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Hypoglycemic and anti-lipemic effects of the aqueous extract from *Cissus sicyoides*

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Abstract

Background: *Cissus sicyoides* (Vitaceae) is a medicinal plant popularly known in Brazil as "cipó-pucá, anil-trepador, cortina, and insulina". The plant is used in several diseases, including rheumatism, epilepsy, stroke and also in the treatment of diabetes. In the present work, we studied the hypoglycemic and anti-lipemic effects of the aqueous extract prepared from fresh leaves of the plant (AECS), in the model of alloxan-induced diabetes in rats. In addition, hepatic enzyme levels were also determined.

Results: Results showed that the daily treatment of diabetic rats with AECS for 7 days (100 and 200 mg/kg, p.o.) significantly decreased blood glucose levels in 25 and 22% respectively, as compared to the same groups before AECS treatment. No significant changes were seen in control diabetic rats before (48 h after alloxan administration) and after distilled water treatment. While no changes were seen in total cholesterol levels, a significant decrease was observed in plasma triglyceride levels, in the alloxan-induced diabetic rats after AECS treatment with both doses, as compared to the same groups before treatment. Significant decreases in blood glucose (25%) and triglyceride levels (48%) were also observed in the alloxan-induced diabetic rats after 4 days treatment with AECS (200 mg/kg, p.o.). Aspartate (AST) and alanine (ALT) aminotransferases levels, in diabetic controls and AECS-treated rats, were in the range of reference values presented by normal rats.

Conclusions: The results justify the popular use of *C. sicyoides*, pointing out to the potential benefit of the plant aqueous extract (AECS) in alternative medicine, in the treatment of type 2 diabetes mellitus.

Background

There are several species of medicinal plants popularly used in the treatment of diabetes mellitus, a disease responsible for serious complications, affecting a large number of people worldwide. *Cissus sicyoides* (L.) belongs to the Vitaceae family, and is a medicinal plant popularly used in Brazil in several diseases, such as epilepsy, stroke, as well as in abscesses and in the treatment of diabetes. Anti-inflammatory and anti-rheumatic activities are also attributed to the plant. Other species (*C. succicaulis*) presented anti-ulcer activity in the model of ethanol-induced gastric ulcer in rats [1]. A survey carried out in Nigeria identified *C. populnea*, among other species, as being largely used for the treatment of trypanosomiasis [2]. The aqueous extract from *C. rubiginosa* was shown to possess prominent antibacterial activity, which supports the ethno-medical use of this plant as an anti-diarrhea agent [3].

Earlier work [4] showed that the aqueous extract from *C. sicyoides* contracts isolated guinea pig aortic rings, in a dose dependent manner. These authors concluded that the extract acts at the membrane level, increasing the calcium entry through the membrane as well as acting in the internal calcium deposits, such as the sarcoplasmic reticulum. In a recent work [5], the aqueous extract from *C. sicyoides* was shown to present an anti-inflammatory effect, as determined by the carrageenan-induced rat paw edema (a model for general inflammation), and in the mouse ear edema (a model of topical inflammation). These authors also observed a decrease in the level of myeloperoxidase in tissue samples from the inflammation area. While Saenz et al. [6] demonstrated a cytostatic activity in *C. sicyoides* against Hep-cells, others [7] did not detect any antiviral activity against the influenza type A virus, in this species. According to these authors, the main chemical components of the aerial part of *C. sicyoides* were tannins, steroid-triterpenes, aminoacids, lipids and flavonoids.

