.

Results: The extract exposition demonstrated hypoglycemic effect like the drug control glibenclamide. The extract was able to increase the HDL levels. Histopathological study on diabetic rats' pancreas after extract treatment showed morphological alterations in STZ-induced diabetes rats, which were apparently restored after extract treatment.

Conclusions: This work demonstrates the potential use of *R. cuspidata* as hypoglycemic agent in the treatment of diabetes.

1. Introduction

Brazil is known for its biodiversity with 40–55 thousand plant species distributed across several biomes with a large number of plants used by the population in folk medicine [1].

The Connaraceae family comprises twenty genera and about 350 species distributed in Africa, Southeast Asia and tropical America [2]. In tropical America, this family is formed by five genera and among them *Connarus* and *Rourea* are the most

representative ones. *Rourea* is a pantropical genus with about 100 species, 48 of them are in the Neotropics [3,4]. In Brazil, Connaraceae family native species are found mainly in the Amazonic region [5].

Few species of *Rourea* genus have been screened so far for their biological activities. Among them, the antidiabetic and antimalarial potential of *Rourea minor* (*R. minor*), antioxidant and hepatoprotective activities of *Rourea induta* and anti-inflammatory and hepatoprotective activities of *Rourea coccinea* can be noticed [6–8].

According to phytochemical investigations on *Rourea* species, it was described that two glycoside derivatives (rourosinose and rouremin) and derivative [1-(26-hydroxyhexacosanoyl)-glycerol], actives as antimalarial, as well as nor-sesquiterpene (dihydrovomifoliol-9-β-D glucopyranoside) were present in *R. minor* [8]. It was also described that the compounds quinone (rapanone) and cyanidine (leucopelargonidine) were from

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Rourea santaloides [9]. From *Rourea microphylla*, flavonoids (quercetin, quercetin 3-O- β -L rhamnopyranoside, astilbin, hyperin, rutin, and kaempferol), anthraquinones (physcion and erythroglauconin), triterpenes (23-hydroxybetulinic acid, ursolic acid and hederagenin), coumarin (daphnetin), phytosteroids (β -sitosterol, β -sitosteryl- β -D-glucopyranoside), besides fatty acids, alkane, alcohol, and glycery derivative were found [10,11]. From the leaves of *Rourea induta*, flavonoids were found (such as quercetin, hyperin, quercetin 3-O- α -L-arabinofuranoside, and quercetin 3-O- β -D-xylopyranoside) [3].

Rourea cuspidata (*R. cuspidata*) Benth ex. Baker popularly known in Brazil as ‘miraruíra’, ‘cipó miraruíra’, and ‘muiraruíra’, is a shrub of the Connaraceae family, common in Amazonic region [12], which is used for diabetes treatment in folk medicine. The same activity was reported in *R. minor* [7]. Although these studies involved *Rourea* species, there are few scientific papers focusing on *R. cuspidata*. Therefore, the present work aimed to evaluate the chemical composition of *R. cuspidata* stem extracts as well as the hypoglycemic activity of the stem hydroalcoholic extract of the species.

2. Material and methods

2.1. Chemical reagents

Streptozotocin and hyperin were purchased from Sigma-Aldrich (Saint-Louis, Missouri, USA). Accu-check active monitor and test stripes were purchased from Roche Diagnostics (Mannheim, Germany). Acetonitrile, sulphuric acid, phosphoric acid, formic acid, ammonia hydroxide, hexane, chloroform, ethyl acetate, ethanol, haematoxylin, eosin, formaldehyde and paraffin were supplied by Merck (São Paulo, SP, Brazil). All chemicals were of analytic grade.

2.2. Plant material

R. cuspidata plant material was collected in Uaicurapa river, in Santo Expedito community, Parintins (02° 37' 42" S, 56° 44' 09" W), Amazonas, Brazil, under authorization from Ibama number 02001.004236/2013-63, and identified by Dr. Juan David Revilla Cardenas, from Herbarium of INPA (Instituto Nacional de Pesquisas da Amazônia), where a voucher specimen was deposited.

