

# HEPATIC ALTERATIONS AND GENOTOXIC EFFECTS OF *Croton cajucara* BENTH (SACACA) IN DIABETIC RATS

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**ABSTRACT** – *Context* - *Croton cajucara* Benth is a plant found in Amazonia, Brazil and the bark and leaf infusions of this plant have been popularly used to treat diabetes and hepatic disorders. *Objectives* - This study investigated effects hepatics alterations and genotoxic and antidiabetic effect of *Croton cajucara* Benth bark extracts treatment in streptozotocin-induced diabetic rats. *Methods* - Male Wistar rats were divided into six groups: control rats; control rats treated with *Croton cajucara* Benth extract during 5 and 20 days; diabetic rats, and diabetic rats treated with *Croton cajucara* Benth during 5 and 20 days. Diabetes was induced by a single intraperitoneal injection of streptozotocin (70 mg/kg). Eight weeks later we measured glucose, triglyceride, cholesterol and hepatic transaminases on blood. The bone marrow micronucleus assay was used to assess the genotoxic activity of *Croton cajucara* Benth. *Results* – Treatment with aqueous extrat of Croton cajucara was able to significantly reduce levels of triglycerides in diabetic animals, however, did not modify significantly the levels of glucose and cholesterol in these animals. There was no significant elevation in liver transaminases in the control group treated with Croton cajucara Benth, as there was no genotoxic effect of treatment in this model. Our results did not show a significant effect on glucose and cholesterol reduction, the treatment was able to significantly reduce triclycerides plasmatic level. There was no significant alterations on hepatic transferase in the animals from the control group treated with *Croton cajucara* Benth. It was observed no genotoxic effect of the treatment in the model studied. *Conclusion* – In this study *Croton cajucara* bark extract showed absence of hepatotoxicity in this animal model and presented a hypolipidemic activity, and could be used to reverse dyslipidemia associated with diabetes and to prevent the cardiovascular complications that are very prevalent in diabetic patients.

**HEADINGS** – Drug-induced liver injury. Diabetes mellitus. Croton, toxicity. Rats.

## INTRODUCTION

The Amazonian region, because of its large biodiversity and popular culture, is characterized by the use of medicinal plants for treatment of various diseases. However, the indiscriminate use of some plant species may lead to serious problems to the human health.

*Croton cajucara* Benth - CcB (of the Euphorbiaceae family), commonly known as Sacaca, is a shrubby plant found in Amazonia, North Brazil<sup>(5, 28)</sup>, whose bark and leaf infusions have been popularly used to treat diabetes, diarrhea, malaria, fever, gastrointestinal, renal and hepatic disorders, as well as in the control of cholesterolemia<sup>(11, 15)</sup>. The wide use of Sacaca for medical purposes in North Brazil and, now, in the Southeast and South regions, is associated with the appearance of numerous clinical cases where side effects are seen, many of which connected with the plant's hepatotoxicity due to continued intake.

In Belém, PR, north of Brazil, it is popularly known that the steady intake of CcB leaves is beneficial because it causes a healthy weight loss. It is also known that the plant can be toxic. Nevertheless, such awareness is not strong enough to prevent its use, and people feel encouraged to take it, feeling that the benefits outweigh the damages<sup>(18, 19, 20, 29)</sup>.

Indeed, cases of acute, chronic and fatal hepatitis were reported in the Amazonian region and in other regions of the country in patients who made use of Sacaca to lose weight and reduce cholesterol levels<sup>(19,20)</sup>.

In the School Hospital of the “Universidade Federal do Pará”, Belém, PR, there is a significant documented history of people afflicted by liver disorders who were drinking CcB leaf teas in long-lasting diets for weight loss. The connection between hepatitis and Sacaca became remarkable in the Amazonian region, particularly after this plant began to be sold for the purposes of weight loss and cholesterol reduction<sup>(4, 14)</sup>.

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The possibility of developing hepatotoxicity may be related to the susceptibility of the liver to external agents, mainly chemical ones, to the high level of these substances in the organ, its high metabolic activity and its anatomic localization, as an intermediate agent between the places of absorption and systemic circulation, working as a filter and as an entry point of several substances ingested<sup>(22)</sup>.

A study performed in 2004, Amazonian people who used Sacaca for 36 months documented 25 cases of hepatotoxicity ascribed to Sacaca consumption, with evidence that 21 people had acute hepatitis, 3 chronic hepatitis, and 1 fulminating hepatitis<sup>(27)</sup>.

