Giuseppe Antonio Presta

EFEITO DE UM EXTRATO DE *Chrysobalanus icaco* NA MARCAÇÃO DE CONSTITUÍNTES SANGUÍNEOS COM TECNÉCIO-99m, NA MORFOLOGIA DE HEMÁCIAS, NA TOPOLOGIA PLASMIDIAL E NA AÇÃO DO CLORETO ESTANOSO NO DNA PLASMIDIAL.

Tese apresentada à Universidade Federal do Rio Grande do Norte, para a obtenção do título de Doutor em Ciências da Saúde.

Natal, RN

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A todos que direta ou indiretamente auxiliaram na realização deste trabalho.

LISTA DE ABREVIAÇÕES, SIGLAS E SÍMBOLOS.

DNA	ácido desoxirribonucléico
O_2	oxigênio
ANOVA	análise de variância
ATP	adenosina trifosfato
Bq	Bequerel (unidade de atividade de amostra radioativa no Sistema Internacional, sendo que 1 Bq equivale a uma desintegração por segundo)
BC	Blood Cell (célula sanguínea)
Ca^{++}	íon cálcio
Cl^-	íon cloreto
CO_2	dióxido de carbono
FI-C	fração insolúvel da célula
FS-P	fração solúvel da célula
FI-P	fração insolúvel do plasma
FS-P	fração solúvel do plasma
γ	radiação gama
Hb	hemoglobina
HCO_3^-	íon bicarbonato
keV	quiloeletronvolt
kBq	quilobequerel
MBq	megabequerel
µg	micrograma
µl	microlitro
mg	miligrama
Mo-99	molibdênio
NaCl	cloreto de sódio
nm	nanometro
P	plasma
PP	proteína plasmática
%ATI	porcentagem de radioatividade
rpm	rotações por minuto

Sn^{++}	íon estanoso
$SnCl_2$	cloreto estanoso
99mTc	tecnécio-99m
TCA	ácido tricloroacético
TcO_4^-	íon pertecnetato
$Na^{99m}TcO_4$	pertecnetato de sódio
UI	Unidade internaciomal
IPEN	Instituto de Pesquisas Energéticas e Nucleares de São Paulo
CNEN	Comissão Nacional de Energia Nuclear
RBC	Célula Vermelha do Sangue

RESUMO

O uso de radionuclídeos tem contribuído para avanços relevantes em Ciências da Saúde, seja para pesquisa ou seja para o diagnóstico e/ou tratamento de doenças. Muitos desses avanços têm sido possíveis com a utilização de radiofármacos marcados com tecnécio-99m (99mTc). A marcação desses radiofármacos necessita de um agente redutor e o cloreto estanoso tem sido o mais empregado. Tem sido descrito que diversas drogas naturais ou sintéticas são capazes de interferir na marcação de constituintes sanguíneos com 99mTc, assim como na morfologia das hemácias. O objetivo deste estudo foi avaliar possíveis interferências de um extrato de *Chrysobalanus icaco* (*C. icaco*) na marcação dos constituintes sanguíneos com 99mTc, na morfologia de hemácias do sangue de ratos Wistar, na quebra de DNA de plasmídios e na ação do cloreto estanoso no DNA plasmidial. Os resultados indicam que o extrato de *C. icaco* altera a marcação de constituintes sanguíneos significativamente ($p<0,05$) com 99mTc, a morfologia e a morfometria (relação perímetro/área) das hemácias, talvez por ação quelante ou antioxidante e/ou por efeitos em estruturas da membrana envolvidas no transporte de íons. Além disso *C. icaco* altera o perfil eletroforético e diminui significativamente ($p<0.05$) os efeitos do cloreto estanoso no DNA plasmidial. Estes últimos resultados sugerem uma ação protetora dose-dependente contra a ação do cloreto estanoso e um efeito genotóxico do extrato de *C. icaco* no DNA plasmidial. O estudo tem caráter multidisciplinar com a participação das seguintes áreas do conhecimento: Radiobiologia, Botânica, Genética, Fitoterapia e Hematologia.

Palavras chave: constituintes sanguíneos, tecnécio-99m, cloreto estanoso, morfologia, DNA plasmidial, Chrysobalanus icaco.

SUMÁRIO

Lista de abreviações……..……..vii

Resumo……....ix

Introdução.....01

Revisão de literatura.....03

Artigos anexados.....

Manuscrito aceito para publicação I.....08

Manuscrito aceito para publicação II.....18

Comentários, críticas e conclusões.....25

Anexos.....27

Anexo 1 Carta de Aceite do Manuscrito 1.....27

Anexo 2 Carta de Aceite do Manuscrito 2.....28

Referências.....29

Apêndice 1

Abstract……....34

1. INTRODUÇÃO

Ao longo do tempo, plantas medicinais foram progressivamente introduzidas na medicina alternativa, tendo seu uso aumentado nos últimos anos, mesmo com o número limitado de estudos científicos. Dessa forma, as possibilidades de efeitos tóxicos e/ou genotóxicos não podem ser descartadas, o que tem estimulado estudos desses efeitos para tentar garantir segurança e eficácia na utilização de plantas medicinais. Abajeru (*Chrysobalanus icaco*) é utilizado popularmente em várias regiões do mundo. O extrato de suas folhas tem sido empregado como agente diurético e também como hipoglicemiante. Extrato de abajeru mostrou efeito hipoglicemiante, corrigindo a hiperglicemia de jejum da diabetes mellitus aloxânica e apresentando proteção contra doses tóxicas da aloxana (1,2). O extrato de abajeru contém polifenóis, substâncias que apresentam amplo espectro de atividades biológicas incluindo efeitos antinflamatórios, antivirais, antibacterianos, antiaterogênicos e também anticancer (3,4). Estas atividades estão associadas em grande extensão às suas propriedades antioxidantes, embora outros mecanismos possam também estar envolvidos (5).

O estudo das plantas medicinais através da pesquisa básica é relevante na aplicação clínica das mesmas. Neste trabalho procurou-se utilizar modelos de estudo de efeitos biológicos de plantas medicinais com utilização de radionuclídeos. Um dos modelos experimentais utiliza constituintes sanguíneos marcados com o radionuclídeo tecnécio-99m (99mTc) e tem sido um método importante para a avaliação de efeitos produzidos pelos produtos naturais em testes *in vitro* e *in vivo*.(6,7,8,9) Nos processos de marcação com 99mTc normalmente um agente redutor é utilizado e o de uso mais freqüente é o cloreto estanoso.

Estudos têm demonstrado que cloreto estanoso apresenta efeito citotóxico, sendo capaz de inativar culturas de *Escherichia coli* (6,7) e células K562 (10). Além disso, também é capaz de induzir quebra da cadeia simples do DNA através da geração de radicais livres (efeito genotóxico) *in vitro* (7,11 ,12).

O objetivo deste trabalho foi verificar os efeitos do *Chrysobalanus icaco* na marcação de constituintes sanguíneos com 99mTc, na morfologia de hemácias, na topologia plasmidial e na ação do cloreto estanoso no DNA plasmidial.

O extrato de abajerú foi capaz de (i) apresentar máximo valor de densidade óptica no comprimento de onda de 500nm, (ii) alterar a marcação de constituintes sanguíneos com 99mTc *in vitro* e (iii) modificar qualitativamente (morfologia) e quantitativamente (morfometria) a forma de hemácias; (iv) produzir quebras na cadeia simples do DNA de plasmídeo (efeito genotóxico) e (v) proteger o DNA plasmidial da ação do cloreto estanoso. Estes resultados propiciaram o envio de 2 manuscritos que foram aceitos para publicação em periódicos indexados:

A *Chrysobalanus icaco* extract alters the plasmid topology and the effects of stannous chloride on the DNA strand of plasmids. Aceito para publicação no periódico “Revista Brasileira de Farmacognosia”.

Effects of *Chrysobalanus icaco* on the labeling of blood constituents with technetium-99m and on the shape of the red blood cells. Aceito para publicação periódico ”Brazilian Archives of Biology and Technology”.

2. REVISÃO DA LITERATURA

O desenvolvimento da Ciência tem gerado conhecimentos importantes para o controle das substâncias radioativas, permitindo que os processos envolvidos na sua produção, armazenamento e uso se tornassem mais seguros. A origem do fenômeno radioativo é nuclear, portanto, os nuclídeos que emitem radiação são chamados de radionuclídeos. A emissão radioativa altera profundamente a estrutura atômica do elemento emissor, pois modifica a composição e o balanço energético do seu núcleo. (13,14,15). As aplicações dos radionuclídeos em medicina nuclear podem ser como fontes de irradiação e como traçadores. (14,15,16).

Radiofármacos são moléculas ou células marcadas com radionuclídeos (radiobiocomplexos) (17). A primeira etapa na preparação de radiofármacos é a produção de um radionuclídeo apropriado. Existem duas fontes principais para a produção de radionuclídeos as quais são usadas nos procedimentos em medicina nuclear: as fontes primária e secundária (14,16,18,19,20).

