Full Length Research Paper

Antidiabetic and genotoxic effects on Wistar rats treated with aqueous extract from *Chrysobalanus icaco* L.

S. C. Ferreira-Machado¹,², R. F. Gagliardi³, A. P. M. Nunes², M. P. Rodrigues², F. J. S. Dantas², J. C. P. De Mattos², C. A. F. Peregrino⁴, E. G. Moura⁵ and A. Caldeira-de-Araujo²*

¹Departamento de Biologia Geral, Universidade Federal Fluminense, Niterói, RJ, Brasil.
²Departamento de Biofísica e Biometria, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brasil.
³Núcleo de Biotecnologia Vegetal, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brasil.
⁴Laboratório Universitário Rodolpho Albino, Universidade Federal Fluminense, Niterói, RJ, Brasil.
⁵Departamento de Ciências Fisiológicas, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brasil.

*Corresponding author. E-mail: caldeiradearaujo@gmail.com. Tel: (5521) 2334-0061.

Chrysobalanus icaco L. is a medicinal plant, used to treat diabetes and dyslipidemia in Brazil. The biological effects may vary depending on the source of plant. Experiments were performed to assess these effects from plants collected in the field and those obtained from dried herbs market. Glycemia, cholesterol and triglycerides serum concentrations were measured in healthy and diabetic rats treated with aqueous extract of leaves. Diabetic rats treated with the extract showed lower serum triglycerides, but there was no significant difference (P>0.05) in glycemia and cholesterol levels, compared to the control group without diabetes. Also, the genotoxic effects of these extracts were evaluated using the comet assay in total blood cells obtained from healthy rats ingested with extracts instead of drinking water. This assay showed that the extracts from either free market or endemic area were genotoxic. However, the extract obtained from the popular market was more genotoxic than that prepared from field plants. This study demonstrates that though the extract has therapeutic property that lowers the rate of triglycerides, it is not free of deleterious effects; this calls for precaution in its use as a phytotherapeutical agent.

Key words: Chrysobalanus icaco, cholesterol, comet assay, genotoxic potentiality, triglycerides, diabetes.

INTRODUCTION

The use of plants for therapeutic purposes is probably as old as human civilization (Akinboro and Bakare, 2007). The herbs used in alternative therapy have been intensively marketed in Brazil; they are found in free markets, pharmacies, supermarkets and specialty stores. When tagged, plant materials are called "natural products";
they have greater efficacy and fewer collateral effects compared to phytochemicals produced industrially (Gadano et al., 2006). Many of therapeutic activities attributed to certain plant drugs have no scientific support (Costa et al., 2008) and often times its ethnobotanic knowledge that directs phytochemical research of different metabolites and their biological effects.

*Chrysobalanus icaco* L. (*Chrysobalanaceae*) is a medium sized shrub known as abajeru, in Rio de Janeiro (Brazil). This species occurs in coastal areas of the American continent (Dahlgren, 1980; Ferreira-Machado et al., 2004) and is used as diuretic and hypoglycemic agent in the treatment of diabetes (Costa, 1977; Vargas-Simon et al., 1997; De Paulo et al., 2000). In folk medicine, this species is used in the form of tea prepared from various parts of the plant, especially leaves. In Northern Brazil, root is also used for this purpose (Coelho-Ferreira, 2009). There are pharmacological studies that show the antihyperglycemic property of some species of *Chrysobalanaceae*, as *C. icaco* (Presta and Pereira, 1987) and *Parinari excelsa* (Ndiaye et al., 2008), proving the effects reported in folk medicine. Phytochemical studies have evidenced the presence of myricetin-3-O-glucuronide, quercetin, and rutin, as well as other minor myricetin and quercetin derivatives in the hydroalcoholic extract of *C. icaco* leaves. It was shown that myricetin has both a hypoglycaemic and hypotriglyceridemic effect in diabetic rats (Ong and Khoo, 2000) and could thus have therapeutic value in the treatment of diabetes. These data could explain the traditional use of this species.