Many traditional plants have been claimed to be useful for the control of problems caused by hyperglycemia<sup>(3, 21)</sup>.

The pharmacological activity of the bark extract main component, trans-dehidrocrotinine (t-DCTN), has been extensively studied<sup>(17, 26)</sup>.

Rats treated with t-DCTN (50 mg/kg) showed a significant reduction in the streptozotocin-induced increase in blood glucose levels as well as in the ethanol-induced increase in blood triglycerides<sup>(26)</sup>. This compound demonstrated a significant hypoglycemic activity in alloxan-induced diabetic rats but not in normal rats. The drug also effectively lowered the blood sugar levels in glucose fed normal rats<sup>(7)</sup>. Hyperlipidemic mice treated with CcB bark aqueous infusion had the level of triglycerides reduced and the level of cholesterol redistributed<sup>(3)</sup>. In vivo studies confirmed that t-DCTN is not genotoxic nor cytotoxic to mice bone marrow cells<sup>(1, 25)</sup>.

The aim of this work was to determine hepatic alterations, genotoxic activities and the potential effects of CcB bark extract in long term (chronic) diabetic rats.

## METHODS

### Plant material

Bark fragments of *Croton cajucara* Benth were collected in Santarém, North Brazil. The bark (5 g) was ground and mixed with boiling water (100 mL) to provide a 5% aqueous extract. After 10 minutes, the mixture was filtered with filter paper and the extract was administered to the rats.

### Animals

The experimental procedures complied with the rules established by the "Research in Health and Animal Rights" according to the Commission of Research and Ethics in Health of the Research and Postgraduate Group of the "Hospital de Clínicas de Porto Alegre"<sup>(9)</sup> (HCPA), Universidade Federal do Rio Grande do SUL (UFRGS), Porto Alegre, RS, Brazil.

Fifty male Wistar rats weighing 200-300 g were used. They were obtained from the Experimental Animals Breeding Colony of the "Instituto de Ciências Básicas da Saúde, UFRGS" (CREAL). They were kept at the Animal Experimentation Unit of the Research Center of the HCPA, in plastic boxes measuring 47x34x18cm lined with wood chips, in a 12-hour dark/light cycle (light from 7 a.m. to 19 p.m.) at a temperature of 22 ± 4C.

### Groups and treatment protocols

The rats were randomly divided into six groups. In three groups, diabetes was induced by a single intraperitoneal (i.p.) injection of streptozotocin (70 mg/kg body weight; Sigma Chemical) in freshly prepared 10 mmol/L sodium citrate, pH 4.5. Five days after streptozotocin injection, plasma glucose concentration was measured using retro-orbital blood samples obtained from rats after overnight food deprivation. A plasma glucose level >250 mg/dL was considered indicative of diabetes.

The experimental groups comprised: I - normal control group (Co: n = 10) received 1,5 mL of distilled water administered intragastric (I.G.); II - group treated with CcB for 5 days (CcB-5D: n = 10): 1,5 mL of the CcB extract I.G. during the last 5 days before killed; III - group treated with CcB for 20 days (CcB-20D: n = 10): 1,5 mL of the CcB extract I.G. for 20 days before killed; IV - diabetic group (DM: n = 10): 1,5 mL of distilled water I.G.; V - diabetic group treated with CcB for 5 days (DM + CcB-5D: n = 10): 1,5 mL of the CcB extract I.G. during the last 5 days before killed; and VI - diabetic group treated with CcB for 20 days (DM + CcB-20D: n = 10) 1,5 mL of the CcB extract I.G. for 20 days before killed.

### Biochemical analysis

All animals were killed 8 weeks after streptozotocin administration. The rats were anaesthetized with 2% xylazine hydrochloride (50 mg/kg) and ketamine hydrochloride (100 mg/kg) i.p., and a sample of venous blood was collected in two aliquots from the orbital net for determination of glycemia, triglycerides, and cholesterol levels (Labtest kits) and for aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AP) activities (performed by the Laboratory of Pathology of HCPA).

