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Evaluation of the Effect of an Extract of *Sabugueiro (Sambucus australis)* on the Labeling of Blood Constituents with Technetium-99m

Camila Godinho Ribeiro [1,2]*, Bernardo Machado Rebello[1,2], Rosane de Figueiredo Neves[1,2], Sebastião David Santos-Filho[1,2], Adenilson de Souza da Fonseca[1], Aldo da Cunha Medeiros[2], Mario Bernardo-Filho[2,3] and Maria Teresa Jansem de Almeida Catanho[4]

[1]Laboratório de Radiofarmácia Experimental; Departamento de Biofísica e Biometria; Instituto de Biologia Roberto Alcantara Gomes; Universidade do Estado do Rio de Janeiro; Av. 28 de setembro, 87; 20551-030; cacagr@yahoo.com.br; Rio de Janeiro - RJ - Brasil. [2]Programa de Pós-Graduação em Ciências da Saúde; Centro de Ciências da Saúde; Universidade Federal do Rio Grande do Norte; Av. Gal. Gustavo Cordeiro de Farias, s/n; 59010-180; Natal - RN - Brasil. [3]Coordenadoria de Pesquisa; Instituto Nacional do Câncer; Praça Cruz Vermelha, 23; 20230-130; Rio de Janeiro - RJ - Brasil. [4]Universidade Federal de Pernambuco; Departamento de Biofísica e Radiobiologia; Av. Prof. Moraes Rego s/n; 50670-420; Recife - PE - Brasil

ABSTRACT

Sambucus australis (sabugueiro) has been used to treat inflammatory and rheumatologic disorders. *Blood constituents labeled with technetium-99m (99mTc) have been used in nuclear medicine to obtain diagnostic images. The aim of this work was to evaluate the effect of a sabugueiro extract on the labeling of blood cells with 99mTc. Blood samples from Wistar rats were incubated with sabugueiro extract and the radiolabeling assay of blood constituents was carried out. After centrifugation, samples of plasma and blood cells were separated. Aliquots of plasma and blood cells were precipitated with trichloroacetic acid and centrifuged to isolate soluble and insoluble fractions. The radioactivity in each fraction was counted and the percentage of activity (%ATI) was determined. Incubation with sabugueiro extract altered significantly ($p<0.05$) the %ATI incorporated to the blood constituents. These results could be explained due the presence of chemical substances in the sabugueiro extract that present redox and/or chelating action altering the labeling of the blood constituents with 99mTc.*

Key words: *Sambucus australis*, blood constituents, technetium-99m

INTRODUCTION

Epidemiological and clinical studies have proved the beneficial effect of bioactive compounds of medicinal plants used in phytotherapy on prevention of several diseases (Kery et al., 2004). Moreover, investigations in basic research have permitted to understand better some properties related with the medicinal plants (Fonseca et al., 2005; Bernardo-Filho et al., 2005).

Sambucus australis, known as sabugueiro in Brazil, is used in folk medicine as diuretic, to treat inflammation, burn, pain and rheumatologic disorders (Guarrera et al., 2005; Jorge et al., 1999). In Brazil, sabugueiro is found from the Bahia State up to Rio Grande do Sul State.

* Author for correspondence

Technetium-99m (99mTc) has been the most utilized radionuclide in nuclear medicine to diagnosis procedures (Saha, 2004). Several compounds and cells, as red blood cells and white blood cells, are used as radiopharmaceuticals (radiobiocomplexes) (Bernardo-Filho et al., 2005). Blood constituents labeled with 99mTc have also been used as experimental model to evaluate the redox properties of synthetic and natural drugs (Abreu et al., 2006; Fonseca et al., 2007).

The labeling of blood constituents with 99mTc is based on the utilization of a reducing agent, and the stannous chloride ($SnCl_2$) is commonly used for this purpose. The radionuclide is used as sodium pertechnetate ($Na^{99m}TcO_4$). In the labeling of red blood cells, the band-3 proteins and calcium channels seem to have strong importance to these ions reach cellular compartment (Callahan and Rabito, 1990; Gutfilen et al., 1992). The factors, as natural drugs, that can influence the labeling of blood constituents have been reported (Bernardo-Filho et al., 2005).