Another phytochemical analysis of the plant showed the presence of alkaloids, triterpenes and/or steroids, flavonoids, tannins and saponins [3]. Other compounds, namely onocer-7-ene 3 alpha 21 beta-diol, delta-amyrin, delta-amyrone, and 3,3', 4,4'-tetrahydroxybiphenyl were also isolated from *C. quadrangularis*. These compounds are used for plant extract standardization purposes [8]. An earlier study [9] detected the presence of steroids, terpenes, quinones and phenolic compounds in *C. sicyoides*, while others showed no alkaloid in the plant, and cyanidins only in the fruit [10,11]. Recently [12,13], a new coumarin glycoside, 5,6,7,8 tetrahydrocoumarin-5-beta-xylo-pyranoside was isolated from the aerial parts of *C. sicyoides*, together with a known coumarin, sabadin, two flavonoids, kaempferol 3-rhamnoside and quercetin 3-

rhamnoside, and two steroids, sitosterol and 3-beta-O-beta-glupyranosylsitosterol.

We showed [14,15] that the aqueous extract from *C. sicyoides* (AECS) presents an anti-nociceptive activity, as demonstrated by the tests of acetic acid-induced abdominal contractions, formalin, and hot plate in mice. Besides, AECS also has anticonvulsant properties as evidenced in the models of pentylenetetrazol and strychnine-induced convulsions in mice. Although the plant is popularly used, in Brazil, for the treatment of diabetes mellitus, there are only two reports in the literature on this subject [16,17]. Furthermore, several plant constituents, including flavonoids (also detected in AECS), are known to decrease triglycerides and transaminases [18,19], which are usually increased in the serum of diabetic patients [20]. Thus, in order to validate the popular use of the plant as an anti-diabetic, the objectives of the present work were to study the possible effects of the aqueous extract from leaves of *C. sicyoides* on glycemia, lipid profile, as well as on levels of ALT and AST hepatic enzymes, in the model of alloxan-induced diabetes, in rats.

Results

Table 1 shows the results of blood glucose values in alloxan-induced diabetic rats, after the daily treatment with the aqueous extract of *C. sicyoides* (AECS 100 and 200 mg/kg, p.o.), for 7 days. Significant decreases in the blood glucose levels, of 25 and 22% respectively, were observed in the groups treated with both doses of AECS, as compared to the same group before treatment. No significant changes were seen in diabetic (48 h after administration with alloxan) controls, before and after distilled water treatment.

While no changes were seen in total cholesterol levels in any group (AECS treated and controls), a significant decrease was observed in plasma triglyceride levels in the alloxan-induced diabetic rats, after AECS treatment for 7 days with the doses of 100 mg/kg (50% decrease) and 200 mg/kg (42% decrease) (Table 2).

Table 3 shows the results of AECS (100 and 200 mg/kg) treatments, for 4 days, on biochemical parameters in the blood of alloxan-induced diabetic rats. Even after this shorter treatment, there was a significant decrease (25%) in the blood glucose levels after treatment with the higher AECS dose. AST and ALT levels were also significantly increased after distilled water and with the lower (100 mg/kg), but not with the higher dose of AECS (200 mg/kg). However, these enzyme alterations are still in the range of reference values shown in Table 6. Although triglyceride levels were significantly decreased after 4-day treatment in the three groups, the decrease was much

Table 1: Effects of the aqueous extract from leaves of *Cissus sicyoides* (AECS) on blood glucose levels in alloxan-induced diabetic rats

Group	Glucose levels (mg/dL)	
	(Before treatment)	(After treatment)
Control (11)	313.1 ± 15.04	281.0 ± 15.07
AECS		
100 mg/kg, p.o. (20)	300.1 ± 10.47	224.7 ± 13.13*
200 mg/kg, p.o. (13)	292.7 ± 8.05	227.8 ± 12.90*

Values are means ± SEM of the number of animals (in parenthesis). The blood was collected from the orbital sinus, 48 h after alloxan administration, 60 mg/kg, i.v., before and 1 h after the last administration of distilled water (control group) or of AECS, administered daily for 7 days. * $p < 0.05$ as compared to the same group, 48 h after alloxan administration (ANOVA and Tukey as the *post hoc* test).

