Effect of a *Syzygium jambolanum* (jamelão) extract on the labeling of blood elements with sodium pertechnetate ($Na^{99m}TcO_4$)

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RESUMO: O tecnécio-99m (^{99m}Tc) é utilizado em vários procedimentos de diagnóstico em medicina nuclear. As características físico-químicas e biológicas desse radionuclídeo têm favorecido a marcação de estruturas de interesse biomédico. Dentre as estruturas marcadas estão as hemácias que podem ser utilizadas para estudo do volume sangüíneo, de sangramento gastrointestinal e de seqüestro esplênico. A marcação de hemácias com ^{99m}Tc necessita de um agente redutor e a substância comumente usada é o cloreto estanoso ($SnCl_2$). Entretanto, muitos fatores, incluindo o tratamento com drogas e/ou radiação, alimentação e ainda processos patológicos podem afetar a biodistribuição e/ou a marcação de radiofarmácos. A falta de conhecimento desses fatores pode levar a repetição do exame com desnecessária irradiação do paciente ou até a um diagnóstico equivocado. Tem sido descrito que extratos de produtos vegetais podem alterar a marcação dos elementos sangüíneos com ^{99m}Tc. A árvore do Jamelão atinge grande altura, sendo originária da Índia e aclimatada no Brasil. Na medicina popular, o extrato de folhas do jamelão (Syzygium jambolanum) é empregado para redução da glicemia no tratamento do diabetes mellitus. No presente trabalho, investigamos se o extrato das folhas do referido vegetal é capaz de alterar a marcação dos elementos sangüíneos com ^{99m}Tc. Amostras de sangue foram obtidas de ratos Wistar e incubadas com diferentes concentrações do decocto da folha do vegetal (2; 1; 0,50; 0,25; 0,125 mg/ml). Cloreto estanoso na concentração de 1,2 mg/ml e ^{99m}Tc, como pertecnetato de sódio, foram adicionados. O sangue foi centrifugado e plasma (P) e células sangüíneas (CS) isolados. Amostras de P e CS também foram precipitadas com ácido tricloroacético 5%, centrifugadas e as frações solúveis (FS) e insolúveis (FI) separadas. As atividades (%ATI) em P, C, FI-P, FI-CS, FS-P e FS-CS foram calculadas. Os resultados demonstraram: (i) um decréscimo na %ATI de CS (93,68±3,18) com as concentrações do extrato, 2 mg/ml (24,17±4,93), 1 mg/ml (17,92±0,95), 0,5 mg/ml (26,37±3,69), 0,25 mg/ml (27,53±1,45) e 0,125 mg/ml (31,41±1,79), (ii) um decréscimo na %ATI de FI-CS (95,81±1,64) com as concentrações de 2 mg/ml (79,44±1,65) e 0,25 mg/ml (85,73±4,92) e (iii) um acréscimo na % ATI de FI-P em todas as concentrações utilizadas. Como o extrato de jamelão foi capaz de alterar a ligação do pertecnetato de sódio aos elementos sangüíneos, sugerimos, como possíveis mecanismos de ação: (i) inibição direta (ação quelante) dos íons envolvidos, (ii) danos na membrana eritrocitária, (iii) competição com os mesmos sítios de ligação do ^{99m}Tc, (iv) possível geração de espécies reativas de oxigênio que poderiam oxidar o íon estanoso e/ou (v) direta oxidação do íon estanoso.

Palavras-chave: *Syzygium jambolanum*, tecnécio-99m, células sangüíneas, oxidantes, proteínas sanguíneas.