The aim of this work was to evaluate the influence of a sabugueiro extract on the labeling of blood constituents with 99mTc.

MATERIALS AND METHODS

Commercial S*ambucus australis* was obtained from the Rodomonte Laboratório Vegetal (Belo Horizonte, Brazil).

To experimental procedures the commercial sabugueiro extract (1000 mg/mL) was diluted in 0.9% NaCl solution (saline) to obtain the different concentrations (500, 250, 125, 62.5 and 31 mg/mL). A spectrophotometric analysis (Analyser, 800M, São Paulo, Brazil) of the extract was carried out (400 up to 700nm) with intervals of 10nm. The absorbance at 500 nm was considered the marker of preparation of this extract. It was also determined the pH of this prepartion by standard indicator of pH (Merck, Germany). All the prepared extracts to be used in the experiments must have the optical density of 1.34±0.028 at 500 nm (Fig. 1) and the pH 5.

Wistar male rats (250-300 g) from *Universidade do Estado do Rio de Janeiro*, food and water *ad libitum*, maintained under constant environmental conditions (23±2ºC, 12h/12h of light/dark cycle) were used in experiments. The protocol was approved by the Ethical Committee to handle animals in experiments of the *Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro*, with the number CEA/126/2006.

In vitro labeling of blood constituents with 99mTc was carried out as described in Bernardo-Filho et al. (1983).

Blood samples were (0.5 ml) were incubated with 100µl of different concentrations of sabugueiro extract (500, 250, 125, 62.5 and 31 mg/mL) for 1 hour at room temperature. Blood samples were incubated with 0.9% NaCl solution as control. Then, 0.5ml of stannous chloride (Sigma Chemical Co., USA) (1.2µg/ml) solution freshly prepared was added and the incubation continued for another 1 hour. After this period of time, 99mTc (0.1ml), as sodium pertechnetate ($Na^{99m}TcO_4$), recently milked from a 99Molybdenium/99mTechnetium generator (*Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear*, São Paulo, Brazil), was added and the incubation continued for 10 minutes. These samples were centrifuged (clinical centrifuge, 1500 rpm, 10 minutes) and plasma (P) and blood cells (BC) were separated. Samples (20µl) of P and BC were also precipitated with 1ml of trichloroacetic acid (5%) and soluble (SF) and insoluble fractions (IF) were separated after centrifugation (clinical centrifuge, 1500 rpm, 10 minutes). The radioactivity in P, C, IF-P, SF-P, IF-BC and SF-BC were determined in a well counter (Automatic Gamma Counter, C5002, Packard, USA) and percentage of radioactivity (%ATI) was calculated, as previously described (Bernardo-Filho et al., 1983).

Data are reported as media ± standard deviation of %ATI and absorbance. Statistical analysis was performed by One-way ANOVA followed by Bonferroni post-test with $p<0.05$ as significance level.

RESULTS

The Fig. 1 shows the absorption spectrum of the sabugueiro extracts used in the experiments. The pattern of the absorption spectrum presents the highest measure of the optical density (1.34±0.028) at 500 nm. This parameter has permitted to control the conditions of the extracts being used as a marker.