### Micronucleus assay (MN)

The animals of all groups were killed by cervical dislocation. The MNs were prepared according to the method of MacGregor et al.<sup>(16)</sup>. Briefly, the femurs of each animal were dissected out, and the bone marrow was flushed out into fetal calf serum (FCS) separately. The cells were pelleted by centrifugation and the excess of supernatant was discarded. The pellet and 0.5 mL of supernatant were mixed thoroughly. Smears were drawn onto precleaned coded slides using a drop of the resultant suspension in FCS. The slides were air-dried for 24 hours and stained with Leishman solution. Two thousand polychromatic erythrocytes were analyzed per rat. The slides were scored blindly using a light microscope with a 100x immersion objective. Data regarding the polychromatic and normochromatic erythrocyte (PCE/NCE) ratio were also collected, in which a minimum of 400 erythrocytes per animal were scored.

The results were expressed as mean values ± SEM. The data were compared by analysis of variance (ANOVA); when the analysis indicated the presence of a significant difference, the means were compared with the Student Newmann Keuls test. For micronucleus test, the means were compared with the Tukey test and expressed as mean ± SDM. Significance was accepted at P<0.05.

## RESULTS

### Biochemical analysis

Blood plasma glucose, triglycerides, and cholesterol levels in streptozotocin-treated rats were significantly higher than in the Co and in CcB-treated animals (CcB-5D and CcB-20D). Glucose and cholesterol levels were not affected by CcB treatment in diabetic animals. On the other hand, triglyceride concentration in the plasma of streptozotocin-treated rats was significantly lower after treatment with CcB for 5 and 20 days (Table 1).

TABLE 1. Glucose, triglycerides and cholesterol in the blood plasma

Group	Glucose (mg/dL)	Triglycerides (mg/dL)	Cholesterol (mg/dL)
Co	215,97 ± 9,86a	91,58 ± 12,41b	51,85 ± 5,69a
CcB-5D	211,8 ± 17,09a	90,2 ± 8,11b	55,6 ± 4,86a
CcB-20D	232,08 ± 24,49a	89,66 ± 12,12b	56,08 ± 3,65a
DM	468,8 ± 36,12b	227,3 ± 54,22a	85,1 ± 4,86b
DM+CcB-5D	406 ± 15,77b	83,3 ± 9b	72,8 ± 2,50b
DM+CcB-20D	418,5 ± 22,82b	91,34 ± 24,34b	71,6 ± 6,12b

Values are mean ± SEM, n = 10. Means without a common letter differ, P<0.05

The biochemical analysis showed that the diabetic group had higher ALT and AP levels than the control group. Treatment reduced ALT level only in diabetic rats. ALT, AST and AP were not affected by CcB treatment in non-diabetic animals (Table 2).

TABLE 2. Effect of streptozotocin induced diabetes and *Croton cajucara* bark extract (CcB) on serum enzymes activities: ALT, AST and AP

Group	AST (U/L)	ALT (U/L)	AP (U/L)
CO	153,90 ± 12,30 <sup>a</sup>	53,20 ± 1,86 <sup>a</sup>	136,20 ± 10,07 <sup>a</sup>
CcB-5D	149,4 ± 11,30 <sup>a</sup>	66,5 ± 10,63 <sup>a</sup>	168,2 ± 18,75 <sup>a</sup>
CcB-20D	131,73 ± 12,60 <sup>a</sup>	59,45 ± 5,28 <sup>a</sup>	137,45 ± 9,96 <sup>a</sup>
DM	201,8 ± 41,63 <sup>a</sup>	154,1 ± 38,85 <sup>b</sup>	411,3 ± 46,08 <sup>b</sup>
DM+CcB-5D	127,3 ± 15,53 <sup>a</sup>	71,33 ± 8,57 <sup>a</sup>	330,4 ± 93,49 <sup>b</sup>
DM+CcB-20D	143,3 ± 21,95 <sup>a</sup>	99,8 ± 10,82 <sup>a</sup>	436,2 ± 46,74 <sup>b</sup>

Values are mean ± SEM, n = 10. Means without a common letter differ, P<0.05

### Genotoxic potential

Table 3 displays the micronucleus frequency in the animals treated in vivo. Although the diabetic rats increased PCEs as compared to the controls, significant differences were not observed between the groups. Similarly, no difference in the PCE/NCE ratio was found in any of the groups analyzed.