Table 2: Effects of the aqueous extract from *Cissus sicyoides* (AECS) on blood total cholesterol and triglyceride levels in alloxan-induced diabetic rats

Group	Cholesterol (mg/dL)		Triglycerides (mg/dL)	
	Before	After	Before	After
Control (7)	87.0 ± 5.15	74.0 ± 2.93	244.0 ± 29.65	286.8 ± 58.23
AECS				
100 mg/kg, p.o. (20)	76.8 ± 3.94	78.1 ± 5.18	222.1 ± 26.40	111.5 ± 9.51*
200 mg/kg, p.o. (13)	86.4 ± 2.96	73.2 ± 3.27	294.0 ± 32.89	171.1 ± 19.13*

Values are means ± SEM of the number of animals (in parenthesis). The blood was collected from the orbital sinus, 48 h after alloxan administration, 60 mg/kg, i.v., before and 1 h after the last administration of distilled water (control group) or AECS, administered daily for 7 days. * $p < 0.05$ as compared to the same group, 48 h after alloxan injection (ANOVA and Tukey as the *post hoc* test).

Table 3: Effects of the aqueous extract from *Cissus sicyoides* (AECS) on biochemical parameters in the blood from alloxan-induced diabetic rats

Parameter	Group		
	Control	AECS 100 mg/kg	AECS 200 mg/kg
Glucose (mg/dL)			
Before	264.8 ± 12.66	252.2 ± 8.44	250.8 ± 10.74
After	248.1 ± 11.65	218.1 ± 16.21	188.0 ± 16.22*
Cholesterol (mg/dL)			
Before	58.4 ± 3.92	56.6 ± 3.19	63.0 ± 2.55
After	55.4 ± 2.91	50.1 ± 5.89	54.6 ± 2.29
Triglycerides (mg/dL)			
Before	169.8 ± 8.61	256.4 ± 24.60	165.4 ± 12.87
After	124.1 ± 8.02 *	132.5 ± 9.02 *	86.4 ± 7.56 *
ALT (IU/l)			
Before	20.0 ± 1.33	28.6 ± 2.15	20.8 ± 1.62
After	34.7 ± 2.89 *	41.2 ± 2.71 *	29.8 ± 2.22
AST (IU/l)			
Before	60.5 ± 3.90	62.2 ± 5.52	75.9 ± 4.74
After	85.5 ± 4.35 *	89.3 ± 7.03 *	88.3 ± 3.73

Values are means ± SEM of 8 to 18 animals per group. The blood was collected from the orbital sinus, 48 h after the alloxan administration, 60 mg/kg, i.v., before and 1 h after the last administration of distilled water (controls) or AECS, daily for 4 days. * $p < 0.05$ as compared to the same group, 48 h after the alloxan injection (ANOVA and Tukey as the *post hoc* test).

higher (48%) in the diabetic rats after AECS (100 and 200 mg/kg) treatments, as compared to diabetic control rats (27%) at the same period of time (Table 3).

Under the conditions of the present work, the alloxan treatment (60 mg/kg) caused a 22% body weight loss,

after 7 days, while in the alloxan plus AECS-treated group the body weight decrease ranged from 8.5 to 14%. The percentage death was between 33 and 53%, at the same period of time (Table 4). On the other hand, AECS alone and in the absence of alloxan did not alter the biochemical profile of the parameters studied when administered

Table 4: Body weights of alloxan-induced diabetic rats before and after treatment with the aqueous extract from *Cissus sicyoides* (AECS)

Group	Body weight (g)		% Death
	Before	After	
Control	218.3 ± 4.30 (15)	169.3 ± 5.41 (10)*	33
AECS			
100 mg/kg, p.o.	288.8 ± 6.63 (15)	264.3 ± 14.45 (8)	47
200 mg/kg, p.o.	243.7 ± 7.63 (15)	208.7 ± 9.61 (7)	53

Values are means ± SEM. Animals were administered daily with distilled water (controls) or AECS, for 7 days (see Methods, for details). *p < 0.05, as compared to the same group, 48 h after the alloxan injection (ANOVA and Tukey as the *post hoc* test).