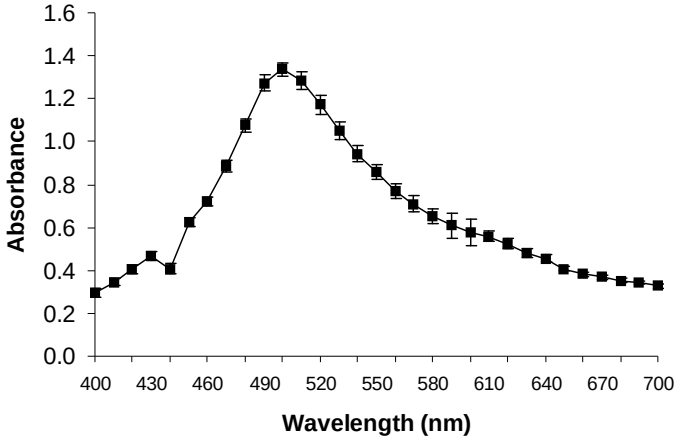

Figure 1 - Absorption spectrum of sabugueiro extract

Table 1 shows the distribution of the radioactivity between plasma and cellular compartments of blood incubated with sabugueiro extract. The data presented in this table suggest that the incubation with sabugueiro extract induces a significant (p<0.05) alteration of the distribution of radioactivity between these compartments.

Table 2 shows the fixation of the radioactivity in the insoluble fraction of plasma obtained from whole blood treated with sabugueiro extract. The analysis of these results indicates that the extract used is capable of significantly (p<0.05) modify the fixation of 99mTc on the insoluble and soluble fractions of plasma.

Table 1- Effect of a sabugueiro extract on the distribution of 99mTc between plasma and cellular compartments.

Sabugueiro extract (%)	%ATI	
	Cells	Plasma
0.00 (control)	96.50±1.03	3.50±1.03
31(*)	92.14±0.69	7.86±0.69
62.5(*)	91.28±1.14	8.72±1.14
125.0(*)	87.30±3.27	12.70±3.27
250.0(*)	65.52±3.08	34.48±3.08
500.0(*)	27.47±4.95	72.53±4.95

Blood samples were incubated with a sabugueiro extract for 1 hour. The radiolabeling of blood constituents was performed; the samples were centrifuged to plasma (P) and blood cells (BC) separation. The radioactivity in plasma (P) and blood cells (BC) was counted and the percentage of radioactivity (%ATI) was calculated. (*) p<0.05 when compared with control.

Table 2 - Effect of a sabugueiro extract on the fixation of 99mTc on insoluble and soluble fractions of plasma.

Sabugueiro extract (%)	%ATI	
	IF-P	SF-P
0.00 (control)	69.01±1.35	30.99±1.35
6.25(*)	55.51±4.52	44.49±4.52
12.5(*)	57.04±4.44	42.96±4.44
25.0(*)	52.43±1.89	47.57±1.89
50.0(*)	51.49±9.65	49.51±9.65
100.0(*)	51.62±1.97	48.38±1.97

Blood samples were incubated with a sabugueiro extract for 1 hour. Radiolabeling of blood constituents was performed; the samples were centrifuged to plasma (P) and blood cells (BC) separation. Aliquots of P were precipitated with trichloroacetic acid and insoluble (IF) and soluble fraction (SF) were separated. The radioactivity in SF-P and IF-P was counted and the percentage of radioactivity (%ATI) was calculated. (*) p<0.05 when compared with control.

Table 3 shows the effect of sabugueiro extract on the fixation of the radioactivity on the insoluble and soluble fractions of blood cells. The results indicate a significant (p<0.05) modification of fixation of 99mTc on insoluble and soluble fractions of the blood cells from blood samples incubated with sabugueiro extract.

Table 3 - Effect of a sabugueiro extract on the fixation of 99mTc on insoluble and soluble fraction of blood cells.

Sabugueiro extract (%)	%ATI	
	IF-BC	SF-BC
0.00 (control)	78.48±0.29	21.52±0.29
6.25(*)	51.78±2.02	48.22±2.02
12.5(*)	54.38±2.68	45.62±2.68
25.0(*)	52.77±8.02	47.23±8.02
50.0(*)	52.15±8.74	47.85±8.74
100.0(*)	53.74±4.81	46.26±4.81

Blood samples were incubated with a sabugueiro extract for 1 hour. Radiolabeling of blood constituents was performed; the samples were centrifuged to plasma (P) and blood cells (BC) separation. Aliquots of BC were precipitated with trichloroacetic acid and insoluble (IF) and soluble fraction (SF) were separated. The radioactivity in SF-BC and IF-BC was counted and the percentage of radioactivity (%ATI) was calculated. (*) p<0.05 when compared with control.