ABSTRACT: Technetium-99m (^{99m}Tc) is used in many clinical evaluations in nuclear medicine. The physical chemical and biological characteristics of this radionuclide permit the labeling of several structures of biomedical interest. Besides these structures there are the red blood cells (BC) that are used to determine the blood volume, to locate the sites of gastrointestinal hemorrhage, to imaging of blood pool and to evaluate splenic sequestration. When whole blood is employed in the labeling of BC with ^{99m}Tc, radioactivity is found in blood cells, however, it is also bound to plasma proteins. This labeling process depends on a reducing agent and stannous chloride is used for this purpose. However many factors, including drug therapy, radiation therapy, dietary conditions, besides pathological processes, could affect the bio-distribution and/or the labeling of the radio-pharmaceuticals. If unknown, such factors may lead to poor visualization, requiring the repetition of the examination procedure resulting in unnecessary irradiation to the patient or even misdiagnosis. It has been described that vegetal extracts alter the labeling of blood elements with ^{99m}Tc. Jamelan trees reach a high length, being originated from India and acclimated in Brazil. In popular medicine, it is utilized in order to reduce the glucose level in blood in the treatment of *diabetes mellitus*. We have investigated if an extract of this vegetal (leaves) is capable of modifying the labeling of blood elements with ^{99m}Tc. Blood was obtained from *Wistar* rats and incubated with different concentrations of the decoct (2; 1; 0.50; 0.25; 0.125 mg/ml). Stannous chloride ($SnCl_2$) 1.2 µg/ml and ^{99m}Tc, as sodium pertechnetate, were added. After blood centrifugation, plasma (P) and blood cells (BC) were isolated, also precipitated with 5% trichloroacetic acid and soluble (SF) and insoluble fractions (IF) were separated. The percentages of radioactivity (%ATI) in P, C; IF-P and IF-BC were calculated. The results show that there is: (i) a decrease on the labeling of BC (93.68±3.18) with the concentrations: 2 mg/ml (24.17±4.93), 1 mg/ml (17.92±0.95), 0.50

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mg/ml (26.37±3.69), 0.25 mg/ml(27.53±1.45) and 0.125 mg/ml (31.41±1.79), (ii) a decrease in the IF-BC (95.81±1.64) on the concentrations of 2 mg/ml (79.44±1.65) and 1 mg/ml (85.73±4.92) and (iii) an increase on the labeling of IF-P in all the concentrations used. As the Syzygium jambolanum extract was capable of modifying the binding of sodium pertechnetate to blood elements, we suggest, as possible action mechanisms: (i) a direct inhibition (chelating action) of the referred ions, (ii) damages induced in the plasma membrane, (iii) competition of the cited ions for the same binding sites, (iv) possible generation of reactive oxygen species that could oxidize the stannous ion and/or (v) direct oxidation of the stannous ion.

Key words: *Syzygium jambolanum*, technetium-99m, blood cells, oxidants, blood proteins.

INTRODUCTION

Nuclear medicine techniques are worthwhile due to their ability to provide information on physiology rather than anatomy (Srivastava, 1987; Perkins & Frier, 1996). Technetium–99m (^{99m}Tc) is the most frequently used radionuclide in diagnostic nuclear medicine procedures (Hladik *et al.*, 1987; Early & Sodee, 1995; Saha, 1998) and is also used to label biological structures in basic scientific research (Bernardo-Filho *et al.*, 1992, 1993; Gutfilen *et al.*, 1993; Plotkowski *et al.*, 1993). ^{99m}Tc labeling of erythrocytes (^{99m}Tc–RBC) has come into wide use in clinical nuclear medicine for various applications, as (i) the image of the cardiovascular system (particularly gated wall-motion studies), (ii) detection and localization of gastrointestinal hemorrhage, (iii) measurement of red cell volume and (iv) spleen imaging. Erythrocyte labeling with ^{99m}Tc can be carried out with *in vitro* technique, *in vivo* method, or a combination of these two, sometimes called *in vitro/in vivo* labeling (Early & Sodee, 1995; Harbert *et al.*, 1996). These labeling methods require a reducing agent, usually stannous ion (Sn^{+2}), for effective labeling. The presence of medications (synthetic or natural products) in the patient's blood (Hesslewood & Leung, 1994; Oliveira *et al.*, 1997; Vidal *et al.*, 1998, Oliveira *et al.*, 2000), as well as the labeling conditions (Hladik *et al.*, 1987; Gutfilen *et al.*, 1992; Harbert *et al.*, 1996), can have an effect on the labeling of the blood elements with ^{99m}Tc. Thus, the presence of the disease may be missed and/or underestimated (Hladik *et al.*, 1987).