O Radionuclídeo 99mTc

O 99mTc é um dos nuclídeos do elemento tecnécio que é classificado como um metal de transição do grupo VII. Ele possui uma meia-vida de 6 horas, uma emissão de radiação gama de 140 keV de energia e apresenta estados de oxidação que variam de –1 a +7. Estas características físico-químicas e reações radioquímicas apropriadas têm permitido a marcação de inúmeras estruturas celulares e moleculares, de interesse biomédico, com esse radionuclídeo. Na obtenção desses

radiobiocomplexos marcados com 99mTc, um agente redutor é quase sempre necessário e o cloreto estanoso tem sido largamente utilizado.

O 99mTc é obtido em gerador de Mo-99/99mTc com tecnologia desenvolvida no Instituto de Pesquisas Energéticas e Nucleares da Comissão Nacional de Energia Nuclear do Brasil, que o distribui para todos os Serviços de Medicina Nuclear do país, tornando sua disponibilidade de baixo custo, e com atividade diluída, justificando a sua utilização na rotina médica e em pesquisa (17).

O Agente Redutor

A utilização de um agente redutor nos processos de marcação com o 99mTc é de grande relevância, visto que o eluído obtido no gerador como pertecnetato de sódio ($Na^{99m}TcO_4$) não se liga facilmente a outras espécies químicas. Por essa razão, torna-se necessária a redução deste radionuclídeo da valência +7 para valências mais baixas (+3, +4, +5) (20,21).

A redução do íon pertecnetato pode ser obtida através de diferentes agentes químicos, sendo o cloreto estanoso ($SnCl_2$) o agente redutor mais freqüentemente utilizado para esta finalidade (22,23), pois possui uma eficiência de marcação do traçador radioativo, superior a de outros agentes redutores, justificando sua preferência, não só na medicina nuclear, mas também na marcação de diversas estruturas de interesse biomédico (20, 24).

Tem sido descrito que o $SnCl_2$ apresenta importantes efeitos biológicos, como a inativação de culturas bacterianas e a alteração da mobilidade eletroforética de molécula de DNA plasmidial (10,11).

Radiofármaco $Na^{99m}TcO_4$ (Pertecnetato de Sódio*)*

Com administração intravenosa, o $Na^{99m}TcO_4$ é distribuído no compartimento vascular. Cerca de 75% dos íons pertecnetato ligam-se inicialmente às proteínas plasmáticas e esta ligação é reversível (25). A eliminação plasmática é muito rápida e o equilíbrio entre o compartimento vascular e o fluido intersticial é completado em um curto tempo, entre 2 a 3 minutos. A meia–vida de eliminação do plasma é de aproximadamente 30 minutos (20,26). A dose administrada varia com o tipo de estudo a ser realizado, alternando de 37 kBq a 925 kBq.

Modelo de transporte do $SnCl_2$ e $Na^{99m}TcO_4$ para o interior das hemácias

As proteínas integrais da membrana da hemácia através das regiões hidrofóbicas existentes nas suas cadeias polipeptídicas se ligam fortemente aos fosfolipídios da bicamada, fixando as proteínas à membrana plasmática. Uma dessas proteínas é denominada banda-3 e cada hemácia tem cerca de 10^6 exemplares dessa proteína. Além de desempenhar um papel estrutural na fixação do citoesqueleto à membrana, a banda-3 constitui a principal proteína no transporte de ânions das hemácias, estando envolvida na difusão facilitada de íons cloreto que atuam durante o transporte de O_2 e CO_2 (27, 28, 29). Estudos realizados demonstraram que na marcação de hemácias com 99mTc, o ânion pertecnetato atravessa a membrana utilizando o sistema de transporte iônico da banda-3 por troca com os íons cloreto e/ou bicarbonato (24,30). Estes fatos indicam que o processo de marcação ocorre no meio intracelular. Somando-se a isso, o agente redutor, $SnCl_2$, também parece ser transportado para o interior da hemácia por um sistema de transporte específico, o canal de cálcio (19, 31, 32,33).

Hemácias marcadas com 99mTc

A marcação de hemácias com 99mTc representa uma das técnicas de relevância na medicina nuclear, e é aperfeiçoada em resposta ao grande número de exames clínicos em que é utilizada (33,19,21). A marcação de hemácias também tem servido como um modelo simples, conveniente e útil no estudo do fenômeno de transporte, estrutura e função da membrana (22,29). Existem diferentes metodologias para a marcação de hemácias com 99mTc (18,21,24,30). Nos métodos que empregam a marcação *in vitro*, o sangue isolado é incubado com o íon estanoso e posteriormente com íon pertecnetato. Embora a hemoglobina seja o sítio preferencial, outros componentes intracelulares e as proteínas da membrana celular também correspondem a sítios de ligação do 99mTc às hemácias (22,34).

O mecanismo que envolve a ligação 99mTc-hemácias não é completamente entendido. Ainda assim, encontra-se na literatura evidências que sustentam algumas conclusões: (i) o íon estanho ligado ao citrato ou a outros agentes é difusível para o interior das células e liga-se ao componente celular; (ii) o íon pertecnetato se difunde livremente dentro e fora da célula; (iii) ao mesmo tempo, o íon pertecnetato, dentro da célula, na presença do Sn^{++} é reduzido e se liga principalmente a fração globina da hemoglobina; (iv) a ligação do 99mTc com a globina é predominantemente na cadeia-beta (34).

Hemácias marcadas com 99mTc são utilizadas em medicina nuclear para a obtenção de imagens através do *pool* sangüíneo, avaliação do sistema cardiovascular, volemia, hemorragias gastrintestinais e outros (19,21). Além disso, novas aplicações têm sido realizadas com este radiofármaco incluindo a determinação

do fluxo sangüíneo no miocárdio e no cérebro, de imagens do osso e para o diagnóstico da função excretora do fígado e dos rins (21).

A marcação de constituintes sanguíneos com 99mTc pode ser alterada por produtos naturais e sintéticos (17,22,33,36,37).

O interesse em extratos vegetais

O crescente aumento do interesse da comunidade acadêmica em estudar os medicamentos naturais tem dado respaldo cada vez mais sólido às drogas à base de ervas (35). Existem alguns estudos sobre o efeito de plantas medicinais na marcação de hemácias com 99mTc (36,37).

O extrato aquoso das folhas de *C. icaco* é comumente usado para controlar a glicemia de pacientes diabéticos (38,39). A atividade redutora da glicemia foi verificada em modelos experimentais com plantas (1, 40).

Os efeitos tóxicos do extrato aquoso do abajerú foram estudados em plasmídios. Foi observada uma diminuição do efeito letal induzido pelo $SnCl_2$ na presença do extrato de abajerú, sem redução na sobrevivência bacteriana. A eficiência na transformação de plasmídeos também foi reduzida. Os resultados sugerem que o extrato pode apresentar potencial efeito genotóxico. Também apresenta ação antioxidante (12).

Vários triterpenóides foram isolados do abajerú. Tais substâncias foram capazes de inibir crescimento e induzir apoptose de K562, uma linhagem eritroleucêmica. Tambem inibiu a proliferação de Lucena1, derivada de K562, resistente à múltiplas drogas. Tais achados enfatizam atividade antitumoral destes

triterpenos e também a probabilidade de serem agentes anti-resistência a múltiplas drogas (40).

3 ARTIGOS ANEXADOS

3.1 MANUSCRITO ACEITO PARA PUBLICAÇÃO NA REVISTA BRASILEIRA DE FARMACOGNOSIA – QUALIS INTERNACIONAL C

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

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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 μg/ml), after that,

the agarose gel electrophoresis procedure was carried out. Plasmid incubated only with $SnCl_2$ 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: plasmid DNA, stannous chloride, genotoxic effect, antioxidant, *Chrysobalanus icaco*.

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, 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).

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).

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.

Materials and Methods

Drugs

Commercial *C. icaco* was used in this study and it was 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 μg/ml) of *E. coli* DH5αF'Iq (rec-) strain hosting this plasmid was carried out (18 hours, 37^{o} 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 µg/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 µg/ml) and the DNA bands were visualized by fluorescence under an ultraviolet transilumination system. The assay was repeated at least three 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).

<Figure 1>

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 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, i.e., there were significant differences in every lane when the control group was compared with the various concentrations of *C. icaco,* with or without $SnCl_2$.

<Figure 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

(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.

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REFERENCES

-Barbosa-Filho JM, Vasconcelos THC, Alencar AA, Batista LM, Oliveira RAG, Guedes DN, Falcão HS, Moura MD, Diniz MFFM, Modesto-Filho J 2005. Plants and their active constituents from South, Central, and North America with hypoglycemic activity. *Rev Bras Farmacogn 15,* 392-413.

