Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
17(3): 331-335, Jul./Set. 2007

Received 06/17/07. Accepted 08/02/07

Artigo

A *Chrysobalanus icaco* extract alters the plasmid topology and the effects of stannous chloride on the DNA of plasmids

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RESUMO: "Um extrato de *Chrysobalanus icaco* altera a topologia de plasmídios e os efeitos do cloreto estanoso sobre o DNA de plasmídios". Folhas de *Chrysobalanus icaco* (*C. icaco*) são usadas na medicina popular (conhecido como Abajeru no Brasil) para controlar a glicemia em pacientes diabéticos. Cloreto estanoso ($SnCl_2$) é um agente redutor potente usado para diferentes propostas e apresenta efeitos citotóxico e genotóxico. O objetivo deste trabalho foi investigar os efeitos de um extrato aquoso de *C. icaco* na topologia de DNA plasmidial e nos efeitos do cloreto estanoso sobre o DNA plasmidial. Plasmídios pBSK foram incubados com um extrato de *C. icaco* na presença ou ausência do $SnCl_2$ (200 mg/mL), em seguida, o procedimento de eletroforese em gel de agarose foi realizado. Plasmídios incubados somente com $SnCl_2$ foram usados como controle positivo e, como controle negativo, plasmídios incubados com tampão Tris. Os géis foram corados com brometo de etídio e as bandas de DNA foram semiquantificadas por densitometria. Os dados mostraram que o extrato de *C. icaco* altera o perfil eletroforético e diminui significativamente ($p < 0,05$) os efeitos do $SnCl_2$ sobre DNA plasmidial. Os resultados obtidos neste trabalho indicam uma ação protetora dependente da dose e um efeito genotóxico de extrato de *C. icaco* sobre o DNA plasmidial.

Unitermos: *Chrysobalanus icaco*, Chrysobalanaceae, DNA plasmidial, cloreto estanoso, efeito genotóxico, antioxidante.

ABSTRACT: *Chrysobalanus icaco* (*C. icaco*) leaves are used in folk medicine (known as Abajeru in Brazil) to control the glycaemia in diabetic patients. Stannous chloride ($SnCl_2$) is a powerful reducing agent used for different purposes and presents cytotoxic and genotoxic effects. The aim of this work was to investigate the effect of an aqueous *C. icaco* extract on the plasmid DNA topology and on the effects of the stannous chloride on DNA plasmid. Plasmid pBSK was incubated with a *C. icaco* extract in the presence or absence of $SnCl_2$ (200 mg/mL), after that, the agarose gel electrophoresis procedure was carried out. Plasmid incubated only $SnCl_2$ was used as positive control and, as negative control, plasmid incubated with Tris buffer. The gels were stained with ethidium bromide, DNA bands were semiquantified by densitometry. The data showed that *C. icaco* extract alters the electrophoretic profile and decreases significantly ($p < 0.05$) the effect of $SnCl_2$ on plasmid DNA. The results obtained in this work could indicate a dose-dependent protective action and a genotoxic effect of *C. icaco* extract on plasmid DNA.

Keywords: *Chrysobalanus icaco*, Chrysobalanaceae, plasmid DNA, stannous chloride, genotoxic effect, antioxidant.

INTRODUCTION

Chrysobalanus icaco. (*C. icaco*), also known as "coco plum", "icaco", "agirú", is an evergreen, medium-sized shrub or, rarely, a small tree with leathery, dark-green, round to oval leaves belonging to Chrysobalanaceae family (Mendez et al., 1995; Coradin et al., 1985).

The species are native to coastal areas of southern Florida, the Bahamas and through the Caribbean. It is also found through Central and South America, including Mexico, Ecuador and Northern Brazil as well as tropical Africa (Little et al., 1974). In Brazil,

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aqueous extracts of *C. icaco* leaves (Chrysobalanaceae family) are commonly used in Brazilian traditional medicine to control the glycaemia of diabetic patients (Costa, 1977; Barbosa-Filho et al., 2005; Agra et al., 2007).

Earlier studies have reported the trienoic, tetraenoic acids and their oxo derivatives (Gunstone; Subbarao 1967) and catechol tannins (Verma; Raychaudhuri, 1972) in seed oil of *C. icaco*. The presence of diterpenes and triterpenes in the leaves of *C. icaco* were also reported (Fernandes et al., 2003; Gustafson; Munro, 1991). Phytochemical investigations of *C. icaco* extracts have reported the presence of myricetin in *C. icaco* leaf (Mendez et al., 1995; Fernandes et al., 2003; Gustafson; Munro, 1991; Barbosa et al., 1996).

Stannous chloride ($SnCl_2$) is a powerful reducing agent used for packing canned food, in dental amalgams and for preparing ^{99m}Technetium-radiopharmaceuticals. Previous studies have demonstrated that stannous chloride is capable to inactivate *Escherichia coli* cultures (Melo et al., 2001) and K562 cells (Dantas et al., 2002) as well as to induce single strand breaks in plasmid DNA through generation of free radicals *in vitro* (Dantas et al., 1999; Ferreira-Machado et al., 2004).