2.3. Extraction

The plant samples were dried in air oven at 45 °C. The stem was separated from the bark and it was powdered. Stem was extracted under reflux (10 g of plant material with 200 mL of solvent for 2 h) in ascending polarity order. The extracts were nominated according to the following condition: S = under reflux extract, s = stem, 1 = hexane, 2 = chloroform, 3 = ethyl acetate, 4 = ethanol, 5 = ethanol/water (1: 1). After the extraction process, the solvent was evaporated under reduced pressure. Each extract resulted in powder, and was stored in the dark.

2.4. Phytochemical characterization

The *R. cuspidata* powered extracts were dissolved in a solution 50% (v/v) chromatographic grade acetonitrile (Tedia, Fairfield, OH, USA), 50% (v/v) ultrapure water (Milli-Q[®]) and 0.1% formic acid or 0.1% ammonia hydroxide for ESI (+) or ESI (–),

respectively. The samples were separated by liquid chromatography (UFLC system), consisted of a LC-20ADXR pump, a SIL-30AC autosampler (Shimadzu[®]). Chromatographic separations were performed on a Shim-pack XR-ODS (30 mm × 2.0 mm, 2.2 μ m) column. A hybrid high-resolution and high accuracy microTof (Q-TOF) (Bruker[®] Scientific) was used for detection, with electrospray ionization (ESI) source (MicroTOF-QII Bruker[®] Scientific) in positive and negative mode. The range of mass was 50–1 200 *m/z* with two scans per second, providing the resolution of 50 000 (FWHM). The drying temperature was 200 °C and nitrogen was used for drying gas, in a 10 L/min flow. The ionization energy was 3.0 eV and the capillary voltage was 4 500 eV. The software was used to read the spectrum, Compass DataAnalysis version 4.3 was used along with the following tools: Smart formula, Smart formula 3D and Send formula to Compound Crawler (Compound Crawler version 3.0). MetFrag 2010 was also used to predict fragments with mass spectra in order to compare with the practice results, corroborating with the identification of the compound.

2.5. Quantitative analysis by HPLC-UV

The study was conducted according to Kalegari *et al.* [6] with slight modifications. Analytical HPLC experiments were performed with Shimadzu LC-20AD fitted with an analytical column (Agilent LiChrosfer 100 RP C18, 5 μ m, 250 × 4.6 mm) and UV–vis detector (λ 356 nm). The mobile phase consisted of water buffer acid solution (H₂SO₄ 0.01 mol/L; H₃PO₄ 0.02 mol/L)-solvent A, and acetonitrile-solvent B, with 1 mL/min flow rate following linear gradient over a total run time of 25 min, initially 98%: 2% for A: B and at 25 min, 74%: 26% for A: B.

2.6. Animals

The study was conducted according to Abeeleh *et al.* [13] with slight modifications, and it was previously approved by Ethics Committee on Animal Use (CEUA)-University of Caxias do Sul (Project number: 002/2013). Healthy, young male adult Wistar rats, weighing 250–350 g, purchased from Technology and Science Foundation (Santa Maria, Brazil), were used in the study. The animals were housed under standard conditions, kept on a 12 h light: 12 h dark cycle, and fed with a commercial rodent diet (Nuvital[®]) and water *ad libitum*.

2.7. In vivo study

For the experiment, male Wistar rats were randomly distributed into 4 groups (*n* = 5). Diabetes was induced through *i.p.* administration of 55 mg/kg streptozotocin (STZ). After 24 h, blood glucose concentration was measured to confirm the development of diabetes mellitus. During a 28 days period of diet, normal control rats (G1) were orally administrated 0.6 mL of phosphate buffer (PBS) only. STZ-induced diabetic rats were randomly divided into three groups that orally administrated glibenclamide (G2) 0.7 mg/kg, *R. cuspidata* hydro alcoholic stem extract (Ss5) 200 mg/kg (G3) or PBS (G4) using gavage. Basal glycemia was measured in T0 (first measurement), T1 (7 days), T2 (14 days), T3 (21 days) and T4 (28 days). Total cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and high density lipoprotein

(HDL) was measured before the administration of STZ and at the 28th day. The total cholesterol, triglycerides and HDL content in plasma were estimated by enzymatic colorimetric method [14], while ALT and AST levels were measured by ultraviolet kinetic method [14,15]. The measurements were processed with Labmax 240 biochemical analyser (Labtest, Japan). The animals were euthanized at the end of the experiments.