However, despite its popular use and beneficial biological effects, some cytotoxic effects have been found in this species. This way, *in vitro* experiments with aqueous extract revealed a potential genotoxic effect, as demonstrated either by induction of single strand breaks in plasmid DNA or by transformation efficiency reduction in bacteria (Ferreira-Machado et al., 2004). On this basis, it is also important to determine the biological effects by *in vivo* bioassays. In this context, the alkaline version of Single Cell Gel Electrophoresis (SCGE) or Comet Assay has been used by many investigators to evaluate the genotoxicity of several chemicals *in vitro* and/or *in vivo* (Tice et al., 2000; Santos et al., 2008; Costa et al., 2008; Verschaeye and Van Staden, 2008; Moretti et al., 2013). The unique design of the comet assay provides direct determination of the single and double-strand DNA breaks, alkalilabile lesions and indirect excisions caused by repair enzymes in individual cells (Rojas et al., 1999; Kim et al., 2002; Collins et al., 2008; Trzeciak et al., 2008).

In the present work, the effects of the aqueous extract of *C. icaco* from the endemic area of Rio de Janeiro, Brazil, were evaluated in rodents. Glycemia, cholesterol and triglycerides serum concentrations were measured in healthy and diabetic rats treated with the aqueous extract of leaves to assess the influence of plant origin (leaves) medicine in the form of tea. These effects have been reported to occur with the use of plants from different regions (Barbosa et al., 2013). Also, the genotoxic effects of these extracts were evaluated using the comet assay in total blood cells obtained from healthy rats that received the extract.

**MATERIALS AND METHODS**

**Plant and preparation of extracts**

*C. icaco* leaves were collected in the endemic area of Parque das Dunas, Cabo Frio City, 22°54'26.4''S - 42°02'05.5''W, Rio de Janeiro State, Brazil, between 9:00 and 10:00 am in spring. To minimize variations induced by environmental factors in plant chemistry, the leaves were processed once to obtain the total extract used during this work. The sample was identified and a voucher was deposited at the Herbarium Bradeanum, at the Rio de Janeiro State University under registration number 85422.

The leaves from these plants were dried at 37°C for six days in B.O.D. (FANEN, SP, Brazil). After this time, the leaves were minced/chopped and boiled in distilled water (10 g/L) at 100°C for 5 min. This concentration was based on the information gathered among users of Cabo Frio city. Then, tea was filtered, lyophilized and stored at -20°C. At the time of use, the extract was dissolved in water at the desired concentration (0.7 mg/ml) to be given to rats. The same procedure was performed with the dried plant bought from free market.

**Treatment of animals**

Adult male Wistar rats, clinical healthy with 220 to 330 g body weight, were used in this study. They were obtained from the Central Botery of the Rio de Janeiro State University. The rats were divided into five groups (7/group): group 1 (control), healthy rats; group 2, healthy rats treated with aqueous extract of abajeru from endemic area; group 3, healthy rats treated with aqueous extract of abajeru from the free market; group 4, diabetic rats; group 5, diabetic rats treated with aqueous extract of abajeru from endemic area. Each group contained seven animals. All groups were maintained in appropriate plastic cages with cycle of brightness of 12 h and controlled temperature (22°C). Animals could drink daily changed water/extract (from free market or endemic area) *ad libitum*, for 35 days. Diabetes was induced in rats by injecting intraperitoneally a single dose (45 mg/kg body weight) of streptozotocin (Sigma, St Louis, USA) freshly dissolved in 40 mM citrate buffer (pH 4.5) (Hulstijn et al., 2003). Diabetes was confirmed by blood glucose rate above 200 mg/dl, measured 12 days after administration. After confirmation of the hyperglycemia, treatment with the extract was started. The study protocol was approved by the Rio de Janeiro State University ethical committee for animal use.

**Biochemistry analysis**

Glucose, triglycerides and cholesterol rates were measured in healthy and diabetic rats after treating with *C. icaco* extracts. The blood glucose was measured at 1st, 15 and 30th days during the
treatment; this was done always at 8:00 a.m., before meals; the tails of the rats (groups 1, 4 and 5) were punctured. The glucose concentration was measured using a reflectance apparatus based on glucose-glucose oxidase reaction (Glucometer Elite XL Diabetes Care System - Bayer, Tarrytown, NY).