-Coradin L, Giannasi DE, Prance AT 1985. Chemosystematic studies in the Chrysobalanaceae. Flavonoids in Parinari. *Brittonia* 37, 169-178.

-Costa OA 1977. Brazilian plants with hypoglycaemic effects.*Leandra 7*, 63-75.

-Dantas FJS, Moraes MO, De Mattos JCP, Bezerra RJAC, Carvalho EF, Bernardo-Filho M, Caldeira-de-Araújo A 1999. Stannous chloride mediates single strand breaks in plasmid DNA. *Toxicol Lett 110*, 129-136.

-Dantas FJS, De Mattos JCP, Viana ME, Lage CAS, Cabral-Neto JB, Leitão AC, Bernardo-Filho,

M, Bezerra RJAC, Carvalho JJ, Caldeira-de-Araújo A 2002. Genotoxic effects of stannous chloride ($SnCl_2$) in K562 cell line. *Food Chem Toxicol 40*, 1493-1498.

-De Mattos JCP, Dantas FJS, Bezerra RJAC, Bernardo-Filho M, Cabral-Neto JB, Lage CAS, Leitão AC, Caldeira-de-Araújo A 2000. Damage induced by stannous chloride in plasmid DNA. *Toxicol Lett 116*, 159-163.

-Fernandes J, Castilho RO, Costa MR, Wagner-Souza K, Kaplan MAC, Gattass CR 2003. Pentacyclic triterpenes from Chrysobalanaceae species: cytotoxicity on multidrug resistant and sensitive leukemia cell lines. *Cancer Lett 190*, 165-169.

-Ferreira-Machado SC, Rodrigues MP, Nunes APM, Dantas FJS, De Mattos JCP, Silva CR, Moura EG, Bezerra RJA, Caldeira-de-Araújo A 2004. Genotoxic potentiality of aqueous extract prepared from *Chrysobalanus icaco* L. leaves. *Toxicol Lett 151*, 481-487.

-Guedes AP, Cardoso VN, De Mattos JC, Dantas FJ, Matos VC, Silva JC, Bezerra RJ, Caldeira-de-Araujo A.2006. Cytotoxic and genotoxic effects induced by stannous chloride associated to nuclear medicine kits. *Nucl Med Biol 33*, 915-21.

-Gunstone FD, Subbarao R 1967. New tropical seed oils. Part I. conjugated trienoic and tetraenoic acids and their oxoderivatives in the seed oils of *Chrysobalanus icaco* and *Parinarium laurinum*. *Chem Phys Lipids 1,* 349-359.

-Gustafson KR, Munro MHG 1991. HIV inhibitory natural products: 3. Diterpenes from *Homalanthus acuminatus* and *Chrysobalanus icaco*. *Tetrahedron 47,* 4547-4554.

-Javor T, Tarnok F, Past T, Nagy S 1986. Cytoprotective effect of free radical scavengers against mucosal damage produced by different antirheumatic drugs. *Int J Tissue React 8*, 35-40.

-Little EL-Jr, Woodbury RO, Wadswort FH 1974. *Trees of Puerto Rico and the Virgin Islands*, Agriculture Handbook 449, Vol.2. Washington, DC: U.S. Department of Agriculture.

-Melo SF, Soares SF, Costa R F, Silva CR, Oliveira MBN, Bezerra RJ, Caldeira-de-Araújo A, Bernardo-Filho M 2001. Effect of the *Cymbopogon citrans, Maytenus ilicifolia* and *Baccharis genistelloides* extracts against the stannous chloride oxidative damage in *Escherichia coli*. *Mutat Res 496*, 33-38.

-Mendez J, Bilia AR, Morelli I 1995. Phytochemical investigations of *Licania* genus. Flavonoids and triterpenoids from *Licania pittieri*. *Pharma Acta Helv* 223-226.

-Simoes C, De Mattos JC, Sabino KC, Caldeira-de-Araujo A, Coelho MG, Albarello N, Figueiredo SF. 2006. Medicinal potential from in vivo and acclimatized plants of Cleome rosea. *Fitoterapia 77,* 94-9.

FIGURE 1

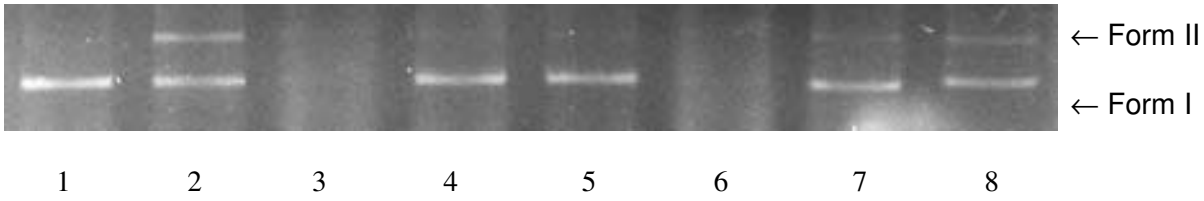
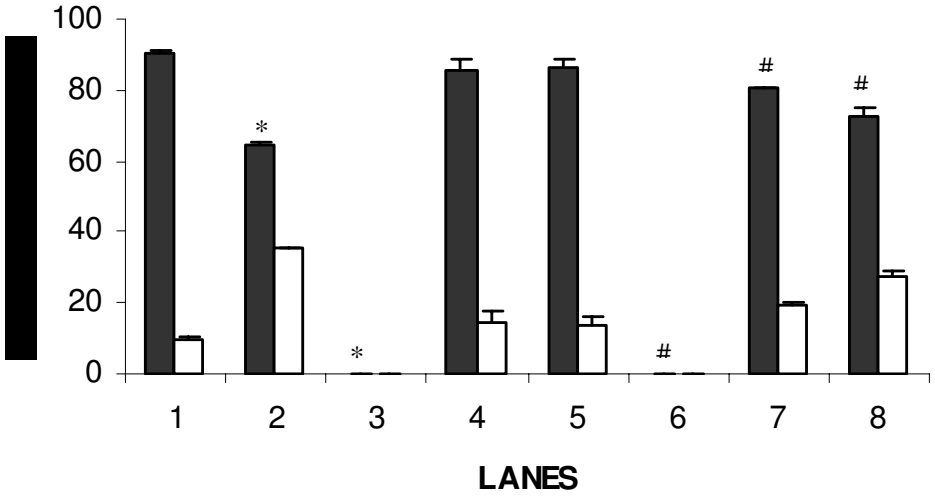


FIGURE 2



FIGURES LEGENDS

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 (5mg/ml); (5) pBSK + *C. icaco* extract (0.5mg/ml); (6) pBSK + *C. icaco* extract (50 mg/ml) + $SnCl_2$; (7) pBSK + *C. icaco* extract (5mg/ml) + $SnCl_2$; (8) pBSK + *C. icaco* extract (0.5mg/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 (0.5mg/ml); (6) pBSK + *C. icaco* extract (50mg/ml) + $SnCl_2$; (7) pBSK + *C. icaco* extract (5mg/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).

3.2 MANUSCRITO ACEITO PARA PUBLICAÇÃO NA REVISTA BRAZILIAN

ARCHIVES OF BIOLOGY AND TECHNOLOGY – QUALIS INTERNACIONAL B

Effects of *Chrysobalanus icaco* on the labeling of blood constituents with technetium-99m and on the shape of the red blood cells

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ABSTRACT

Chrysobalanus icaco (abajeru; C.icaco) is recommended in the treatment of diabetes and other clinical disorders. Blood constituents labeled with technetium-99m (99mTc) are used in nuclear medicine. The aim of this study was to verify the effects of an abajeru extract on the labeling of blood constituents with 99mTc and on the shape of red blood cells (RBC). Blood samples(Wistar rats) were incubated with abajeru extract and the labeling of blood constituents with 99mTc and morphology of RBC were carried out. The results showed significant (P<0.05) alteration of labeling of blood constituents with 99mTc and the morphometry (perimeter/area ratio) of the RBC in presence of the extract. These data suggest that this abajeru extract could alter the labeling of blood constituents with 99mTc by its chelating/antioxidant action and/or effects on membrane structures involved in the ion transport.

Key-words: Red blood cells, Technetium-99m, Morphometry, *Chrysobalanus icaco*.

INTRODUCTION

Medicinal herbs with different properties are used in a therapeutic way to treat various undesirable clinical conditions (Hart, 2005). Their use has considerably increased among populations, as they are believed to be beneficial and have few relevant side effects. In addition, the amount of information available on the toxicity and therapeutic properties of several medicinal herbs in the human organism is still quite limited and most of such information does not have sufficient scientific support. Medicinal herbs have their use as medicines, in general based only on traditional folk use, which has been passed from generation to generation (Ernst, 2002; Rotblatt & Ziment, 2002).