The use of natural products, as medicinal plants, is very frequent all over the world. Jamelan (*Syzygium jambolanum – S. jambolanum*), a vegetal of *Myrtacea* family, is native of India. The tree reaches a high length and is used for ornamental and medicinal purposes in Brazil. The most common medicinal use of *S. jambolanum* (mainly the leaves, but the bark and the seeds are also used) is in order to reduce the glucose level in blood. It is also reported that the bark is astringent and antidysenteric; the fruits are astringent, antidysenteric, antiblennorrheal and antileucorrhoeal (Bragança, 1996).

In this work, we studied the influence of different concentrations of *S. jambolanum* decoct on the labeling of blood cells (BC) and plasma proteins with ^{99m}Tc using an *in vitro* technique (Bernardo-Filho *et al.*, 1983, 1990).

MATERIAL AND METHOD

These experiments were performed without sacrificing the animals. Heparinized whole blood was obtained from *Wistar* rats. A decoct was prepared with 20 g of jamelan in 1000 ml of 0.9%NaCl solution and after filtration, this decoct preparation was considered 2 mg/ml. Blood samples (0.5 ml) were incubated with 100µl of different concentrations of *S. jambolanum* (Bragança, 1996) decoct (2; 1; 0.50; 0.25; 0.125 mg/ml) for 1 h at room temperature. Leaves of *S. jambolanum* collected in Macaé city, Rio de Janeiro (Brazil), gathered in May, 2001, were identified by Ana Maria Donato from the Department of Botany, Unicersidade do Estado do Rio de Janeiro, Brazil. A voucher specimen (no. 5865) has been deposited in the Herbarium of the Universidade do Estado do Rio de Janeiro (H.RJ). A sample of heparinized whole blood was incubated with 0.9% NaCl solution as control. Then, 0.5 ml of stannous chloride (1.2 µg/ml), as $SnCl_2.2H_2O$ (Sigma, USA) was added and the incubation continued for another 1 h. After this period of time, ^{99m}Tc (0.1ml), as sodium pertechnetate (3.7 MBq/ml), recently eluted from a $^{99}Mo/^{99m}Tc$ generator (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, Brazil), was added and the incubation continued for another 10 minutes. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Samples (20 µl) of P and BC were also precipitated with 1ml of trichloroacetic acid (TCA) (5%) and soluble (SF) and insoluble fractions (IF) were separated. The radioactivity (% ATI) was calculated, as previously described (Bernardo-Filho *et al.*, 1983, 1994). A statistical analysis (ANOVA test) was utilized to compare the experimental data.

RESULT

Figures 1A and 1B show the distribution of the radioactivity in blood cells (BC) and plasma (P) isolated from blood treated with different concentrations of *S. jambolanum* decoct. The

analysis of the results indicates that there is a significant decrease ($P < 0.05$) in radioactivity uptake by the BC in presence of *S. jambolanum* extract from 93.68±3.18 to 31.41±1.79.

Figures 2A and 2B show the distribution of the radioactivity in insoluble fractions of blood cells(IF-BC) and plasma (IF-P) isolated from blood treated with different concentrations of *S. Jambolanum* decoct. The analysis of the results indicates that there is a significant decrease ($P < 0.05$) in the fixation of ^{99m}Tc in IF-BC when the concentrations of 2 mg/ml (from 95.81±1.64 to 79.44±1.65) and 1 mg/ml (from 95.81±1.64 to 85.73±4.92) of the referred extract are used. There is a significant increase ($P < 0.05$) in the radioactivity in IF-P in all the concentrations used.

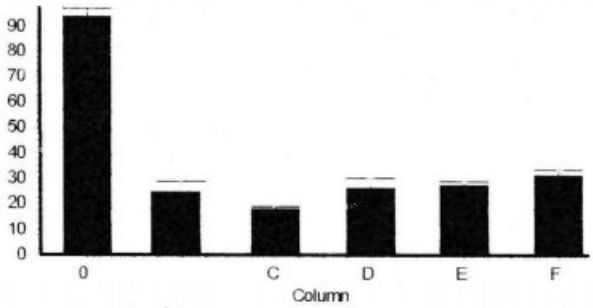

FIGURA 1A and FIGURA 1B - Samples of heparinized blood were incubated with *S. Jambolanum* solution (2; 1; 0.50; 0.25; 0.125 mg/ml). Then, stannous chloride and ^{99m}Tc were added. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. The %ATI in BC (Fig. 1A) and in P (Fig 1B) was calculated. (ANOVA test, n= 4, p <0.05).

