

Hypoglycaemic effect of *Croton cuneatus* in streptozotocin-induced diabetic rats

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RESUMO: “Efeito hipoglicêmico de *Croton cuneatus* em ratos diabéticos induzido por estreptozotocina”. A ação hipoglicemiante do extrato aquoso das cascas do caule de *Croton cuneatus* Klotz (Euphorbiaceae) foi investigada em ratos com diabetes induzida pela estreptozotocina (STZ). Doses crescentes do extrato aquoso (6,5, 13, 26 e 52 mg/kg i.p.) foram administradas separadamente a grupos de animais normais e diabéticos em jejum. Foram avaliadas as concentrações plasmáticas de glicose e colesterol, assim como mudanças no peso corporal. A administração crônica intraperitoneal (i.p.) do extrato durante 22 dias induziu uma redução significativa nos níveis de glicose sanguínea. Foi feita uma comparação entre o extrato aquoso de *C. cuneatus* e a droga de referência glibenclamida. Os resultados desse experimento indicam que esta planta possui atividade antidiabética em modelo com animais hiperglicêmicos.

Unitermos: *Croton cuneatus*, estreptozotocina, açúcar no sangue, efeito antihiperglicêmico.

ABSTRACT: Aqueous extract of the stem barks of *Croton cuneatus* Klotz (Euphorbiaceae) was investigated for hypoglycaemic activity in streptozotocin(STZ)-induced diabetic rats. Increasing doses of aqueous extract (6.5, 13, 26 and 52 mg/kg i.p.) were separately administered to groups of fasted normal and diabetic rats. Plasma glucose concentration, cholesterol and changes in body weight were evaluated. The chronic intraperitoneal (i.p.) administration of the extract for 22 days was found to induce significant reduction in blood glucose level. A comparison was made between the action of the aqueous extract of *C. cuneatus* and the reference standard drug glibenclamide. The results of this experimental animal study indicate that this plant has an antidiabetic activity in hyperglycaemic rat models.

Keywords: *Croton cuneatus*, streptozotocin, blood glucose, antihyperglycemic effect.

INTRODUCTION

Diabetes mellitus (DM) is an endocrine disorder characterized by hyperglycemia and glycosuria due to absolute or relative lack of insulin. In 2004 according to WHO, more than 150 million people worldwide suffer from diabetes. Its incidence is increasing with alarming mortality and morbidity, and it is estimated that by the year 2025 the number of people with this disease will be double (WHO 1999, Boyle et al., 2001, Wild et al., 2004). There is an increasing demand of new antidiabetic products due to the drawbacks associated with insulin and oral hypoglycemic agents actually available (Fertig et al., 1995, Yariura-Tobias et al., 2001). Lowering the concentration of glucose in blood is the best defense against complications due to diabetes such as: blindness, renal failure and limb amputation (Mayfield, 1998). In folk medical practice around the world, many plants have been used to treat diabetes (Bayley; Day 1989; Ivorra et al., 1989; Barbosa-Filho et al., 2005; Agra et al., 2007).

Most of these medicinal plants are not scientifically validated, for their therapeutic efficacy and safety uses. The World Health Organization has also recommended the evaluation of the effectiveness of the numerous medicinal plants used by the people in different countries to get relief from diabetes mellitus (WHO 1980).

C. cuneatus is commonly known in Venezuela as “arapurina” and “caferana”. The plant is attributed with medicinal properties for the indigenous people, such as: relief of gastrointestinal disorders, rheumatism and diabetes. The isolation of three new alkaloids from the organic extracts of the leaves of *C. cuneatus* has been reported (Suárez et al., 2004). In the essential oil obtained from the leaf, 43 compounds has been described, being the major ones: α -11 eudesmene, methyleugenol, 4- α -seleniol, cedryl propyl ether, τ -cadinol and cubenol (Suárez et al., 2005a). Recently, we have determined the anti-inflammatory activity of an aqueous extract of the aerial parts of this plant (Suárez et al., 2005b), and to the best of our knowledge, no other information

about the pharmacological properties have yet appeared. The present study was undertaken to evaluate the hypoglycemic and antidiabetic properties of *C. cuneatus* stem bark aqueous extract in normal and streptozotocin-induced diabetic rats. The results were also compared with glibenclamide as a reference drug.