The concentrations of cholesterol and triglycerides were determined enzymatically with cholesterol oxidase-peroxidase 4-aminophenazone (CHOD-PAP) and glycerophosphate oxidase-peroxidase-4-aminophenol-zone (GPO-PAP) methods, respectively, on a Cecil CE spectrophotometer (Maatra et al., 1997). For these analyzes samples (2 ml) of total blood from each animal (groups 1, 4 and 5) treated for 35 days with aqueous extract (endemic area) or just drinking water was obtained by heart puncture.

**Single cell gel electrophoresis assay (SCGE)**

Genotoxic potential was studied using the SCGE assay by *in vivo* assays of an aqueous extract prepared with *C. icaco* L. from free market or endemic area on healthy Wistar rats. To detect DNA lesions in healthy rats treated with or not with the extract, a blood sample (10 μl) of each animal was removed every 7 days, for 35 days, and mixed to 120 μl of low-melting point agarose (0.5%). The cell suspension was then placed on microscope slides that were pre-gelatinized with normal melting point agarose (1.5%). The slides were then coverslipped and kept refrigerated at 4°C to gelify. After 20 min, the slides were immersed in ice-cold alkaline lysing solution (2.5M NaCl, 10 mM Tris, 100 mM ethylenediaminetetraacetic acid (EDTA), 10% dimethyl sulfoxide (DMSO), 1% triton X-100, final pH>13) for at least 1 h, protected from light and refrigerated (4°C). The slides were then placed in an electrophoresis chamber, covered with buffer (300 mM NaOH and 1 mM EDTA, pH>13) and maintained in the dark at 4°C, for 25 min. The electrophoresis was run at 300 mA and 1.6 V/cm for 25 min. The slides were then neutralized with 0.4 M Tris-HCl buffer, pH 7.5, three times for 5 min each; they were air dried and fixed in absolute ethanol for 10 min. Before being examined, the slides were stained with ethidium bromide (20 μg/ml). DNA of individual cells was analyzed under fluorescence microscopy, with an excitation filter of 516 to 560 nm from a 50 W mercury light source and barrier filter, and quantified as described subsequently. For each slide, 100 nuclei were randomly chosen and classified in agreement with the intensity of the tail based on four categories: class 0 (absence of tail); class 1 (tail of up to 1×the diameter of the nucleus of class 0); class 2 (tail of up to 2×the diameter of class 0); class 3 (tail of more than 3×the diameter of the nucleus of class 0). To quantify the lesions produced (arbitrary units), the mean score of the damage was calculated by multiplying the number of cells showing damage in each class (n) by the value of the class. Therefore, the final sum of the classes of the 100 comets can vary between 0 (no harmed) and 300 (all harmed to the maximum).

**Statistical analysis**

Seven replicates were taken in each treatment to detect the differences between the control and treated groups in the same period of analysis. The data were analyzed by ANOVA with repeated measures followed by the Student-Newman-Keuls multiple comparison test through the statistical program InStat version 3.01 (GraphPad Software, San Diego, CA, USA). Differences were considered significant when p value < 0.05.

**RESULTS**

The results presented in Figures 1 and 2 showed that the abajeru extract from endemic area did not promote modifications in the glucose or cholesterol serum
concentrations in diabetic rats. On the other hand, there was a significant decrease (p<0.05) of the triglycerides serum concentration of diabetic treated rats, which was at the same level with the control animals (Figure 2).

The comet assay used to evaluate the genotoxic potential of the abajeru aqueous extract (free market or field) was analyzed based on the presented categories and indicated that the groups that received the extract had a larger amount of lesions in DNA than those that received drinking water (control) (Figure 3).

Regarding the group that received the endemic area aqueous extract, the results indicate that the number of DNA lesions in total blood cells was reduced in the course of time. During the periods of analysis (7, 14 and...
21 days) after beginning treatment with these extracts, there were significant differences in relation to the control (p<0.05); while at the last time intervals (28 and 35 days), there were no observed differences (p>0.05). On the other hand, the rats treated with the extract from samples of free market presented an opposite profile; the number of lesions became more expressive in elapsing of the treatment, in 21, 28 and 35 days after treatment.