2.8. Oral glucose tolerance test (GTT)

Glucose tolerance test was performed in all animals before the diabetes induction, in order to evaluate the glucose tolerance. A 25% glucose solution was intraperitoneally administered in a 2 g/kg body weight on the last day of the experiment after an overnight fast. Blood samples were collected from the tail vein at 30, 60, 120, 180 and 240 min after injection.

2.9. Histopathology of pancreas

After euthanizing the animals, the whole pancreas of each rat was removed for histological studies. Pancreatic tissue was fixed in 10% neutral formalin solution and after, fixation tissues were embedded in paraffin. Solid sections were cut at 5 μ m and further stained with haematoxylin and eosin [16].

2.10. Statistical analysis

For *in vivo* experiment, statistical analysis for glucose level was performed through two-way ANOVA followed by

Bonferroni test, in order to assess differences between treatment groups and sampling times. For total cholesterol, triglycerides, AST, ALT, and HDL levels statistical analysis was performed employing Student's *t*-test. In all analyses the IBM SPSS 21.0 was used and $P < 0.05$ was considered statistically significant for all tests.

3. Results

3.1. Chemical characterization

The *R. cuspidata* extracts chemical composition was performed by High Resolution Mass Spectrometry (HRMS). Based on the complex composition of the plant extracts, the HRMS has been used as a powerful tool for identification of natural metabolites [17]. In agreement with the expected chemical classes, each extract was analyzed in positive ESI (+) and negative ESI (–) mode as can be seen in Table 1.

As a marker compound of the extract, hyperin was quantified by HPLC-UV according to the method described above. The standard stock solution (0.5 mg/mL) was prepared and further diluted to the desired test concentrations. Quantitative estimation of hyperin presented in each extract was made using the calibration curve of the standard solution and plotted concentration versus area. The calibration curve of hyperin was found to be linear $R^2 \geq 0.992$ ($y = 5E + 07x - 8E + 07$) in the concentration range (0.53–218.00 μ g/mL), limit of detection 0.10 μ g/mL and limit of quantitation 0.48 μ g/mL. The results are expressed in content of hyperin (% m/m) as following: hexane extract (Ss1) not detected, chloroform extract (Ss2) 0.013%,

Table 1

Chemical compounds identified in *Rourea cuspidata* extracts by HRMS in positive mode.

Entry	Precursor ion <i>m/z</i>	Ext.	Fragmentation pathway	Identification	Element. Comp.	Diff. Ppm	Ref.
1	195.1007	Ss1	177.0889[M-OH]; 163.0768[M-CH ₃ O]; 135.0841[M-C ₂ H ₃ O ₂]; 134.0696[M-C ₂ H ₄ O ₂];	Zingerone	C ₁₁ H ₁₄ O ₃	4.6	[17]
2	284.2932	Ss1	116.1086[M-C ₁₂ H ₂₃]; 102.0890[M-C ₁₃ H ₂₅];	Octadecanamide	C ₁₈ H ₃₇ NO	4.5	[18]
3	291.0864	Ss5	123.0424[M-C ₈ H ₇ O ₄]; 139.0385[M-C ₈ H ₇ O ₃];	Epicatechin or Catechin	C ₁₅ H ₁₄ O ₆	–0.1	[6]
4	435.0922	Ss5	303.0538[M-C ₅ H ₇ O ₄]; 304.0502[M-C ₉ H ₆ O];	Quajaverin (quercetin 3-O- α -L-arabinoside) or quercetin 3-O- β -xiloside)	C ₂₀ H ₁₈ O ₁₁	0.1	[6]
5	465.1014	Ss5	151.0372[M-C ₁₃ H ₁₃ O ₃]; 153.1249[M-C ₁₁ H ₃ O ₁₁]; 303.0496[M-C ₆ H ₉ O ₅]; 304.0554[M-C ₆ H ₇ O ₅];	Hyperin (quercetin-3-O-beta-D-galactopyranoside)	C ₂₁ H ₂₀ O ₁₂	2.8	[5]
6	577.1388	Ss5	426.0883[M-C ₈ H ₇ O ₃]; 425.0893[M-C ₈ H ₆ O ₃]; 287.0570[M-C ₁₅ H ₁₃ O ₆];	Proanthocyanidin A2	C ₃₀ H ₂₄ O ₁₂	4.7	[19]