MATERIAL AND METHODS

Plant material

Stem-barks of the plant *C. cuneatus* were collected in May 2003 from the Barinas State of Venezuela. The plant was identified and authenticated by Dr. Anibal Castillo, and a voucher specimen (AC-6483) has been deposited in the Herbarium Ovalles of the Facultad de Farmacia of the Universidad Central of Venezuela.

Preparation of the plant extract

The aqueous extract was prepared by decoction. Stem-barks air-dried at room temperature were cut into small pieces, and powdered in a blender. 250 g of powder were mixed with 500 mL of distilled water and boiled for 15 min. The aqueous extract was filtered and freeze-dried. The lyophilized, a dark-brown material was stored in a refrigerator at 5 °C. Portions of this residue were weighed and suspended in distilled water daily, just before administration.

Animals

Young adult male albino rats (Sprague-Dawley strain) weighing 170 - 180g were used. The animals were housed in polypropylene cages in standard environmental conditions, 12 h light and 12 h dark cycle at 25 ± 2 °C. Before and during the experiments, the rats were fed with standard laboratory pellet diet and water *ad libitum*. Animals were treated according to international standards of animal's welfare (National Institutes of Health, 1996).

Induction of diabetes

Experimental diabetes was induced in rats by intraperitoneal administration of streptozotocin (Sigma, St Louis, MO, USA) at a dose of 50 mg/kg body weight (Verspohl, 2002). After 48 h of streptozotocin injection, blood glucose levels were estimated. Animals with blood glucose concentrations increasing by more of 40% were considered diabetic and were included in this study.

Experimental design

The study was conducted on 30 rats divided into six different groups of 5 rats each described as follows. Group I: Non-diabetics control. Rats maintained on

standard diet and water *ad libitum*; Group II: Diabetic control rats without treatment; Group III: Diabetic rats were given intraperitoneally dose of ($1/2 TD_{50}$) 52 mg/kg body weight of aqueous extract; Group IV: Diabetic rats were given intraperitoneally dose of ($1/4 TD_{50}$) 26 mg/kg body weight of aqueous extract; Group V: diabetic rats were given intraperitoneally dose of ($1/8 TD_{50}$) 13 mg/kg body weight of aqueous extract; Group V: Diabetic rats were given intraperitoneally dose of ($1/16 TD_{50}$) 6.5 mg/kg body weight of aqueous extract; Group VI: Diabetic rats were given intraperitoneally dose of glibenclamide 5 mg/kg.

The effects of the administration of the aqueous extract of *C. cuneatus* in the animals under study were determined in collected blood samples from the tail vein, the blood glucose lowering activity was observed after 2, 4, and 6 h of administration of single dose, this experiment was considered as an acute treatment.

The evaluation of the blood glucose levels was done each other day during 22 days after daily treatment with the different doses. Levels of blood glucose and serum cholesterol were determined in each sample. The blood samples were centrifuged at 5 °C for ten minutes at 5000 rpm for serum separation. Serum samples were stored at -20 °C for later determination of blood glucose and cholesterol. Blood glucose was estimated spectrophotometrically using a commercial kit. (Wiener Lab®). Determination of total cholesterol was done according to colourimetric method (Stat Fax® 1904 Plus).

Statistical analysis

All the data reported are expressed as mean \pm S.E.M.; statistical evaluation was performed using one-way analysis of variance (ANOVA), using computerized, software Statitix® followed by Student's *t*-test. The values were considered significantly different when *P*-value was less than 0.05 compared to baseline values.