Table 5: Biochemical parameters determined in normal rats administered daily with the aqueous extract from *Cissus sicyoides* (AECS)

Parameter	Result	Reference Values
Glucose (mg/dL)	88.5 ± 1.55	63.3 – 86.4
Total cholesterol (mg/dL)	54.3 ± 2.74	51.1 – 71.5
Triglycerides (mg/dL)	54.2 ± 0.87	42.9 – 64.4
AST (IU/L)	78.0 ± 4.46	72.6 – 105.8
ALT (IU/L)	29.4 ± 1.20	19.7 – 31.7

Values are means ± SEM of 8 normal rats treated daily with 100 mg/kg, p.o., of AECS, for 7 days. In Reference Values, the intervals are means ± SD of 22 animals (male Wistar rats), from the Animal House of the Faculty of Medicine of Juazeiro do Norte – FMJ.

Table 6: Phytochemical profile of the fresh leaves from *Cissus sicyoides*

Chemical Group	<i>Cissus sicyoides</i>
Hydrolyzable tannin	+
Coumarin	-
Flavonoid	+
Saponin	-
Anthraquinone	-
Alkaloid	-
Steroid	-

For the phytochemical profile, specific reactions for chemical groups or thin layer chromatography (TLC) were performed. + = presence; - = absence.

with the dose of 100 mg/kg, p.o., daily, for 7 days, as compared to reference values from normal animals (Table 5).

The phytochemical analysis of the aqueous extract prepared from the fresh leaves of *C. sicyoides* revealed the presence of flavonoids and hydrolyzable tannins, and the absence of coumarin, anthraquinones, alkaloids, saponins and steroids (Table 6).

Discussion

In the present work, we investigated the hypoglycemic and anti-lipemic effects of the aqueous extract from *C. sicyoides* in the model of alloxan-induced diabetes in rats. Alloxan causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas [24,25]. *In vitro* studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis [26]. The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells [27].

We showed that the aqueous extract from *C. sicyoides* (AECS, 100 and 200 mg/kg), administered orally for 7 days, produced significant decreases in plasma glucose in the model of alloxan-induced diabetes in rats, by comparing the results before and after the AECS treatment. A similar effect was observed after a shorter (4 days) treatment with the higher dose of AECS (200 mg/kg). However, AECS had no effect on glycemia in normal rats. Besides, no significant decrease was detected in diabetic animals administered with distilled water, for the same period of time (controls). Although the alloxan group (controls) presented a dramatic body weight reduction, weight losses were lower in the alloxan plus AECS-treated group, indicating another potential benefit of AECS.

Our findings that the extract of *C. sicyoides* reduces the glycemia of alloxan-induced diabetic rats, but had no effect on that of normal rats, are in agreement with a very recent work [16]. These authors also showed that the leaf decoction from *C. sicyoides* significantly reduced the intake of both food and fluid, and the volume of urine excreted, as well as the levels of blood glucose, urinary glucose and urinary urea, as compared to controls. Others [17] showed that, after oral administration, the leaf extract from *C. sicyoides* presented a potential hypoglycemic activity in hereditary diabetic mice, normal rats and rats with streptozotocin-induced diabetes. The authors showed that the extract, administered for 4 weeks, significantly lowered the mean plasma glucose level of mice, under feeding

conditions. Besides, a single oral administration significantly lowered the plasma glucose level, 1 h after sucrose loading, in normal rats and rats with streptozotocin-induced diabetes.