Chrysobalanus icaco (C. icaco; abajeru) leaf infusions are used popularly as diuretic and hypoglycemic agents. These ethnopharmacological indications have been experimentally suggested for *C. icaco*. The *C. icaco* (50mg/ml) has shown a distinctive hypoglycemic effect, correcting the fasting hyperglycemia caused by alloxan, and presenting a protection effect against alloxan toxic doses. The chemical features of *Chrysobalanaceae* include flavonoids, terpenoids (triterpenes and diterpenes), steroids and tannins (Castilho et al., 2000).

The interest in polyphenols (flavonoids) has increased because many of them exhibit a broad spectrum of biological activities including anti-inflammatory, antiviral, antiatherogenic, antibacterial, as well as anticancer effects (Cos et al., 2000; Middleton et al., 2000). These activities are associated to a great extent to their antioxidant properties, though other mechanisms may also be involved (Ling-Yih Hsu, 2005).

Fernandes et al (2003) demonstrated that a methanol extract from *C. icaco* leaves has a drastic inhibitory effect in HeLa cells and causes a modification of the protein profile for high concentrations (100 and 200 µg/ml) after 48h of incubation. The antimicrobial activity was determined for the abajeru extract using the disk diffusion method. Analgesic and anti-inflammatory activities were found in studies published by Castilho, et al. (2000).

The antiangiogenic potentials were obtained in corioalantoid membrane model and an average reduction of about 44% of the new vessels formation has been reported (Alves de Paulo et al., 2000). Further studies were carried out in which the *C. icaco* extract was utilized (i) to identify the citotoxic activity on multidrug resistant and sensitive leukemia cell lines (Fernandes et al., 2003), (ii) as well as the potential genotoxic effect demonstrated by induction of DNA single-strand breaks in plasmid or by transformation efficiency (Ferreira-Machado et al., 2004).

Radionuclides have been used in several investigations (clinical and basic sicences) and the technetium-99m (Tc-99m) has been a worthwhile tool in these studies (Bajc et al., 2004; Cicek et al., 2006; Cwikla et al., 2000; Das et al., 2002; Joseph et al., 2006; Saha, 2004) .An experimental model based on the labeling of blood constituents with a technetium-99m (Tc-99m) has been used to assess some properties of medicinal herbs (Abreu et al., 2006). Moreover, some authors have reported that some medicinal herbs are capable of altering the labeling of blood constituents with 99mTc (Abreu et al., 2006; Moreno et al., 2005). The 99mTc has been the most utilized radionuclide to label cells or molecules used as radiobiocomplexes (Bernardo-Filho et al., 2005) in the single photon emission computed tomography (SPECT) (Harbert et al., 1996; Saha, 2004). This radionuclide has also been used in basic research (Abreu et al., 2006; Burke et al., 2005; Pettersson et al., 2005).

Red blood cells labeled with 99mTc are used for measurement of red cell volume detection, recognition of gastrointestinal bleeding, identification of hemangyomas, gated blood pool study and other purposes (Saha, 2004). This labeled process depends on an optimal stannous chloride concentration and can be performed using either *in vivo* or *in vitro* methods, or by a combination of both (Gutfilen et al., 1992; Harbert et al., 1996 Kuehne & Reuter, 1999; Saha, 2004).

The extract of *C. icaco* has been used by human beings and some biological effects about it are not fully understood yet. These facts have stimulated us to evaluate the effects of an aqueous extract of *Chrysobalanus icaco* leaves on the labeling of blood constituents with 99mTc and on the shape of the red blood cells.

MATERIALS AND METHODS

Animals

All the experimental procedures have followed the Ethical Guidelines of the *Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro* with the protocol number CEA/116/2006.

The animals were kept under environmental conditions (25±2°C, 12h of light/dark cycle), water *ad libitum* and normal diet. Heparinized whole blood was withdrawn by cardiac puncture from adult male *Wistar* rats under anesthesia by sodium thiopental, 40mg/kg of weight (12 animals, 3 months of age, 245±35g of weight).

Preparation of abajeru extract

Dried *C. icaco* leaves (Estrella da Terra Produtos Medicinais Ltda, Lot 012, validity March 2009) were triturated and to each 5g, a NaCl 0.9% solution (saline) was added up to 100ml. The mixture was brought up to boil and filtered (Schleicher & Schulle filter paper Lot Nº K 932 Size 11 cm). The volume of filtrate was completed to 100ml with saline. The final solution was considered to be 50mg/ml and denominated 100%.

Labeling of blood constituents with 99mTc

The 99mTc, as sodium pertechnetate was freshly milked from a 99Mo/99mTc generator (*Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear,* São Paulo, Brazil) of the *Hospital Universitário Pedro Ernesto, Universidade do Estado do Rio de Janeiro,* RJ, Brazil.

Heparinized blood samples (0.5ml) were incubated and gently mixed with 100µl of different dilutions of the *C. icaco* extract (6.25, 12.5, 25.0,

50.0 and 100.0%) for 60 minutes. Blood samples incubated with saline were used as control. After this period of time, 0.5ml of a freshly prepared stannous chloride solution ($SnCl_2$, 1.2µg/ml, Sigma Chemical Co. St Louis, USA) was added. Then, 100µl of 99mTc (3.7 MBq) were added and the incubation was continued for another 10 minutes. These samples were centrifuged (clinical centrifuge, 1500 rpm) for 5 minutes and plasma (P) and blood cells (BC) were separated. Samples (20µl) of P and BC were also precipitated with 1 ml of trichloroacetic acid (5%) and soluble (SF) and insoluble (IF) fractions were obtained. The radioactivity (% ATI) in P, BC, IF-P, SF-P, IF-BC and SF-BC was determined in a well gamma counter (Clinigamma, gamma counter, Packard Instrument Company, mod C5002, USA). After that, the percentual of incorporated radioactivity (% ATI) was calculated as described previously (Bernardo-Filho et al, 1986).

Morphological evaluation

One drop of the samples incubated with abajeru extract at different concentration (0, 6.25, 12.5, 25, 50 and 100%) was smeared in slides (5 slides for each sample) and the May-Grünwald-Giemsa (MGG) method was performed. The smear blood was fixed with methanol (Vetec, Brazil) for 5 min, then stained with Giemsa (azure eosin methylene blue solution, Isofar, Brazil) for 10 min and washed in methanol to remove excess of stain. The slides stayed at room temperature to dry. The stained slides with MGG were analyzed by optical microscopy and for morphometric measurements a total of five fields per each slide were evaluated. A spherical shape and normal size distribution were assumed to RBC on control samples. The following morphometric parameters were obtained: area (μm^2); diameter (µm); perimeter (µm). A perimeter/area ratio was calculated ("Software" image pro plus, media Cibernetics, USA).

Statistical analysis

The data are presented as mean ± standard deviation of %ATI and perimeter/area ratio. The comparison between treated and control groups were performed by ANOVA followed by Bonferroni post-test with and $p<0.05$ considered significant level. GraphPad InStat version 3.01 for Windows (GraphPad Software, USA) was used.

RESULTS

Table 1 shows the distribution of the radioactivity in plasma and cellular compartments from blood treated with different concentrations of the abajeru extract. The radioactivity is mainly found in the cellular compartment and there was a significant decrease ($p<0.05$) in the distribution of 99mTc in this compartment from 95.69±1.71 (control) to 50.18±2.59 (12.5%) due to the treatment with the extract. It also shows a significant and unexpected increase ($p<0.05$) in the distribution of the radioactivity in the cellular compartment from 50.18±2.59 (12.5%) to 88.82±4.07 (100%).

Table 1 - Effect of abajeru extract on the distribution of the radioactivity in plasma and cellular compartments.

Abajeru extract (%)	P	BC
0.0	4.31±1.71	95.69±1.71
6.25	34.04±1.81	65.96±1.81
12.5	49.82±2.59	50.18±2.59
25	29.53±8.88	70.46±8.88
50	27.38±5.90	72.61±5.90
100	11.18±4.07	88.82±4.07

Blood samples from *Wistar* rats were incubated with abajeru extract for 1 hour and the labeling of blood constituents with 99mTc. Plasma (P) and blood cells (BC) were isolated, radioactivity was counted and the percentage of incorporated radioactivity (%ATI) was calculated.

Table 2 shows the fixation of the radioactivity in the insoluble and soluble fraction of plasma obtained from whole blood treated with different concentrations of the abajeru extract. A significant decrease ($p<0.05$) on the fixation of 99mTc in insoluble fraction of plasma from 78.71±1.68 (control) to 59.65±5.90 (100%) was found.

Table 2 - Effect of abajeru extract on the labeling of the insoluble and soluble fractions of plasma

Abajeru extract (%)	IF-P	SF-P
0.0	78.71±1.68	21.29±1.68
6.25	79.85±0.36	20.15±0.36
12.5	77.96±1.13	22.04±1.13
25	79.88±0.90	20.12±0.90
50	77.33±0.41	22.67±0.41
100	59.65±5.90	40.35±5.90

Blood samples from *Wistar* rats were incubated with abajeru extract for 1 hour and labeling of blood constituents with 99mTc was performed. Insoluble (IF) and soluble (SF) fractions from plasma (P) were isolated, radioactivity was counted and the percentage of incorporated radioactivity (%ATI) was calculated.