RESULTS

Body weight

The effect of the intraperitoneal administration of aqueous extract of *C. cuneatus* on body weight during the chronic treatment for 22 days was not significant. Basal body weights of all groups were not significantly different. In STZ-diabetic rats, the treatment did not affect the body weight values. After 22 days of treatment, the body weights of all animals were not significantly different from the control group.

Total cholesterol

The plasma was separated from the blood samples after the collection from the tail vein. The chronic

administration of the increasing doses of *C. cuneatus* aqueous extract did not show important changes in the levels of total cholesterol in the animals under study.

Single administration of increasing doses on blood glucose levels

Effects of a single intraperitoneal injection of the different doses of the aqueous extract of *C. cuneatus* on blood glucose levels, in normoglycaemic and STZ-diabetic rats, are shown in Table 1. Induction of diabetes in the experimental rats was confirmed by the presence of high glucose levels; animals with blood glucose levels above 250 mg/dL were considered diabetics. After two, four and six hours following administration of the treatment, the blood glucose levels were determined. In STZ rats, the administration of the aqueous extract caused a significant progressive reduction on the blood glucose levels after 2h of the administration. The maximal reductions in the blood glucose levels were observed at the plant extract dose of 13.0 mg/kg. The doses of 13 and 6.5 mg/kg were as effective as the sulfonylurea drug, glibenclamide. At the dose of 13 mg/kg the blood glucose levels dropped from 344.57 ± 63.32 to 210.17 ± 10.20 mg/dL (41.61 %) after 2h, and from 344.57 ± 63.32 to 136.17 ± 3.18 mg/dL (60.48 %) after 6h. The dose of 6.5 mg/kg reduced the glucose levels from 309.67 ± 1.66 to 195.9 ± 19.1 mg/dL (36.73 %) after 2h and, from 309.67 ± 1.66 to 153.77 ± 26.61 mg/dL (50.34 %) after 6h. The reference antidiabetic drug glibenclamide reduced the blood glucose levels from 330.33 ± 29.42 to 220.17 ± 64.36 mg/dL (33.37%) after 2h and, from 330.33 ± 29.42 to 153.77 ± 26.61 mg/dL (53.45 %) after 6h. These results show that the effectiveness of the aqueous extract of *C. cuneatus* at the mentioned doses was similar to the glibenclamide. The blood glucose levels of the untreated diabetic rats increased during 6 hours treatment.

Chronic administration of graded doses

Figure 1 shows the change in blood glucose levels by the chronic administration of *C. cuneatus* aqueous extract (52, 26, 13 and 6.5 mg/kg i.p.) for 22 days in STZ rats. Significant reduction in blood glucose levels was observed with all the administered doses, however this reduction became more pronounced with the lower doses. The antidiabetic effect was not found to be dose dependent as there was not significant differences between the 26, 13 and 6.5 mg/kg extract treated groups.

DISCUSSION

In the present study, the antihyperglycemic activity of increasing doses of aqueous extract of *C. cuneatus* was assessed in STZ-diabetic rats. The model of diabetic rats induced with STZ, is one of the most useful experimental to represent type II diabetes mellitus and is