The aim of this work was to investigate the effect of an aqueous *C. icaco* extract on the plasmid DNA topology and also on the effects of the stannous chloride on plasmid DNA.

MATERIAL AND METHODS

Drugs

Commercial *C. icaco* was used in this study and it is purchased from Estrella da Terra Produtos Naturais Ltda, Rio de Janeiro (lot 12, validity March 2009) and stannous chloride ($SnCl_2$) was purchased form Sigma Chemicals Co. (USA).

Plasmid DNA

To obtain pBSK plasmid, cultures in LB medium (Miller, 1992) with ampicillin (100 mg/mL) of *E. coli* DH5aF'Iq (rec-) strain hosting this plasmid was carried out (18 hours, 37 °C). The pBSK plasmid, carrying an ampicillin resistance gene, was obtained through alkaline cell lysis method (Sambrook et al., 1989).

Plasmid treatment with *C. icaco* extract

To evaluate the action of aqueous *C. icaco* extract on DNA topology, plasmid pBSK was incubated at different concentrations of this extract (0.5, 5, 50 mg/mL). To assess the action of *C. icaco* extract on effects of $SnCl_2$, plasmid pBSK was incubated with this extract, at the same concentration, in presence of $SnCl_2$ (200 mg/mL). Plasmid incubated only with $SnCl_2$ was used as positive control and, as negative control, plasmid incubated at 10 mM Tris buffer (vehicle, pH 7.4). The incubations were carried out at room temperature for 40 minutes. After that, each sample was mixed with loading buffer (0.25% xylene cyanol, 0.25% bromophenol blue and glycerol in water) and applied in 0.8% agarose horizontal gel electrophoresis chamber in Tris-acetate-EDTA buffer at pH 8.0 and run at 7 V/cm. The electrophoresis procedure was performed to separate the structural conformations of plasmid DNA as such:1) supercoiled native conformation (form I) and 2) open circle (form II). The gel was stained with ethidium bromide (0.5 mg/mL) and the DNA bands were visualized by fluorescence under an ultraviolet transilumination system. The assay was repeated at least four times, the results were digitalized (Kodak Digital Science 1d, EDAS 120) and the bands semiquantified using the computer program Gimp 2 for Windows.

Statistical analysis

Data are reported as percentage of form I and form II (means ± standard deviation). These were compared between the treated and control groups by One way analysis of variance - ANOVA, followed by Bonferroni post test with a $p < 0.05$ as level of significance. InStat Graphpad software was used to perform statistical analysis (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego, USA).

RESULTS

The Figure 1 shows the photograph of agarose gel electrophoresis of pBSK plasmid treated with *C. icaco* extract in presence and absence of $SnCl_2$. This figure shows that after incubation with *C. icaco* extract at the higher concentration used in absence (lane 3) or presence (lane 6) of $SnCl_2$ the electrophoretic profile of plasmid DNA was altered and the form I (supercoiled) and form II (open circle) of plasmid DNA were not present. When plasmid DNA was incubated with *C. icaco* extract at lowest concentrations (5.0 and 0.5 mg/mL), there was no alteration of electrophoretic profile of plasmid DNA (lanes 4 and 5). Also, *C. icaco* extract, at these lowest concentrations, was capable of protect the DNA plasmid of the electrophoretic profile changes induced by $SnCl_2$ (lanes 7 and 8).

To quantify the changes in the topology of plasmid DNA, the percentage of forms I and II were determined by a semiquantitative densitometric method (Figure 2). This figure shows that the percentage of form I and II of pBSK plasmid could be modified by treatment with the aqueous *C. icaco* extract, at the higher

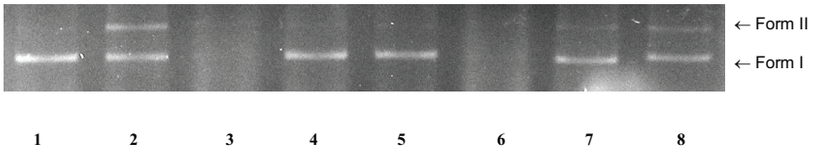

Figure 1. Photograph of agarose gel electrophoresis of plasmid pBSK+ treated with an aqueous *C. icaco* extract in the presence and absence of $SnCl_2$. Plasmids incubated with vehicle (Tris buffer) was used as negative control and plasmid incubated with $SnCl_2$ (200 μg/mL), as positive control. Each sample was mixed with loading buffer and submitted to 0.8% agarose gel electrophoresis. Lanes: (1) pBSK, negative control; (2) pBSK + $SnCl_2$, positive control; (3) pBSK + *C. icaco* extract (50 mg/ml); (4) pBSK + *C. icaco* extract (5 mg/mL); (5) pBSK + *C. icaco* extract (0.5 mg/mL); (6) pBSK + *C. icaco* extract (50 mg/mL) + $SnCl_2$; (7) pBSK + *C. icaco* extract (5 mg/mL) + $SnCl_2$; (8) pBSK + *C. icaco* extract (0.5 mg/mL) + $SnCl_2$. The experiments were performed three times.