The data in table 3 indicate a significant ($p<0.05$) decreasing of the fixation of radioactivity in the insoluble fraction of blood cells obtained from whole blood treated with different concentrations of the abajeru extract from 91.84±4.52 (control) to 66.38±5.21 (50%). A significant and unexpected ($p<0.05$) increase in the fixation of the radioactivity from 66.38±5.21 (50%) to 94.17±0.32 (100%) was also found.

Table 3 - Effect of abajeru extract on the labeling of the insoluble and soluble fractions of the blood cells

Abajeru extract (%)	IF-BC	SF-BC
0.00	91.84±4.52	8.16±4.52
6.25	69.60±2.48	30.40±2.48
12.5	64.64±3.47	35.36±3.47
25	70.80±7.92	29.20±7.92
50	66.38±5.21	33.62±5.21
100	94.17±0.32	5.83±0.32

Blood samples from *Wistar* rats were incubated with abajeru extract for 1 hour and labeling of blood constituents with 99mTc was performed. Insoluble (IF) and soluble (SF) fractions from blood cells (BC) were isolated, radioactivity was counted and the percentage of incorporated radioactivity (%ATI) was calculated.

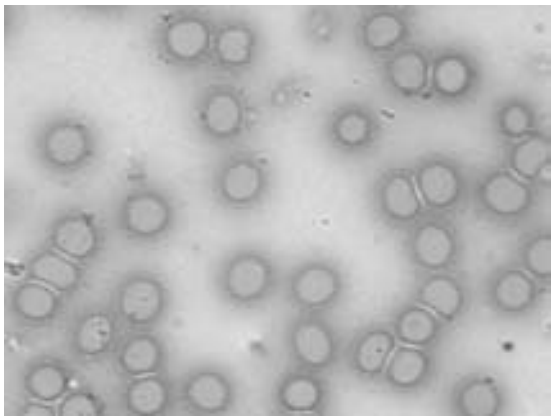


Figure 1: Photomicrography of blood smear from blood incubated with saline (control). Blood samples from *Wistar* rats were incubated with saline (0.9% NaCl) for 1 hour. After that, blood smears were prepared, dried and stained by May-Grünwald-Giemsa method. The slides were analyzed by optical microscopy (x1000).

The qualitative evaluation of the shape of the RBC (not treated and treated with abajeru under optical microscopy is shown in the figures 1, 2 and 3. Alterations on the morphology of the RBC incubated with abajeru extract at 25% were found.

Figure 1 shows the photomicrography of blood smear from whole blood incubated with saline (control). No modifications on the shape of RBC was observed in this figure.

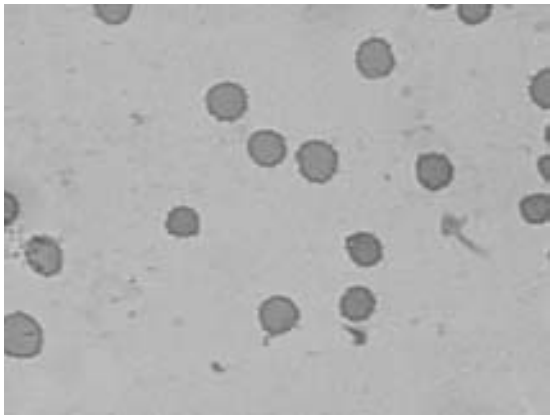


Figure 2: Photomicrography of blood smear from blood incubated with abajeru extract at 25%. Blood samples from *Wistar* rats were incubated with abajeru extract (25%) for 1 hour. After that, blood smears were prepared, dried and stained by May-Grünwald-Giemsa method. The slides were analyzed by optical microscopy (x1000).

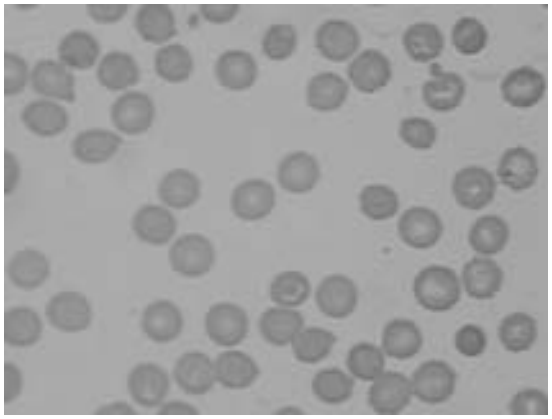


Figure 3: Photomicrography of blood smear from blood incubated with abajeru extract at 100%. Blood samples from *Wistar* rats were incubated

with abajeru extract (100%) for 60 minutes. After that, blood smears were prepared, dried and stained by May-Grünwald-Giemsa method. The slides were analyzed by optical microscopy (x1000).

Figure 2 shows the photomicrography of blood smear from whole blood incubated with the abajeru extract at 25%. The qualitative morphological analysis suggests that this extract altered the shape of RBC.

Figure 3 shows the photomicrography of blood smears from whole blood incubated with abajeru extract at 100%. An aspect similar to the control was found, in which the shape of the RBC seemed to be normal.

Morphometric values obtained to the perimeter/area ratio of the RBC are presented in Figure 4.

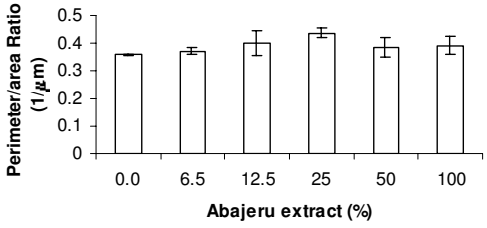


Figure 4: Effect of abajeru extract on the perimeter/area ratio of RBC. Morphometric measurements of perimeter/area of RBC from blood smears with a total of five fields per each slide and five slides to each extract were evaluated. The software Image pro plus, media Cibernetics, USA) was used to these evaluations.

The incubation with abajeru extract at 25% induced a significant ($p<0.05$) alteration on when compared with control cells. Moreover, the 12.5% concentration of abajeru also induced an alteration when compared with control cells, but it was not statistically significant. The findings obtained with RBC isolated from whole blood treated with abajeru in the concentrations of 50 and 100% have not shown quantitative modifications (Figure 4).

DISCUSSION

The development of experimental assays that can contribute to verify some biological properties of the extracts of medicinal herbs are relevant and desirable. Moreover, these findings would be highly worthwhile due to the importance of the use of natural in the world to treat several diseases. Nevertheless, as there are few studies providing evidence, in general, about the efficacy as well as about various properties of the medicinal herbs, the use of alternative experimental models should be encouraged (Rotblatt & Ziment, 2002). Some authors have reported that nuclear medicine procedures could be altered by medication treatments that the patient is undergoing. (Hesslewood & Leung, 1994; Owunwanne et al., 1995; Sampson, 1999). Blood constituents labeled with 99mTc have been used in several clinical examinations (Saha, 2004) and also as an experimental assay on an attempt to verify the effect of drugs (Fonseca et al., 2005). This experimental model has permitted obtaining relevant information about properties of various chemical compounds (synthetic and natural) (Abreu et al., 2006; Fonseca et al., 2007).

The abajeru extract components have exhibited a broad spectrum of biological activities including several activities that could be associated to their antioxidant properties (Ling-Yih Hsu, 2005). Moreover, Ferreira-Machado et al. (2004) have also suggested an antioxidant action of this extract, although a genotoxic effect has been reported. However, our findings presented in the Tables 1, 2 e 3 seem to be probably associated with oxidant properties of the substances of the abajeru extract, at least when the experiments were carried out with the smallest concentrations of this extract. When the highest concentrations of the abajeru extract were used, a possible antioxidant action could be suggested.

Ferreira-Machado et al. (2004) have also suggested a possible chelating property presents in the abajeru extract that could just justify the decrease of the distribution of the 99mTc in the cellular compartment (Table 1), as well as the fixation of the radioactivity on the insoluble fraction of the plasma (Table 2) and blood cells (Table 3). Our findings indicate that this chelating action would be also dependent on the concentration of the studied extract.

The distribution of the 99mTc in the cellular compartment (Table 1), as well as, in the fixation on the insoluble fraction of the blood cells (Table 3) could be also due to the alteration observed in the erythrocyte membrane as shown in the figure 2 and figure 4.