DISCUSSION

The results presented showed that aqueous extract from field plants did not promote modifications in the glucose or cholesterol serum concentrations in diabetic rats. Other authors observed using a similar experimental model that this extract was unable to inhibit the elevation of postprandial glyceremia, in contrast with oral hypoglycemic agent as metformin currently used for the treatment of diabetes (Souza et al., 2009; Barbosa et al., 2013). On the other hand, when chronically administered, it was able to reduce fasting blood glucose of alloxan-induced diabetic mice to similar levels with the metformin (Presta and Pereira, 1987; Barbosa et al., 2013). These different activities in the extracts may be due to the animal model used (rats or mouse), concentrations of the extracts or genotypic differences in plant samples used in these studies. Another hypothesis could be the influence of the environment on the synthesis of special metabolites responsible for the biological effects of the extracts.

The effect of reducing the rate of triglycerides serum concentration in diabetic treated rats, which was at the same level with the control animals, demonstrates the medicinal importance of this species since in diabetic patients high triglycerides serum levels can be a predisposition factor to cardiovascular diseases (Abdel-Maksoud et al., 2008; Farmer, 2008).

Regarding genotoxicity, in spite of its crescent use for the treatment or prevention of diseases, great majority of the phytotherapeutic agents continued without scientific background (Maistro et al., 2004; Costa et al., 2008). The medicinal plants in general synthesize toxic substances, which in nature act as a defense against infections, insects and herbivores (Cavalcanti et al., 2006). The results of this work indicated that the extract from free market can present some substances which are absent in the extract from endemic area, favoring their bioaccumulation and consequently explaining the increased lesions number. Most bio-transformations by a detoxication process may involve many oxidative reactions that produce reactive metabolites which can induce genotoxic effects or metabolites that can protect against mutagens (Hodgson and Levi, 1997). Further, chromatography analysis could explain the real differences between the two extracts.

Pharmacological studies showed that leaves from C. icaco methanolic extract reduced the formation of new blood vessels (antiangiogenic potential) in chicken chorioallantoic membrane (De Paulo et al., 2000). The same extract contains some triterpenoids able to inhibit growth and induce apoptosis of K562, an erythroleukemia cell line (Fernandes et al., 2003). So, C. icaco extract could represent an important tool against the tumorigenesis process. However, potential genotoxic effects have also been demonstrated, like those observed with normal blood cells in this article. In this way, it is necessary to better understand the consequences that the genotoxic products can cause organisms at long-term and whether these substances are linked to the type of extraction performed.

It appears that the beneficial and/or harmful effects of the natural medicinal products typically result from combinations of various components present in the plant (Briskin, 2000; Gilbert and Alves, 2003; Ulrich-Merzenich et al., 2007). Abajeru from Cabo Frio allows better control in the preparation of tea and reproducibility of the experiments. But, the plant from free market lacks information on the origin, conservation and soil where it was grown. Differences as to the origin of plants (genotype, environmental factors), as well as the collection and storage methodologies can affect the composition of the extract, making it dangerous for consumption (Briskin, 2000; Büter et al., 1998; Ksouri et al., 2008). As people have greater access to purchase these herbs in the open market, there exists an urgent need to identify the substances responsible for the therapeutic effect and toxicity so as to enable an orientation of safe use of this plant.

Conclusion

Aqueous extract of abajeru from Cabo Frio region induced a significant reduction in rates of triglycerides in the blood of diabetic rats, although not devoid of genotoxic effects in blood cells of these mice. However, samples of the same plant species purchased from free markets had significantly higher genotoxic effect, highlighting the danger of indiscriminate use of medicinal plants from uncontrolled sources.

ACKNOWLEDGEMENTS

This Research was supported by National Council of Scientific and Technological Development—(CNPq), Foundation for the Coordination of Improvement of Higher Education Personnel - (CAPES), Carlos Chagas Filho Foundation for Research Support at State of Rio de
STZ, Streptozotocin; SCGE, single cell gel electrophoresis; CHOD-PAP, cholesterol oxidase-peroxidase 4-aminophenazone; GPO-PAP, glycerophosphate oxidase-peroxidase 4-aminophenazone.

REFERENCES