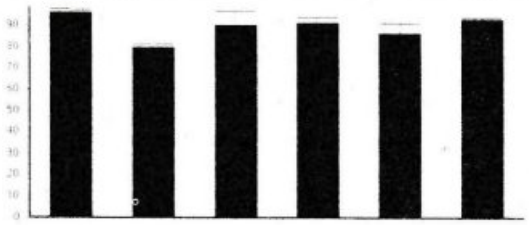

FIGURA 2A and FIGURA 2B - Samples of heparinized blood were incubated with *S. Jambolanum* solution (2; 1; 0.50; 0.25; 0.125 mg/ml). Then, stannous chloride and ^{99m}Tc were added. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Aliquots of P and BC were precipitated with trichloroacetic acid (TCA) 5% and soluble and insoluble fractions (IF) were separated. The %ATI in IF-BC (Fig 2A) and in IF-P (2B) was calculated. (ANOVA test, n= 4, p <0.05).

DISCUSSION

Several authors have reported that a therapeutic drug can modify the nature/amount of the ^{99m}Tc-radio-pharmaceutical bound to blood elements and this may result in a unexpected behavior of the radio-pharmaceuticals. The labeling of the radio-pharmaceuticals can also be altered due to the presence of drugs (Hladik *et al*, 1987, Hesslewood & Leung, 1994). These undesirable conditions could be minimized with the development *of in vitro* tests to evaluate the therapeutic drug/ radio-pharmaceutical interactions (Oliveira *et al.*, 1997; Vidal *et al.*, 1998; Reineger *et al.*, 1999; Oliveira *et al.*, 2000).

As many patients who are submitted to nuclear medicine examinations can make use of the *S. jambolanum* extract, we have decided to study the effect of the *S. jambolanum* extract on the labeling of the blood elements with ^{99m}Tc.

When whole blood is labeled with ^{99m}Tc, radioactivity is found binding to plasma proteins and on BC (Porter *et al.*, 1983; Bernardo-Filho *et al.*, 1983, 1990; Srivastava & Straub, 1992). Sequential stages of the intracellular labeling process include reduction of the ^{99m}Tc pertechnetate by Sn^{+2}, subsequent binding of the reduced ^{99m}Tc to hemoglobin (Dewanjee, 1974; Rehani & Sharma, 1980; Callahan & Rabito,1990) and trans-membrane transport of Sn^{+2} and ^{99m}Tc into the internal compartment of the BC. The band-3 anion transport system (Callahan & Rabito, 1990) and calcium channels (Braga *et al.*, 2000) may be involved in this transport. Plasma proteins (PP) labeled with

^{99m}Tc have been used to evaluate cardiac function and pulmonary perfusion, to determine blood volume and to study gastrointestinal protein loss (Hladik *et al.*,1987; Harbert *et al.*, 1996; Saha, 1998). When *S. jambolanum* decoct was incubated with whole blood, there was an increase in ^{99m}Tc in the insoluble fraction of plasma (plasma proteins).

Some authors have reported that natural products as *Thuya occidentalis* (Oliveira *et al.*, 1997), *Peumus boldus* (Reineger *et al.*, 1999) and *Maytenus ilicifolia* (Oliveira *et al.*, 2000) are able to interfere with the labeling of RBC with ^{99m}Tc and alter the fixation of this radionuclide to the precipitated blood elements. The comparison of the effect of these natural products with *S. jambolanum* is difficult due to the different concentrations used as well as the specific characteristic of the various extracts.

In the labeling of BC and their proteins with ^{99m}Tc, although the exact mechanism of the effect of *S. jambolanum* on the process is not elucidated, we suggest that it might be explained (i) by a direct inhibition (chelating action) of the referred ions, (ii) by damage induced in the plasma membrane, (iii) by competition of the cited ions for the same binding sites, (iv) by possible generation of reactive oxygen species that could oxidize the stannous ion and/or (v) by direct oxidation of the stannous ion.

Care must be taken when attempting to extrapolate these experimental data to the clinical situation. However, the current data suggest that, depending on S. jambolanum decoct concentration, the labeling of red blood cells with ^{99m}Tc can be decreased, at least, when an *in vitro* technique to label red blood cells is used.

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