However, Beltrame et al., 2001 [12] failed to show any anti-diabetic activity in the hydroalcoholic extract obtained from the leaves of *C. sicyoides*, which instead intensified the decreased glucose tolerance promoted by dexamethasone, in rats. It is worthwhile to point out that the plant is popularly used as a decoction, which is similar to the aqueous extract used in the present work. Active principles responsible for the hypoglycemic activity presented by *C. sicyoides* are possibly better extracted by aqueous and more polar solvents.

We found that the aqueous extract is rich in carbohydrate type compounds, which are easily precipitated by ethanol (results not shown). Besides, recently [13] a new coumarin glycoside was obtained from the aerial parts of *C. sicyoides*. Glycosides are sugar derivatives, and an overwhelming number of glycosides occur in nature, mainly in plants, and such compounds have received much attention for possessing a variety of biological activities. Thus, flour prepared from *C. rotundifolia* was shown to contain significant amounts of non-starch polysaccharides [28], the major fraction of which was water-soluble. These authors verified that, in humans, the species elicited significant reductions in plasma glucose levels, at post-prandial time points and for the area-under-the-curve (AUC) values. Significant reductions in plasma insulin levels, at various post-prandial time points and for AUC values, were also seen after *C. rotundifolia* administration. Water-soluble non-starch polysaccharides are certainly one of the components responsible for the glucose and insulin lowering effects.

Although, in the present work, we showed no changes in total cholesterol levels, a significant decrease was observed in plasma triglyceride levels in the alloxan-induced diabetic rats, after 4 and 7-day treatments with AECS administered orally. On the other hand, another work [16] did not find any alteration in lipid metabolism, nor in levels of hepatic glycogen in streptozotocin-diabetic rats, after *C. sicyoides* treatment. According to them, these results indicate that the mechanism responsible for the improvement in carbohydrate metabolism, observed in animals treated with *C. sicyoides* decoction, does not involve inhibition of glycogenolysis and/or stimulation of glycogenesis.

We also measured plasma levels of AST and ALT, hepatic enzyme markers, and showed that these enzyme levels were significantly increased after AECS treatment. However, these effects were also observed in controls and,

under both conditions, the values are in the range of those shown by our normal control rats. Elevated activities of serum aminotransferases are a common sign of liver disease, and are more frequently observed among people with diabetes, than in the general population. Furthermore, diabetic complications such as limited joint mobility, retinopathy and neuropathy are associated with liver enzyme activities, independently of alcohol consumption, body mass index, and metabolic control of diabetes [20]. Besides, it has been shown that the alloxan injection causes a significant increase in the activity of several enzymes, such as beta-glucuronidase, N-acetyl-beta-glucosaminidase, lysosomal acid phosphatase, leucine aminopeptidase, and cathepsin D [29]. Moreover, the activities of the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes, among others, have been used as indicators of tissue toxicity in experimental diabetes. For instance, Mori et al., 2003 [30] reported that levels of AST, ALT and alkaline phosphatase (ALP) were higher in streptozotocin-induced diabetic rats, over a 53-day period.

The duration of the alloxan-induced diabetic state is still a matter of concern. An earlier work [31] showed that alloxan-induced diabetes of 4-day duration produced metabolite changes in the brain, compatible with severe reduction of cerebral metabolism and reduced phosphofructokinase activity. Alloxan-treated animals were also severely dehydrated. A more recent work [32] showed disease-related abnormalities, such as ATPase activities in sciatic nerves, from rats with alloxan-induced diabetes of various durations (2 weeks up to 6 months). Others observed high glucose levels even after 2 weeks of alloxan injection [33]. Under the conditions of the present investigation, diabetes was well maintained up to the 7th-day treatment with AECS, which began 48 h after a single alloxan injection.

Levels of glucose, insulin, triglycerides and total cholesterol were also shown to increase in experimental models of chemically-induced diabetes, including that with alloxan. A recent work [34] reported the reversibility of the diabetic state, 12 days after the alloxan injection, as demonstrated by the reduction of glucose and triglyceride concentrations, and a positive reaction of the anti-insulin antibodies in the pancreatic tissue. In the present investigation, we followed the hepatic enzymes and lipid profile, for 4 and 7 days, when the diabetic state was still well maintained.