Table 2

Levels of blood glucose in normal and diabetic rats after 1, 7, 14, 21 and 28 days of treatment.

Groups	1st day	7th day	14th day	21st day	28th day
G1	92.13 \pm 6.27	82.00 \pm 9.29	92.63 \pm 9.91	106.75 \pm 16.86	86.63 \pm 4.50
G2	330.20 \pm 63.36	299.00 \pm 210.54	168.60 \pm 112.79	227.60 \pm 148.67	257.60 \pm 158.94
G3	325.33 \pm 86.17	221.50 \pm 131.04	182.83 \pm 123.00	202.33 \pm 158.75	218.67 \pm 141.14
G4	349.38 \pm 33.14	343.88 \pm 32.28	414.13 \pm 49.57	427.25 \pm 52.16	438.25 \pm 32.52

Data are expressed as mean \pm SD; $n = 5$ for each group.

ethyl acetate extract (Ss3) 0.016%, ethanol extract (Ss4) 0.011% and ethanol/water extract (Ss5) 0.031%.

3.2. In vivo study

Streptozotocin has its effect in pancreatic β -cells inducing selective cytotoxicity and affecting endogenous insulin release, which results in increase of blood glucose levels taking to diabetes mellitus [18]. Therefore, for the preliminary screening of hypoglycemic agents, the hyperglycemia induced by STZ in animal is considered an experimental model.

In this study, all groups presented differences in glycemic values obtained through time ($P < 0.05$) (Table 2). Statistically

significant difference was found in profiles of glycemic levels between groups ($P < 0.05$), with the exception of values between G2 and G3 ($P > 0.999$).

Possible alterations in the levels of enzymes, metabolic products, hematology, normal functioning and many others parameters can be shown and assessed by measurement of biochemical parameters, revealing the effect of foreign compounds including plant extracts on the blood constituents of animals [19]. In order to evaluate if Ss5 changes total cholesterol, triglycerides, ALT, AST, and HDL levels, the experiment measured the individual values before the administration of STZ and at the end of the experiment. The results are expressed in Table 3. The body weight at the beginning and at

Table 3

Serum levels of cholesterol, triglycerides and other related parameters.

Groups	G1		G2		G3		G4	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Triglycerides (mg/dL)	64.00 \pm 20.82	135.40 \pm 22.60 ^a	88.40 \pm 56.21	122.20 \pm 107.84	76.80 \pm 24.28	147.60 \pm 47.84 ^a	65.80 \pm 13.35	213.60 \pm 13.43 ^a
HDL (mg/dL)	29.40 \pm 6.58	33.00 \pm 8.72	33.40 \pm 6.43	45.50 \pm 9.10 ^a	31.40 \pm 7.96	40.40 \pm 7.64 ^a	26.00 \pm 6.48	38.60 \pm 1.95
Total Chol. (mg/dL)	94.60 \pm 10.97	129.80 \pm 23.97	123.00 \pm 69.09	168.00 \pm 111.51	103.40 \pm 22.94	105.0 \pm 22.67	107.40 \pm 19.75	128.00 \pm 37.97
AST (U/L)	162.00 \pm 82.91	93.00 \pm 48.42	333.20 \pm 43.30	161.80 \pm 111.51	253.00 \pm 156.31	49.00 \pm 26.36	194.60 \pm 27.94	222.00 \pm 82.42
ALT (U/L)	26.00 \pm 8.46	28.00 \pm 10.42	25.80 \pm 17.67	113.00 \pm 8.22 ^a	55.60 \pm 33.62	82.00 \pm 34.89	21.60 \pm 14.33	78.60 \pm 17.74 ^a

^a $P < 0.05$ compared with initial group. Data are expressed as mean \pm SD; $n = 5$ for each group.