In conclusion, the abajeru extract could have the capability of interfering on the labeling of the blood constituents with 99mTc. This action mechanism would be not absolutely clear and it could be possibly dependent on the concentration of the extract used and it could be also associated with the oxidant or antioxidant mechanism. Moreover, a possible chelating property could justify the decrease of the distribution of the 99mTc in the blood constituents. The alteration observed in the red blood cells could be also associated with the effect of the abajeru extract on the labeling of some of the blood constituents. Although the experiments were carried out with animals, precaution is desirable in the interpretation of the examinations in the nuclear medicine that use labeled blood constituents with 99mTc in the patients that are undergoing the abajeru extract.

ACKNOWLEDGEMENTS

We are grateful for the biologist Mario Pereira (uerj) for his technical support and to Mr. Carlos Brown Scavarda (B. A., UNIVERSITY OF MICHIGAN) for the English language revision. Financial support: CNPQ, CAPES and UERJ.

RESUMO

Chrysobalanus icaco (abajeru; C.icaco) é recomendado para tratar diabetes e outras desordens clínicas. Constituintes sangüíneos marcados com tecnécio-99m (99mTc) são usados em medicina nuclear. O objetivo desse estudo foi verificar os efeitos de um extrato de abajetu na radiomarcação de constituintes sangüíneos com 99mTc e na forma de células vermelhas do sangue (RBC). Amostras de sangue retiradas de ratos *Wistar* foram incubadas com extrato de abajeru e a marcação dos constituintes sanguíneos com 99mTc e a morfologia das RBC foram realizadas. Os resultados mostraram alteração significativa ($P<0.05$) da marcação dos constituintes sangüíneos com 99mTc e a morfometria (relação perímetro/área) das RBC na presença do extrato. Esses resultados sugerem que esse extrato de abajeru poderia alterar a marcação de constituintes sangüíneos com 99mTc pela sua ação quelante/antioxidante e/ou seus efeitos nas estruturas de membrana envolvidas no transporte de íons.

REFERENCES

Abreu, P.R.; M.C. Almeida; R.M. Bernardo; L.C. Bernardo; L.C. Brito; E.A. Garcia; A.S. Fonseca and M. Bernardo-Filho.(2006). Guava extract (*Psidium guajava*) alters the labelling of blood constituents with technetium 99m. *J. Zhejiang. Univ. Sci. B*. **7,** 429-35.

Alves de Paulo, S.; Teruszkin Balassiano, I.; Henriques Silva, N.; Castilho, R.O.; Kaplan, M.A.C.; Currie Cabral, M. and da Costa Carvalho, M. G.(2000). Chrysobalanus *icaco* L. extract for antiangiogenic Potential observation. Int. *J. Mol. Med*. **5**, 667-669.

Bajc, M.; Olsson, C.G.; Olsson, B.; Palmer, J.and Jonson, B. (2004).Diagnostic evaluation of planar and tomographic ventilation/perfusion lung images in patients with suspected pulmonary emboli. *Clin. Physiol. Funct. Imaging*. **24**, 249-56.

Bernardo-Filho, M.; M.S. Caniné; R.L.F. Lopes. and E.M. Boasquevisque. (1986). Effect of temperature on the "in vitro" labeling of red blood cells with technetium-99m. *Braz.Arch. Biol. Technol*. **29**, 407-412.

Bernardo-Filho, M.; Santos-Filho, S.D.; Moura, E.G.; Maiworm, A.I.; Orlando, M.M.C.; Penas, M.E.; Cardoso, V.N.; Bernardo, L.C. and Brito, L.C.(2005). Drug Interaction with Radiopharmaceuticals: a Review. *Braz. Arch. Biol. Technol.* **48**, 13-28.

Burke, I.T.; Boothman, C.; Lloyd, J.R.; Mortimer, R.J.; Livens, F.R. and Morris, K.(2005). Effects of progressive anoxia on the solubility of technetium in sediments. *Environ. Sci. Technol*. **39**, 4109-16.

Castilho, R.O.; Souza, I. de; Guimarães, U.P. and Kaplan, M.A.C.(2000). A survey of chemistry and biological activities of Chrysobalanaceae. *An. Acad. Bras. Cienc*. **72** Sessions of the Academia Brasileira de Ciências 293.

Cicek, E.; Yildiz, M.; Delibas, N. and Bahceli, S. (2006).The effects of thyroid scintigraphy studies on oxidative damage in patients *Acta Physiol. Hung.* **93**, 131-6.

Cos, P.; Calomme, M.; Pieters, M.; Vietinck, A.J. and Vanden Berghe, D.(2000). Studies in Natural Products Chemistry. *Atta-Ur-Rahman.* **22**, 307-34.

Cwikla, J.B.; Buscombe, J.R. and Hilson, A.J. (2000). Detection of DCIS using 99m Tc-MIBI Scintimammography in patients with suspected primary breast cancer, comparison with conventional mammography. *Nucl. Med. Rev. Cent. East Eur.* **3**, 41-5.

Das, S.S.; Wareham, D.W. and Britton, K.E. (2002). ^{99m}Tc-Labeled Antimicrobial Peptides for Detection of Bacterial and *Candida albicans* Infections *J. Nucl. Med.* **43**, 1125-1127.

Ernst, E.(2002). The risk-benefit profile of commonly used herbal therapies: ginkgo, St. John's Wort, ginseng, echinacea, saw palmetto and kava. *Ann. Intern. Med.* **136**, 42-53.

Fernandes, J.; Castilho, R.O.; da Costa, M.R.; Wagner-Souza, K.; Kaplan, M.A.C. and Gattass, C.R.(2003) Pentacyclic triterpenes from Chrysobalanaceae species: cytotoxicity on multidrug resistant and sensitive leukemia cell lines. *Cancer Lett.* **190**, 165-169.

Ferreira-Machado, S.C.; Rodrigues, M.P.; Nunes, A.P.; Dantas, F.J.; De Mattos, J.C.; Silva, C.R.; Moura, E.G.; Bezerra, R.J. and Caldeira-de-Araujo, A. (2004). Genotoxic potentiality of aqueous extract prepared from Chrysobalanus *icaco* L. leaves. *Toxicol. Lett*. **151**, 481-488.

Fonseca, A.S.; Frydman, J.N.; Santos, R. and Bernardo-Filho, M.(2005). Influence of antipyretic drugs on the labeling of blood elements with technetium-99m. *Acta Biol. Hung*. **56,** 275-82.

Fonseca, A.S.; Frydman, J.N.; Rocha, V.C. and Bernardo-Filho, M.(2007). Acetylsalicylic acid decreases the labeling of blood constituents with technetium-99m. *Acta Biol. Hung*. **58,** *1*89-98.

Gutfilen, B.; Boasquevisque, E.M. and Bernardo-Filho, M.(1992). Calcium channel blockers: interference on red blood cells and plasma proteins labeling with Tc-99m. *Rev. Esp. Med. Nucl.* **11,** 195-199.

Harbert, J.C.; Eckelman, W.C. and Neumann R. D. (1996), Nuclear Medicine Diagnosis and Therapy. New York: Thieme Medical Publishers

Hart BL. (2005). Review - The Evolution of herbal medicine behavioural perspectives. *Animal Behav*. **70**, 975-989.

Hesslewood, S. and Leung, E. (1994). Drug interactions with radiopharmaceuticals. *Eur. J. Nucl. Med.* **21,** 348-356.

Joseph, B.; Kumaran, V.; Berishvili, E.; Bhargava, K.K. Palestro, C.J. and Gupta, S. (2006). Monocrotaline promotes transplanted cell engraftment and advances liver repopulation in rats via liver conditioning. *Hepatology.* **44**,1411-20.

Kuehne, R. and Reuter, E. (1999). High RBC labeling efficiency by controlling pretinning with the modified in vivo, in vitro labeling method. *J. Nucl. Med. Tec*. **27**, 222-226.

Ling-Yih Hsu.(2005). Evaluation of polyphenolic acid esters as potential antioxidants. *Biol.Pharm.Bull.* **28**, 1211- 1215.

Middleton, E. Jr.; Kandaswami, C. and Theoharides, T.C. (2000) The effects of plant flavonoids on Mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol. Rev*. **52**, 673-751.

Moreno, S.R.F.; Carvalho, J.J.; Nascimento, A.L.; Pereira, M.; Rocha, E.K.; Diré, G.; Arnobio, A.; Caldas, L.Q.A. and Bernardo-Filho, M. (2005). Bioavailability of the sodium pertechnetate and morphometry of organs isolated from Wistar rats: study of possible pharmacokinetic interactions of a Ginkgo biloba extract. *Braz. Arch. Biol. Technol.* **48**, 73-78.

Nigri, F.; Oliveira, M.B. and Bernardo-Filho, M. (2002). Assessment of the effect of antiseizure drugs on the labeling process of red blood cells and plasma proteins with technetium-99m. *Cell. Mol. Biol.* **48**, 793-801.

Owunwanne, A.; Patel, M. and SadeK, S. (1995), The Handbook of Radiopharmaceuticals. London: Chapman & Hall, London.