TABLE 3. Micronucleated polychromatic erythrocytes frequency (MNPCEs) and rate PCE/NCE in the bone marrow of diabetic and non-diabetic male rats treated with *Croton cajucara* Benth stem bark aqueous solution

Group	Number of PCEs analysed	Number of micronucleated PCEs in 2000 PCEs ± ST*	PCE/NCE ratio*
CO	20.000	3,88 ± 2,70 <sup>a</sup>	1,11 ± 0,42 <sup>b</sup>
CcB-5D	20.000	6,11 ± 1,27 <sup>a</sup>	1,13 ± 0,58 <sup>b</sup>
CcB-20D	20.000	3,25 ± 2,25 <sup>a</sup>	1,14 ± 0,43 <sup>b</sup>
DM	20.000	7,25 ± 4,33 <sup>a</sup>	0,93 ± 0,40 <sup>b</sup>
DM+CcB-5D	20.000	6,44 ± 3,47 <sup>a</sup>	1,12 ± 0,46 <sup>b</sup>
DM+CcB-20D	20.000	4,22 ± 2,33 <sup>a</sup>	1,02 ± 0,45 <sup>b</sup>

\*ST: standard deviation. Values with the same letter do not differ statistically (one-way ANOVA; Tukey post-hoc test, P<0.05)

## DISCUSSION

A vegetal species from Amazon region, CcB is widely used by the local population to treat gastrointestinal conditions, but it can cause hepatic dysfunction and in some cases even fatal hepatitis<sup>(12, 22)</sup>. Indeed, cases of toxic hepatitis have been reported in several hospitals in Belém, PA, because of excessive consumption of extremely strong CcB tea<sup>(17)</sup>.

Here the effects of a Sacaca herbal extract were evaluated in rat livers. The administration of Sacaca herbal extract in this trial was not able to cause any kind of alteration in the livers with 14 days of drug administration. However, hepatocellular alterations were identified in some animals when the extract was administered for 28 days, and all the animals treated with the drug for 56 days presented hepatocellular necrosis, similar to acute hepatitis. Therefore, it is concluded that Sacaca toxicity is dose-dependent<sup>(10)</sup>.

The liver plays an important role in the digestion, metabolism, and storage of nutrients. Liver injury due to pharmacological treatment plays a significant role<sup>(8)</sup>. Hepatic damage results in increased concentrations of AST, ALT and AP. The high concentration of serum enzymes such as AST and ALT is generally regarded as one of the sensitive markers of hepatic damage<sup>(30)</sup>. In this study, the increased concentration of ALT, AST and AP in diabetic animals returned to the control index after treatment with CcB bark extract, but AP concentration remained elevated. Similar results are observed in studies with CCl<sub>4</sub> intoxication and treatment with *Rhoicissus tridentate*<sup>(23)</sup> and *Cytisus scoparius*<sup>(24)</sup>. Reduction in the levels of serum aminotransferase (ALT and AST) and lactate dehydrogenase (LH) towards the respective normal values by plant extracts indicates stabilization of the plasma membranes, as well as repair of hepatic tissue damage<sup>(24)</sup>. The non-diabetic animals treated with CcB bark extract did not show increase in transaminases and AP concentration, indicating the absence of hepatotoxicity in this animal model.

In the present study, it was apparent that the CcB bark extract did not have substantial effect on blood glucose and cholesterol levels. This observation differs from the results of Silva et al.<sup>(26)</sup>, who found a hypoglycemic effect of CcB when given to streptozotocin-diabetic rats. These apparently conflicting results

are explained by the fact that, in our study, CcB was given in bark aqueous extract form after diabetes had been established, whereas in the previous study the t-DCTN was administered before the induction of diabetes or 72h after the induction. On the other hand, treatment with CcB bark extract significantly reduced blood triglyceride levels in diabetic rats, and did not have effect on blood triglycerides in non-diabetic rats. Other studies suggest that TG itself is independently related to coronary heart disease<sup>(2,6)</sup>, and most of the anti-hypercholesterolemic drugs do not decrease TG levels<sup>(13)</sup>, but CcB extract treatment returned the triglycerides to control values. This suggests that CcB bark extract has a hypolipidemic activity, and could be used to reverse dyslipidemia associated with diabetes and to prevent the cardiovascular complications that are very prevalent in diabetic patients.

The bone marrow MN was used to assess the genotoxic activity of CcB. The results show that the aqueous extract of CcB bark did not induce any increase in chromosomal damage (chromosomal loss or breakage) when administered to rats with and without diabetes for periods of 5 and 20 days, as compared to the respective control groups (Table 3).