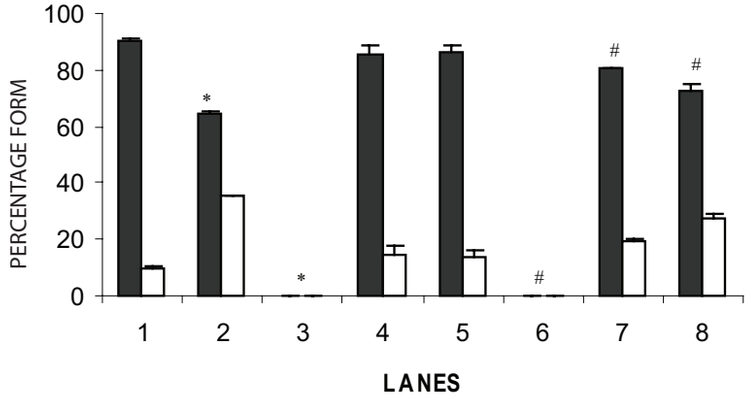

Figure 2. The percentage of plasmid pBSK+ in the form I and II treated with an aqueous *C. icaco* extract in the presence or absence of $SnCl_2$. Plasmids incubated with vehicle (H_2O) were used as negative control while plasmids with $SnCl_2$ (200 μg/mL) used as positive control. Each sample was mixed with loading buffer and submitted to 0.8% agarose gel electrophoresis. Every set of experiments was quadruplicated (n = 4). Lanes: (1) pBSK, negative control; (2) pBSK + $SnCl_2$, positive control; (3) pBSK + *C. icaco* extract (50 mg/mL); (4) pBSK + *C. icaco* extract (5 mg/mL); (5) pBSK + *C. icaco* extract (0.5 mg/mL); (6) pBSK + *C. icaco* extract (50 mg/mL) + $SnCl_2$; (7) pBSK + *C. icaco* extract (5 mg/mL) + $SnCl_2$; (8) pBSK + *C. icaco* extract (0.5mg/mL) + $SnCl_2$. (■) form I (supercoiled), (□) form II (open circle). (*) $p < 0.05$ when compared with negative control (Lane 1). (#) $p < 0.05$ when compared to positive control (lane 2).

concentration used. In addition, the data presented in figure 2 indicate that the effects of $SnCl_2$ could be decreased by *C. icaco* extract at lowest concentrations. Moreover, the semiquantitative analysis presented in figure 2, confirms the qualitative analysis based on figure 1, there were significant differences in every lane when the control group was compared with the various concentrations of *C. icaco,* with or without $SnCl_2$.

DISCUSSION

The genotoxic effect of stannous chloride on DNA has been demonstrated by different experimental models and the mechanism action has been so far related to free radicals generation (Javor et al., 1986; Melo et al. 2001, Dantas et al. 2002, Guedes et al. 2006). In fact, the presence of free radicals scavengers can reduce the changes of electrophoretic profile of plasmid DNA induced by stannous chloride decreasing the DNA strand breaks (Dantas et al. 1999, de Mattos et al., 2000). Moreover, these conformational changes induced by stannous chloride have been used as experimental model to evaluate the either the redoxi, chelating or scavenger potentials of natural products such as *C. icaco* (Simões et al. 2006).

The data obtained in this work indicate that aqueous *C. icaco* extract decreases the genotoxic effect of stannous chloride on plasmid DNA (Figures 1 and 2) suggesting a protective action to this extract. This effect of *C. icaco* extract may well occur at lowest concentrations while, at highest concentrations, this extract could present a strong genotoxic effect (Figure 1, lanes 3 and 6). The data of genotoxic effect of this extract is in according with the results obtained for other authors (Ferreira-Machado et al. 2004) using a different method to prepare the extract of *C. icaco.* As the two extracts were prepared using two different methods, our findings are important due to the extract used in this work represents a situation that is commonly used by the population.

Another relevant finding of our work is that is possible to suggest an important protective effect of *C. icaco* extract against the stannous chloride. Moreover, this result with *C. icaco* and several other natural and synthetic substances have not been described yet by other authors.

The paradoxical results in this work could be explained by presence of different substances in aqueous *C. icaco* extract used. Dependent on the concentration, these compounds could be capable to induce lesions in DNA altering the electrophoretic profile in agarose gels or to protect the same molecule against chemical agents as stannous chloride.

Thus, some compounds (triterpenoids) in *C. icaco* extract demonstrate cytotoxic effect mainly by apoptosis (Fernandes et al. 2003) while other effects could be beneficial on human health. This extract can be used to treat several diseases such as leucorrhea, haemorrhages, diarrhea in folk medicine or as hypoglycemic and antiangiogenic agent (Costa, 1977; Alves-de-Paulo et al., 2000).

In conclusion, the results obtained in this work could indicate a dose-dependent protective action and a genotoxic effect to *C. icaco* extract on plasmid DNA.

ACKNOWLEDGEMENTS

This research was supported by Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Universidade do Estado do Rio de Janeiro (UERJ).

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