Table 4

Body weight and food consume (Mean \pm SD) in different groups.

Groups	G1	G2	G3	G4
Initial body weight (g)	342.87 \pm 32.23	200.80 \pm 17.75	215.0 \pm 15.46	317.63 \pm 27.92
Final body weight (g)	374.37 \pm 30.54 ^a	242.60 \pm 32.59	238.5 \pm 24.24	231.87 \pm 32.91 ^a
Dietary intake (g/d)	30.98 \pm 12.68	33.62 \pm 13.28	31.52 \pm 8.65	36.60 \pm 9.66

^a $P < 0.05$ compared with initial group.

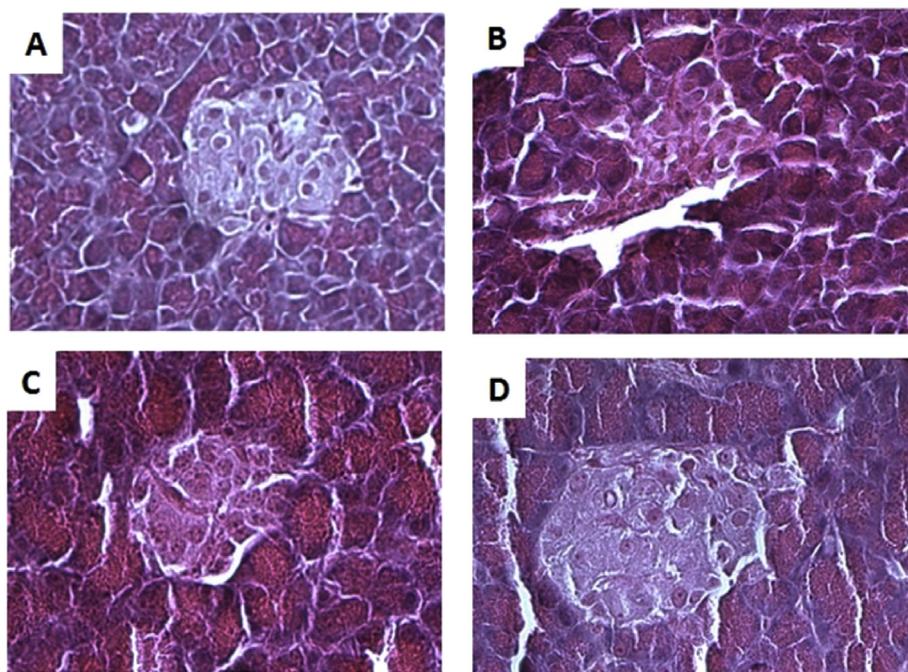


Figure 1. Histopathological changes in the pancreas of different experimental rats stained with Hematoxylin and Eosin.

A) normal control rat (G1); B) Diabetic rat (G4); C) glibenclamide treated (G2) and D) extract exposition (G3). Observe Islet with defined boundary in A, C and D compared to B.

the end of the experiment, and the food intake was also evaluated, and can be seen in Table 4.

Control rats (G1) exhibited normal histological architecture at the morphologic analysis. Prominent nuclei with well-arranged lobules surrounding islet cells were found among normal control rats (Figure 1A). Groups that received STZ demonstrated cellular damage to the pancreatic acini and islets (Figure 1B). Ss5 (G3) and glibenclamide (G2) treated rats showed marked improvement of the cellular injuries (Figure 1C and 1D), as evident from the partial restoration of islet cells, reducing tissue damage.