Pettersson, F.; Vogt, A.M.; Jonsson, C.; Mok, B.W.; Shamaei-Tousi, A. and Bergstrom, S.(2005). Chen Q, Wahlgren M. Whole-body imaging of sequestration of *Plasmodium falciparum* in the rat. *Infect. Immun*.**7**, 7736-46.

Rotblatt, M. and Ziment, I. (2002), *Evidence-Based Herbal Medicine*. Philadelphia: Hanley & Belfus

Saha, G.B.(2004), *Fundamentals in Nuclear Pharmacy*. New York: Springer-Verlag

Sampson, CB. (1999), *Textbook of Radiopharmacy Theory and Practice*. Amsterdam: Gordon and Breach

4 COMENTÁRIOS, CRÍTICAS E CONCLUSÕES

Cheguei ao Laboratório de Radiofarmácia Experimental (LRE) levado pelo amigo Professor Paoli que muito me falava das discussões científicas coordenadas pelo Professor Mario Bernardo Filho e sobre os trabalhos desenvolvidos no laboratório. Fui bem recebido pelo grupo com o qual pude observar algumas atividades com material radioativo e produtos naturais no laboratório. O assunto muito me interessou porque há muito tempo venho utilizando alguns desses produtos, tanto área básica quanto na clínica, sendo o principal, o abajeru. Dessa forma, com a permissão do Professor Mario, eu passei a freqüentar mais ativamente o laboratório e comecei a estudar a técnica de marcação de constituintes sanguíneos com tecnécio-99m, assim como os ensaios envolvendo o uso de plasmídeos. Submetemos um projeto de pesquisa para avaliar efeitos do abajeru em alguns modelos experimentais que tem como título "**Efeito de um extrato de *Chrysobalanus icaco* na marcação de constituíntes sanguíneos com tecnécio-99m, na morfologia de hemácias, na topologia plasmidial e na ação do cloreto estanoso no DNA plasmidial**" ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal do Rio Grande do Norte e começamos a trabalhar com esses modelos experimentais.

A padronização do preparo do extrato de abajeru foi feita utilizando-se como marcador a leitura da absorbância do extrato no comprimento de onda de 500nm. Durante a execução da marcação, concluímos que seria necessário verificar a morfometria das hemácias, o que também foi realizado. Além disso,

através de discussão dos efeitos do abajeru obtidos pelos modelos experimentais testados, percebemos a real necessidade de estudar seus efeitos em plasmídeos, o que proporcionou aprendizado de outra técnica com o Doutor Adenilson da Fonseca, convidado pelo Professor Mario Bernardo Filho a participar do laboratório, cuja convivência muito tem contribuído no aprimoramento de todos.

Acreditamos que o artigo intitulado "**Drug Interation with Radiopharmaceuticals: Effect on the Labeling of Red Blood Cells with Technetium-99m and on the Bioavaliability of Radiopharmaceuticals**" foi uma publicação que muito contribuiu para o manuseio de radiofármacos e seu uso em procedimentos de diagnóstico na área de Medicina Nuclear. A Medicina Nuclear é uma especialidade médica cujo estudo muito nos interessa academicamente e profissionalmente. Além disso, tive oportunidade de participar, a convite do Professor Mario Bernardo Filho, da organização de um encontro científico em Cabo Frio **("SPQV")** cuja experiência pela atividade e convivência com vários cientistas internacionais propiciou aprendizado que servirá para aproveitamento futuro em minha Universidade.

Pela originalidade de nosso trabalho, pois não existe nenhuma publicação com abajeru na área da Medicina Nuclear, e pelo fato do abajeru ser muito utilizado no tratamento da Diabetes Mellitus, nós acreditamos estar contribuindo para o conhecimento de alguns efeitos produzidos por esse produto natural que poderiam levar a erros de diagnóstico em Medicina Nuclear.

A experiência e a aprendizagem acumuladas ao longo do desenvolvimento deste trabalho proporcionaram o gratificante convite da Universidade Federal do Estado do Rio de Janeiro para assumir interinamente o curso de Medicina Nuclear da Faculdade de Medicina e Cirurgia. Desta forma,

aspiramos poder ampliar a Disciplina e até reunir outras áreas de interesse como a Radiobiologia e criar a disciplina ou área de Radiofarmácia, que congregadas à Medicina Nuclear formarão o Departamento de Biociências Nucleares.

5 ANEXOS

Anexo 1

Carta de Aceite do Manuscrito 1

----- Original Message -----
From: José Maria Barbosa Filho
To: giuseppe.presta@gmail.com
Cc: bernardo@uerj.br

Prezado Prof. Giuseppe Presta,

Informo que o manuscrito intitulado "*A Chrysobalanus icaco extract alters the plasmid topology and the effects of stannous chloride on the DNA strand of plasmids*", de autoria de Giuseppe A. Presta, Adenilson S. Fonseca e Mário Bernardo-Filho, foi aceito para publicação na REVISTA BRASILEIRA DE FARMACOGNOSIA.

Atenciosamente,

José Maria Barbosa Filho
Editor Chefe

Anexo 2

Carta de Aceite do Manuscrito 2

Brazilian Archives of Biology and Technology

DECLARAÇÃO

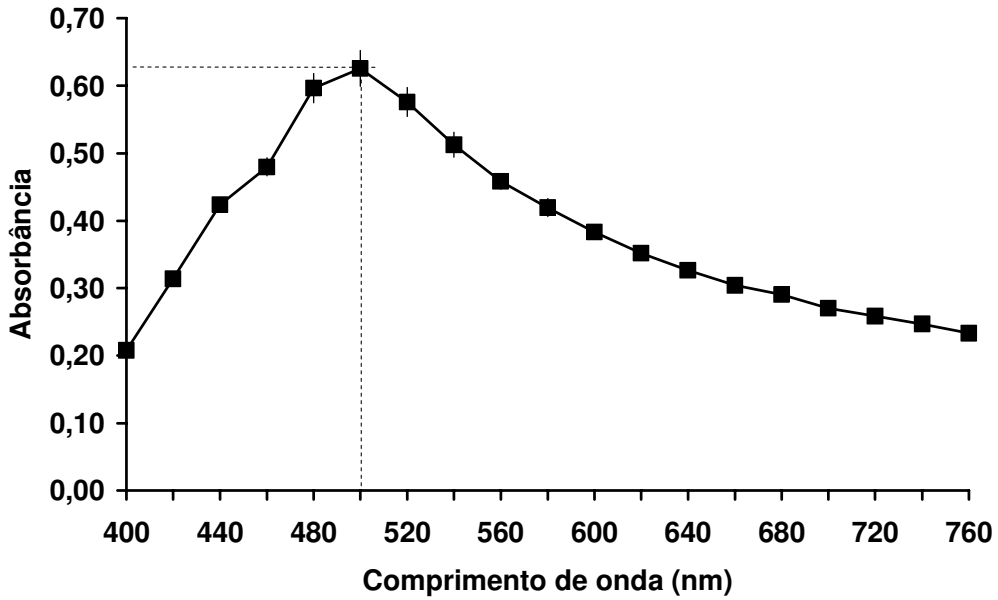
Declaramos para os devidos fins que o artigo: ***"*Effects of Chrysobalanus icaco on the labeling of blood constituents with technetium-99m and on the shape of the red blood cells*",*** de autoria de Giuseppe Antonio Presta, Sebastião David Santos-Filho, Severo de Paoli, Tania Santos Giani, Adalgisa Ieda Maiworm, Adenilson de Souza da Fonseca and Mario Bernardo-Filho, foi aceito e será publicado no ***Brazilian Archives of Biology and Technology.***

Curitiba, 19 de julho de 2007

Prof. Dr. Carlos Ricardo Soccol
Editor

APÊNDICE 1

Espectro de absorção do extrato de abajeru *(C. icaco)*



6 REFERÊNCIAS

1. Presta, GA. Atividade do Abagerú (*Chrysobalanus icaco* L)(*Chrysobalanaceae*) em modelos experimentais para o estudo de plantas hipoglicemiantes. Revista Brasileira de Farmacologia 1987; 68: 91-101.

2. Castilho RO, Souza I de, Guimarães UP and Kaplan MAC. A survey of chemistry and biological activities of *Chrysobalanaceae.* Anais da Academia Brasileira de Ciências 2000; 72: 293.

3. Cos P, Calomme M, Pieters M, Vietinck AJ and Vanden Berghe D. Studies in Natural Products Chemistry. Atta-Ur-Rahman 2000; 22: 307-34.

4. Middleton EJr, Kandaswami C and Theoharides TC. The effects of plant flavonoids on Mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological Reviews 2000; 52: 673-751.

5. Ling-Yih Hsu. Evaluation of polyphenolic acid esters as potential antioxidants. Biological & Pharmaceutical Bulletin 2005; 28: 1211- 1215.