Although the genotoxic activity of the plant species has been little studied, CcB has been described in the literature as not having any genotoxic activity, which corroborates the results obtained in the present study. In fact, Santos et al.<sup>(25)</sup> evaluated the genotoxic activity of methanolic extract of CcB bark using the micronucleus assay in Swiss albino mice treated once a week with three extract concentrations (312.5; 625 and 1,250 mg kg<sup>-1</sup> i.g.) for 28 days. The results revealed the absence of genotoxic activity for the three concentrations tested.

Another study carried out with CcB revealed the absence of genotoxic activity using t-DCTN, the most important diterpene isolated from the plant bark. t-DCTN was administered as a single dose via intraperitoneal injection in Swiss albino mice. The genotoxic analysis was carried out using the micronucleus test and chromosomal aberrations in bone marrow cells<sup>(1)</sup>.

In the light of the negative results of the study above, Agner et al.<sup>(1)</sup> assessed the antimutagenic activity of t-DCTN in the same animals using cyclofosfamide as inducer of genetic damage

and the micronucleus and chromosomal aberration assays as investigation protocols. The authors chose two t-DCTN treatment approaches: intraperitoneal injection and gavage. In both protocols, the modulator agent was administered 30 min before the i.p. injection of the genotoxic agent as pre-treatment. The results obtained in the two bioassays evidenced the antigenotoxic activity of t-DCTN against the genetic damage induced by cyclofosfamide, with both modes of administration.

Based on the results obtained in the present study and those published in the literature, it is possible to conclude that the aqueous and methanolic extracts of CcB and of t-DCTN (its main constituent agent) are not capable to induce chromosomal loss and/or breakage in bone marrow cells of rodents. Apart from this, t-DCTN presented anti-genotoxic action in mice, by protecting against the induction of clastogenic and/or aneugenic events. Although further studies should be carried out to broaden the spectrum of genotoxic events to be evaluated, CcB does not seem to pose hazards of genetic damage to humans who ingest the plant extracts.

## CONCLUSION

In summary, treatment with CcB bark extract did not affect plasmatic glucose and cholesterol levels, but it was able to significantly reduce triglycerides level. There was no significant alteration in hepatic transferase in the control group treated with CcB bark extract. In diabetic rats, the treatment reduced the plasmatic level of these enzymes. No genotoxic effect of the treatment was observed in the model studied.

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Rodrigues G, Marcolino E, Porawski M, Lehmann M, Marroni NP. Alterações hepáticas e efeitos genotóxicos do *Croton cajucara* Benth (SACACA) em ratos diabéticos. *Arq Gastroenterol.* 2010;47(3):301-5.

**RESUMO** – *Contexto* - *Croton cajucara* Benth é uma planta encontrada na Amazônia, Brasil. Infusões da casca e folhas desta planta são utilizadas popularmente no tratamento de diabetes e doenças hepáticas. *Objetivos* - Este estudo investigou as alterações hepáticas e os efeitos genotóxicos da casca do extrato do *Croton cajucara* Benth em animais diabéticos induzidos por estreptozotocina. *Métodos* - Ratos Wistar machos foram divididos em seis grupos: ratos controle, ratos controle tratados com extrato de *Croton cajucara* Benth durante 5 e 20 dias, ratos diabéticos e diabéticos tratados com *Croton cajucara* Benth durante 5 e 20 dias. O diabetes foi induzido por uma única injeção intraperitoneal de estreptozotocina (70 mg/kg). Oito semanas mais tarde foram medidos os níveis de glicose, triglicéridios, colesterol e transaminases hepáticas no sangue. O teste do micronúcleo da medula óssea foi utilizado para avaliar a atividade genotóxica do *Croton cajucara* Benth. *Resultados* - O tratamento com o extrato aquoso do *Croton cajucara* foi capaz de reduzir significativamente os níveis plasmáticos dos triglicéridios nos animais diabéticos, porém, não modificaram significativamente os níveis de glicose e colesterol nesses animais. Não houve elevação significativa nas transaminases hepáticas nos animais do grupo controle tratadas com *Croton cajucara* Benth, assim como também não houve efeito genotóxico do tratamento, no modelo estudado. *Conclusão* – O extrato aquoso da casca do *Croton cajucara* Benth foi hipolipemiante, sugerindo seu uso para prevenir as dislipidemias encontradas em pacientes diabéticos.

**DESCRIPTORIOS** – Doença hepática induzida por drogas. Diabetes mellitus. Croton, toxicidade. Ratos.



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