4. Discussion

In chemical identification using HRMS, a set of information as exact mass and isotopic ratio can be used [20,21], and for unequivocal identification and differentiation of isobaric interferences, the fragmentation pathway is necessary. However, in this work, for epicatechin and catechin, quercetin 3-O- α -L-arabinoside and quercetin 3-O- β -xiloside, were not possible the differentiation due to the small differences between the structures. It was not possible to identify compounds in negative mode, only in positive, as described in Table 1.

The presence of a β -ring catechol group (dihydroxylated β -ring) confers to proanthocyanidins the possibility of being potent antioxidants since it's capable of donating hydrogen (electron) to stabilize the radical specie [22]. Proanthocyanidin A2, founded in Ss5 extract, has antioxidant activity like quercetin and epicatechin and higher activity than the synthetic oxidant BHA and BHT [23].

Zingerone [4-(4-hydroxy-3-methoxy phenyl) butan-2-one], a compound found in Ss1 extract, is an active component of dry ginger rhizome (*Zingiber officinale*), showing a significant effect in reducing the blood glucose level in the treated diabetic rats [24].

The *R. cuspidata* extracts have shown flavonoids compounds and among them, hyperin is one of the active ingredients of *Hypericum perforatum* and has potent antidepressant activity [25]. Hyperin is also present in many plants including *Drosera rotundifolia*, *Stachys byzantine*, *Prunella vulgaris* and *Rumex acetosella*, being an active phytochemical constituent [26]. As a potential therapeutic agent, it has many activities already described like anti-cancer [27], cardioprotective [28], anti-oxidant [29] and anti-inflammatory [30].

Beyond these activities, studies in rodents have suggested that hyperin is also a hypoglycemic agent due to its ability to increase glycolysis (increasing liver hexokinase activity) and decrease the activities of gluconeogenic enzymes in diabetic rats [26].

Between all the evaluated extracts, the Ss5 presented the higher quantity of hyperin and because of that, it was chosen to be tested for further *in vivo* model investigation, in order to evaluate the hypoglycemic activity of *R. cuspidata*.

The inhibition of the enzyme α -glucosidase by the ethyl acetate subfraction of *Parkia roxburghii* methanolic extract containing hyperin and epigallocatechin gallate was demonstrated by Sheikh and coworkers. The authors conducted the same assay with hyperin isolated from this fraction and confirmed the results [31].

It is important to note that diabetes is a chronic metabolism disorder with relative deficiency of insulin secretion and varying degrees of insulin resistance. It is one of the most important clinical and public health problems worldwide [32], and can cause severe complications including blindness, cardiac and kidney diseases [33–35].

The continuous administration of hydroalcoholic extract of *R. cuspidata* (Ss5) at 200 mg/kg or glibenclamide for 28 days significantly reduced the blood glucose concentration in STZ induced diabetic rats. The plant extract showed a comparable activity with the glibenclamide treated group. Glibenclamide, as a standard antidiabetic drug, stimulates insulin secretion from β cells of islets of Langerhans [36]. There was no statistically significant difference of glycemic levels between G2 and G3 ($P > 0.999$).

The hypoglycemic effect of a plant extract depends on the degree of β -cell destruction. The treatment of STZ-diabetic rats with medicinal plant extract can result in the activation of β -cells, presenting the insulinogenic effect [37].

Verma *et al.* isolated hyperin from the flowers of *Rhododendron arboreum* and evaluated the antidiabetic activity in STZ-diabetic rats. The results showed that hyperin enhanced glucose utilization, decreasing glucose level after 30 days of treatment. Beyond that, rats treated with hyperin restored the levels of hepatic glycogen by decreasing activity of glycogen phosphorylase and increasing the activity of glycogen synthase. In the same study, the authors also reported increased activity of liver hexokinase (increased glycolysis) and histopathology results revealed an increase in the number of β -cells in the islets showing regeneration due to administration of hyperin in STZ-rats [38]. Therefore, hyperin is probably one of the compounds responsible for the hypoglycemic activity of Ss5 extract.