widely used in the evaluation of plants extracts (Pepato et al., 2002, Muruganandan et al., 2005, Husen et al., 2004). STZ destroy the pancreatic β cells, which are the insulin secret cells located in the pancreas. The destruction of these β -cells, causes a persistent hyperglycemia (Hofteizer, 1973). Sulfonylureas such as glibenclamide, are the most widely used drugs for the treatment of type 2 diabetes, and appear to function by stimulating insulin secretion. The effect increases the responsiveness of β -cells, which result in more insulin released at all blood glucose concentrations (Groop, 1992); Sulfonylureas usually lower blood glucose concentrations by about 20 percent. The results of blood glucose levels in the STZ-diabetic rats compared with the normal group confirmed the development of the hyperglycemia as result of STZ injection. Experimental evidence obtained in this animal study indicated that the aqueous extract of *C. cuneatus* shows hypoglycaemic properties similar to the used reference drug. The *C. cuneatus* extract significantly reduced the blood glucose levels between 50 and 60% ($p < 0.001$) during the acute treatment, which indicated the strong hypoglycaemic effect. The diabetic animals used in the chronic treatment exhibited significant lowering of the blood glucose levels at the end of 22 days. Our results showed a marked difference between the initial and final levels of blood glucose levels of the different groups treated with the increasing doses of the *C. cuneatus* aqueous extract compared with the diabetic control group. The inter-daily administration for 22 days with the aqueous extract, nearly normalize the glucose levels of the different animals group, which could be interpreted as a cumulative action in the treatment time. The experimental data obtained during the chronic treatment, showed that the antidiabetic activity of *C. cuneatus* aqueous extract was not found dose dependent, as there was no significant differences between the 26, 13 and 6.5 mg/kg extract on the treated groups. The obtained results in our study may suggest that the antihyperglycaemic activity of the aqueous extract of *C. cuneatus*, may be due to a stimulating insulin release from the remnant β cells in the islets of Langerhans. This is the same mechanism exerted by the sulfonyl drugs as glibenclamide which involves an improvement in insulin action at cellular level. Another possible mechanism could also be due however, to a combination of all these effects (Jackson; Bressler, 1981). In order to reveal the possible mechanism of the shown effect a histological analysis of the pancreas, kidneys and liver, is currently under study in our lab.

CONCLUSION

A significant anti-hyperglycaemic activity in STZ diabetic rats was demonstrated, in which the action was exhibited in acute and chronic treatments. The low dose of the extract used 6.5 mg/kg and the duration of the treatment were enough to normalize the blood glucose levels in the diabetic animals. The effectiveness of the

C. cuneatus extract was comparable with the results obtained with glibenclamide which was the drug used as reference in this study. The results of the study revealed the potential of the aqueous extract of *C. cuneatus*, in the treatment of no insulin-dependent diabetes mellitus.

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Table 1. Effect of different doses of aqueous extract of *C. cuneatus* on blood glucose levels (mg/dL) in streptozotocin induced diabetic rats. (Values given represent the mean S.E.M).

Groups (n = 5)	Treatment	plasma glucose levels (mg/dL)			
		0 h	2 h	4 h	6 h
I	Normal Control	172.4 ± 7.66	176.87 ± 16.29	127.57 ± 9.61	127.6 ± 11.40
II	Streptozotocin (diabetic rats)	^{&} 358.93 ± 9.08	^ψ 481.6 ± 40.81	^{&} 464.5 ± 0.00	^ψ 451.2 ± 18.30
III	diabetic rats + 52 mg/kg de <i>C. cuneatus</i>	^ξ 271.17 ± 6.58	^ξ 346.4 ± 40.21 (+ 27.74 %)	^ο 180.67 ± 18.29 (-33.37%)	^φ 160.67 ± 23.88 (-40.75 %)
IV	diabetic rats + 13 mg/kg de <i>C. cuneatus</i>	^{&} 344.57 ± 63.32	^φ 201.17 ± 10.20 (-41.60 %)	^ο 233.13 ± 89.67 (-32.34 %)	^φ 136.17 ± 3.18 (-60.48 %)
V	diabetic rats + 6.4 mg/kg de <i>C. cuneatus</i>	^{&} 309.67 ± 1.66	^ο 195.9 ± 19.1 (-36.4 %)	^ο 224.7 ± 37.35 (-27.66 %)	^φ 153.77 ± 26.61 (-50.34 %)
VI	diabetic rats + Glibenclamide 5 mg/kg	^{&} 330.33 ± 29.42	^φ 220.17 ± 64.36 (-33.5 %)	^ο 167.00 ± 28.11 (-49.44 %)	^φ 153.77 ± 26.61 (-53.44 %)

^ψ p < 0,001 streptozotocin versus control

^φ p < 0,001 treatment versus STZ

[&] p < 0,01 streptozotocin versus control

^ο p < 0,01 treatment versus STZ

^ξ p < 0.05 treatment versus STZ

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