6. Melo SF, Soares SF, da Costa RF, da Silva CR, de Oliveira MB, Bezerra RJ, Caldeira-de-Araujo A and Bernardo-Filho M. Effect of the *Cymbopogon citratus*, *Maytenus ilicifolia* and *Baccharis genistelloides* extracts against the stannous chloride oxidative damage in *Escherichia coli*. Mutation Research 2001; 496: 33-38.

7. Almeida MC, Soares SF, Abreu PR, Jesus LM, Brito LC, Bernardo-Filho M. Protective effect of an aqueous extract of *Harpagophytum procumbens* upon *Escherichia coli* strains submitted to the lethal action of stannous chloride. Cellular and Molecular Biology (Noisy-le-grand) 2007; 53: 923-927.

8. Fonseca AS, Frydman JN, Santos R and Bernardo-Filho M. Influence of antipyretic drugs on the labeling of blood elements with technetium-99m. Acta biologica Hungarica 2005; 56: 275-82.

9. Fonseca AS, Frydman JN, Rocha VC and Bernardo-Filho M. Acetylsalicylic acid decreases the labeling of blood constituents with technetium-99m. Acta biologica Hungarica 2007; 58: *1*89-98.

10. Dantas FJ, de Mattos JC, Moraes MO, Viana ME, Lage CA, Cabral-Neto JB, Leitão AC, Bernardo-Filho M, Bezerraa RJ, Carvalho JJ, Caldeira-de-Araujo A. Genotoxic effects of stannous chloride ($SnCl_2$) in K562 cell line. Food and Chemichal Toxicology 2002; 40: 1493-8.

11. Dantas FJS, Moraes MO, de Mattos JC, Bezerra RJ, Carvalho EF, Bernardo-Filho M and Caldeira de Araújo A. Stannous chloride mediates single strand breaks in plasmid DNA through reactive oxygen species formation. Toxicology Letters 1999; 110:129-36

12. Ferreira-Machado SC, Rodrigues MP, Nunes AP, Dantas FJ, De Mattos JC, Silva CR, Moura EG, Bezerra RJ and Caldeira-de-Araujo A. Genotoxic potentiality of aqueous extract prepared from *Chrysobalanus icaco* L. leaves. Toxicology Letters 2004; 151: 481-488.

13. Garcia AC. Biofísica. São Paulo: Servier 1998.

14. Bushong SC. Radiologic Science for Technologists: Physics, Biology and Protection. 7^{TH}Ed. Missouri, USA: Mosby 2001.

15. Thral JH, Ziessman HA. Medicina Nuclear, Rio de Janeiro: Guanabara Koogan 2003.

16. Perkins A, Frier M. Nuclear Medicine Pharmaceutical Research. London: Taylor & Francis 1999, p. 178.

17. Bernardo-Filho M, Santos-Filho SD, Moura EG, Maiworm AI, Orlando MMC, Pennas ME, Cardoso VN, Bernardo LC, Brito LC. Drug Interaction with Radiopharmaceuticals: a Review. Brazilian Archives of Biology and Technology 2005; 48: 13-27.

18. Owunwanne A, Patel M and SadeK S. The Handbook of Radiopharmaceuticals, London: Chapman & Hall 1995.

19. Bernardo-Filho M. Parâmetros físicos, químicos e biológicos associados à marcação de radiotraçadores com Tecnécio-99m. Tese (Professor Titular), Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Brasil 1999.

20. Saha GB. Fundamentals in Nuclear Pharmacy, New York: Springer Verlag 2004.

21. Arano Y. Recent advances in 99mTc radiopharmaceuticals. Annals of Nuclear Medicine 2002; 16:79-93.

22. Srivastava SC, Straub RF. Blood cell labeling with tc-99m: progress and perspectives. Seminars in Nuclear Medicine 1990; 1: 41-51.

23. Harbert JC, Eckelman WC, Neumann RD. Nuclear Medicine Diagnosis and Therapy. New York: Thieme 1996, p.195-265.

24. Ballinger JR. The influence of carrier on 99mTc radiopharmaceuticals. The Quarterly Journal of Nuclear Medicine 2002; 46:224-32.

25. Nickel RA. Radiopharmaceuticals. In: Early PJ, Sodee DB. Principles and practice of nuclear medicine. 2 nd ed. London: Mosby Year Book Inc1995, p.94-114.

26. Hladik III WB, Saha GB, Study KT. Essentials of Nuclear Medicine Science. Britto Baltimore-London: Williams & Wilkins 1987.

27. Berne RM, Levy MN. Fisiologia. Rio de Janeiro: Elsevier, 2004, p. 303-308.

28. Guyton AC and Hall JE. Tratado de Fisiologia Médica. 11ª ed. Rio de Janeiro: Elsevier 2006, p. 451-468.

29. Stryer L. Bioquímica. 5ª ed. Rio de Janeiro: Guanabara Koogan 2004, p.231-256.

30. Callahan RJ, Rabito AC. Radiobeling of erythrocytes with technetium-99m: role of band-3 protein in the transport of pertechnetate across the cell membrane. Journal of Nuclear Medicine 1990, 31: 2004-2010.

31. Gutfilen, B., Boasquevisque, EM., Bernardo-Filho, M. Calcium channel blockers: interference on red blood cells and plasma proteins labeling with Tc-99m. Revista Espanhola de Medicina Nuclear 1992; 11:195-199.

32. Schatzmann HJ. The red cell calcium pump. Annual Review of Physiology 1993; 45: 303-12.

33. Sampson CB. Complications and difficulties in radiolabelling blood cells: a review. Nuclear Medicine Communications 1996; 17: 648-658.

34. Dewanjee, MK., Rao, SA., Penniston, JT. Mechanism of red blood cell labeling with ^{99m}Technetium-pertecnetate and the role of cation pumps at RBC membrane on distribution and binding of Sn^{+2} and ^{99m} Technetium with membrane proteins and hemoglobin. Journal of Labelled Compounds & Radiopharmaceuticals 1982, 11:1464-1466.

35. Capriles PV, Dias AP, Costa TE, Oliveira MB, Faria MV, Moura E , Abreu BA, Bernardo-Filho, M. Effect of eggplant (*Solanum melongena*) extract on the in vitro labeling of blood elements with technetium-99m and on the biodistribution of sodium pertechnetate in rats. Cellular and Molecular Biology 2002; 48: 771-776.

36. Diré G, Lima E, Gomes M and Bernardo-Filho M. The effect of a chayotte (*Sechium edule*) extracts (decoct and macerated) on the labeling of blood elements

with Technetium-99m and on the biodistribution of the radiopharmaceutical sodium pertechnetate in mice: an *in vitro* and *in vivo* analysis. Pakistan Journal of Nutrition 2003; 2:221-227.

37. Costa OA. Brazilian plants with hypoglycaemic effects. Leandra 1977; 7*:* 63-75.

38. Barbosa-Filho JM, Vasconcelos THC, Alencar AA, Batista LM, Oliveira RAG, Guedes DN, Falcão HS, Moura MD, Diniz MFFM, Modesto-Filho J. Plants and their active constituents from South, Central, and North America with hypoglycemic activity. Revista Brasileira de Farmacognosia 2005; 15: 392-413.

39. Pereira NA. Plants as hypoglycemic agents. Sao Paulo: Ciencia e Cultura 1997; 49: 354-358.

40. Fernandes J, Castilho RO, Costa MR, Wagner-Souza K, Kaplan MAC, Gattass CR. Pentacyclic triterpenes from Chrysobalanaceae species: cytotoxicity on multidrug resistant and sensitive leukemia cell lines. Cancer Letters 2003; 190, 165-169.

ABSTRACT

The use of radionuclides has contributed for advances in Health Sciences, to research or to the diagnosis and/or treatment of diseases. These advances have been possible with the utilization of radiopharmaceuticals labeled with technetium-99m (99mTc). Stannous chloride ($SnCl_2$) has the main reducing agent utilized to obtain radiopharmaceuticals labeled with technetium-99m. It has been reported that several natural or synthetic drugs are capable to alter the labeling of blood constituents with 99mTc, as well as the red blood cells morphology. The aim of this study was to evaluate possible alterations of *Chrysobalanus icaco* extract on the labeling of blood constituents with 99mTc, on the morphology of RBC of blood of Wistar rats, on the breakage of plasmid DNA and on the effects of stannous chloride on plasmid DNA. The results showed significant ($P<0.05$) alteration of the labeling of blood constituents with 99mTc, as well as, modification of the morphology and morphometry (perimeter/area ratio) of the RBC in presence of the extract. These data suggest that this abajeru extract could alter the labeling of blood constituents with 99mTc by its chelating/antioxidant action and/or effects on membrane structures. Moreover *C. icaco* extract altered the electrophoretic profile and decreased 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 against the $SnCl_2$ and a genotoxic effect of *C. icaco* extract on plasmid DNA.

Key words: blood constituents, technetium-99m, stannous chloride, morphology, plasmid DNA, *Chrysobalanus icaco*.