Furthermore, the presence of epicatechin may contribute to the hypoglycemic effect. As a phenolic constituent, it is a moderate α -glucosidase inhibitor [39]. Flavonoids, such as guaijaverin, have been tested and proven for its inhibitory activity against aldose reductase, another enzyme involved in diabetes mellitus [40]. Thus, aldose reductase inhibitors can reduce the hyperglycemia-induced polyol pathway, contributing to the treatment and prevention of diabetic complications such as cataract [41].

Guaijaverin and hyperin are glycosides of quercetin with different sugars. Both compounds, isolated from Guava leaves, presented inhibitory activity against rat intestinal α -glucosidase as well as porcine pancreatic α -amylase [42].

It is well known that diabetes is associated with hyperlipidemia, since insulin activates the enzyme lipoprotein lipase, which hydrolyzes triglyceride under normal condition. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [43–45]. Hypertriglyceridemia is associated with metabolic consequences of hypercoagulability, hyperinsulinemia, insulin resistance and glucose intolerance. In addition, STZ rats show an important lipolytic activity, due to the insulinopenic state, which contributes to maintaining the abnormally elevated plasma triglycerides and cholesterol levels [46]. In STZ-induced diabetes, the increase in blood glucose levels is usually accompanied by an increase in plasma cholesterol and triglycerides, and decreases in HDL, which lead to cardiovascular risk [45]. In this way, the effects on diabetic complication were assessed by measuring the atherogenic lipids (total cholesterol and triglycerides) after chronic feeding of Ss5 to diabetic rats.

Results demonstrated that total cholesterol level was not changed by *R. cuspidata* treatment. In this study, all groups exhibited significantly elevated triglyceride levels at the end of the experiment, except group 2, treated with glibenclamide. The Ss5 extract is not able to control the triglycerides levels, unlike the glibenclamide group.

As follows, repeated extract administration for 28 days, significantly increased HDL levels ($P < 0.05$), as shown in Table 3. The same result was observed in glibenclamide group. HDL is inversely associated with coronary heart disease and its elevation is considered as an anti-atherosclerotic factor [47].

Serum enzymes like AST and ALT are indicators of hepatic disorders. Increases in these enzyme activities express active liver damage like, inflammatory hepatocellular disorders [48,49]. According to Zafar and coworkers, STZ in rats can produce alterations in the hepatic functions as well as structure of hepatocytes [50], but the effect of STZ on the levels of enzymes in the liver has remained unraveled. While some authors reported increased activities of AST and ALT [51,52] in the liver of STZ diabetic rat models, another group reported no alteration in the levels of these enzymes [53]. In this study, ALT was significantly higher (G4 and G2). On the other hand, treatment of the diabetic rats with the Ss5 extract had no significance changes of the ALT enzyme activity in plasma compared to the beginning of the experiment (T0). More studies are necessary in order to evaluate if Ss5 extract can perform a hepatoprotective activity against liver damage in STZ diabetic rats.

The decrease in body weight of STZ diabetic rats (Table 4), as seen in the present study in G4 may be associated to gluconeogenesis, related to the characteristic loss of body weight due to increased muscle wasting and loss of tissue proteins [54]. STZ diabetic rats treated with Ss5 had no significant difference in body weight at the end of the experiment compared to the initial time (G3) as well as the glibenclamide treated group (G2). This can be related to a protective effect in controlling muscle wasting and reversal of gluconeogenesis. To investigate if Ss5 extract has the ability of regulating gluconeogenesis, more studies are necessary.

In the histopathologic study of pancreas (Figure 1), it can be observed on the diabetic control and Ss5 treated group that the extract presented cytoprotective properties.

In conclusion, the chemical composition of *R. cuspidata* showed seven compounds identified herein by HRMS in different extracts. These compounds are described for the first time for this particular specie. Furthermore, the Ss5 extract presented hypoglycemic and anti-atherosclerotic effects, apparently promoting restoration of islet cells at the morphological analysis. However, further studies designed to isolate, characterize, and test the compounds of *R. cuspidata* should provide a better understanding of the mechanisms of action observed in the present study.

Conflict of interest statement

We declare that there is no conflict of interest.

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