AECS administration to normal animals caused no changes in any of the measured parameters, similarly to results observed by others [16]. Their data and ours suggest that the mode of action of AECS in diabetic animals does not resemble those of sulfonyl ureas or insulin. It

may, however, act in a similar way to biguanides, via the inhibition of gluconeogenesis.

Conclusions

In conclusion, we showed anti-diabetic and anti-lipemic effects of the aqueous extract prepared from the fresh leaves of *C. sicyoides*. The plant leaves are rich in polysaccharide type compounds, and some of the effects observed with the AECS were also demonstrated with the carbohydrate fraction. Our results suggest that, at least in part, AECS effects are due to this plant constituents.

Methods

Plant material and preparation of the aqueous extract

The plant was collected at the city of Barbalha, state of Ceará, Brazil, and identified by Prof. A. Fernandes from the Biology Department, of the Federal University of Ceará (UFC). The voucher (No. 32.240-EAC) is deposited at the Prisco Bezerra Herbarium, of the UFC. Three hundred grams of fresh leaves from *C. sicyoides* were blended with approximately 2 L of distilled water, in order to prepare an aqueous extract. The extract was heated for about 2 h at 60°C, filtered in a double layer of gauze, and reduced at 60°C to half of its original volume. A one milliliter sample was completely evaporated in the oven, and the solid residue was weighted, giving a final concentration of 20 to 30 mg per mL. The extract was kept at -20°C until use.

Animals

Male Wistar rats (250–300 g) from the Animal House of the Faculty of Medicine of Juazeiro do Norte (FMJ) were used. Animals were maintained in plastic cages and 12 h light/dark cycle, with free access to food and water. Experiments were performed according to the Guide for the care and use of laboratory animals, from the US Department of Health and Human Services.

Experimental protocol

Diabetes was induced by the intravenous administration of alloxan (60 mg/kg), after anesthesia with ethyl ether. Forty-eight hours later, the blood (1 mL) was collected from the orbital sinus into tubes containing separator gel (from Vacuette, Brazil). The serum was separated by centrifugation at 3,500 rpm for 10 min, and immediately used for biochemical assays. Only animals presenting glycemic levels equal to or above 200 mg/dL were submitted to treatments, which consisted of a daily administration of the AECS (100 and 200 mg/kg, p.o.) or an equivalent volume of distilled water (controls), for 4 or 7 days. The oral treatments (by gavage) of all groups were carried out at the same time (in the morning) and under the same conditions. One hour after the last administration, the blood was collected again for biochemical measurements. In another set of experiments, non-diabetic normal rats

were administered daily with AECS (100 mg/kg, p.o.) for 7 days and, 1 h after the last administration, the blood was collected for biochemical measurements as described above.

Biochemical measurements

Glucose was determined according to a previously described method [21]. Determinations of ALT and AST were carried out by methods described by Bergmeyer et al., 1978 [22], and triglycerides and cholesterol were measured by standard enzymatic methods with a spectrophotometer Selectra II model, from Winner. The phytochemical profile was performed as described by Costa, 1977 [23], through identification reactions based on the chemical group to be determined or by thin layer chromatography.

Statistical analysis

Results were expressed as means \pm SEM. Data were analyzed with One-way ANOVA for the comparison between groups, followed by Tukey as a *post hoc test*. The significance level was set at $p < 0.05$.

Authors' contributions

GSB Viana: coordination of the project, determination of biochemical parameters, discussion of results and manuscript elaboration.

ACC Medeiros and AMR Lacerda: assistance with animal handling and in the determination of biochemical parameters (medical students from FMJ).

LKAM Leal: determination of phytochemical profile.

TG Vale: determination of biochemical parameters.

FJA Matos: identification of the botanical species.

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