DISCUSSION

There are evidences that natural drugs could affect the radiolabeling of red blood cells with 99mTc and some findings have been considered in the development of an experimental model to verify properties of these drugs (Santos-Filho et al., 2005; Abreu et al., 2006, Freitas et al., 2007). Moreover, the possible interference of the medicinal plants on the nuclear medicine procedures based on red blood cells labeled with 99mTc and the consequences in the interpretation of these examinations could be considered.

Investigators have reported that natural products, as *Coffea arabica*, *Mentha crispa* and *Psidium guajava* are able to interfere with the labeling of BC with 99mTc. Furthermore, extracts of *Sechium edule* and *Pfaffia* sp. do not alter the fixation of this radionuclide on the blood constituents (Fernandes et al., 2005; Oliveira et al., 2003; Abreu et al., 2006; Santos-Filho et al., 2004; Diré et al., 2004).

Sambucus australis is one of the plants widely used in popular medicine in Brazil due to its diuretic action, as well as is indicated to inflammation, burn, pain and rheumatologic disorders (Guarrera et al., 2005).

Our data suggest that the sabugueiro extract was capable to modify the distribution of the radioactivity between the cellular and plasma compartments reducing the uptake of 99mTc in the cellular compartment (Table 1). The %ATI was also reduced in the insoluble fractions of plasma and cells form blood samples incubated with this extract (Tables 2 and 3). As the fixation of the 99mTc depends on the presence of a reducing agent, it is possible to suggest that chemical substances in sabugueiro extract could present redox and/or chelating actions that could to alter the labeling of the blood constituents with 99mTc. These properties have also been suggested to other medicinal plant, as *Hypericum perfloratum*, *Coffea arabica*, *Mentha crispa* and *Gingko biloba* (Santos-Filho et al., 2005; Moreno et al., 2004; Oliveira et al., 2003; Santos-Filho et al., 2004). Other possibility could be an alteration on the ion transport systems of Sn^{++} and pertechnetate through of membrane of red blood cells, which could interfere on the labeling of the blood constituents with 99mTc. In fact, alterations on the membrane of red blood cells and reduction of labeling of red blood cells have been reported to other extracts, as *Coffea arabica*, *Mentha crispa* and *Gingko biloba* (Moreno et al., 2004; Oliveira et al., 2003; Santos-Filho et al., 2004).

In conclusion, our experimental data showed that the labeling of blood constituents with 99mTc could be altered in the presence of sabugueiro extract. These findings suggest that chemical compounds in this extract could present redox and/or chelating properties capable to interfere in this labeled assay with 99mTc. Morphological experiments are ongoing to try to verify the action of this extract on the shape of the red blood cells.

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RESUMO

Sambucus australis (sabugueiro) tem sido utilizado para o tratamento de desordens inflamatórias e reumáticas. Constituintes sangüíneos marcados com tecnécio-99m (99mTc) são utilizados na medicina nuclear para obtenção de imagens. O objetivo desse trabalho foi avaliar o efeito de em extrato de sabugueiro na marcação dos constituintes sangüíneos com 99mTc. Amostras de sangue de ratos *Wistar* foram incubadas com um extrato de sabugueiro durante 1 hora. Em seguida, o ensaio de marcação de constituintes sangüíneos com 99mTc foi realizado. Após a centrifugação, plasma (P) e células (C) foram separadas e alíquotas de P e CS também foram precipitadas em ácido tricloroacético e centrifugadas para isolamento das frações solúvel (FS-P e FS-C) e insolúvel (FI-P e FI-C). A radioatividade em cada fração foi contada e a porcentagem de radioatividade incorporada (%ATI) foi determinada. A incubação com o extrato de sabugueiro alterou significativamente ($p<0.05$) a %ATI dos constituintes sangüíneos Esses resultados poderiam ser explicados devido à presença de substâncias químicas no extrato de sabugueiro com ação redox e/ou quelante, que poderiam alterar a marcação dos constituintes sangüíneos.

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