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Studies Involving a Commercial Extract of *three Ballerina*: I- Characterization and the Effect on the Binding of two 99mtechnetium-Radiopharmaceuticals on Blood Constituents

Nelson de Souza Pinto[1], Lúcia de Fátima Amorim[1], Dulciane Nunes Paiva[2], Vilma Gomes Barreto[3], Gláucio Diré Feliciano[4,5], Sebastião David Santos-Filho[6], Mario Bernardo-Filho[6,7]

[1]Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal do Rio Grande do Norte, Natal, RN, Brasil;
[2]Universidade de Santa Cruz do Sul, Santa Cruz do Sul, RS, Brasil;
[3]Universidade Federal da Bahia, Salvador, BH, Brasil;
[4]Universidade Estadual da Zona Oeste, Rio de Janeiro, RJ, Brasil;
[5]Universidade Estácio de Sá, Rio de Janeiro, RJ, Brasil;
[6]Laboratório de Radiofarmácia Experimental, Departamento de Biofísica e Biometria, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brasil;
[7]Coordenadoria de Pesquisa, Instituto Nacional do Câncer, Rio de Janeiro, RJ, Brasil.

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ABSTRACT

We have determined some physicochemical parameters of an aqueous extract of *Three ballerina* (3TB) and its influence on the binding of the radiopharmaceuticals sodium pertechnetate ($Na99mTcO_4$) and 99mTc-methylene diphosphonate (MDP-99mTc) on blood constituents. Whole blood of rats was incubated with a 3TB extract and with $Na99mTcO_4$ or MDP-99mTc. Plasma (P) and blood cells (BC) from *Wistar* rats (control and treated) were separated. P and BC were precipitated with trichloroacetic acid (TCA) and soluble (SF) and insoluble fractions (IF) isolated. The percentage of incorporated radioactivity (%ATI) in each fraction was determined. The treatment not influenced statistically ($p>0.05$) the %ATI of both radiopharmaceuticals on BC and P compartments, as well as on IF-P and SF-P isolated by TCA precipitation. However, 3TB has altered the fixation of the 99mTc-MDP on the IF-BC and SF-BC. We are firstly reported physicochemical parameters (absorption spectrum, viscosimetry and phmetry) about an aqueous 3TB extract. Moreover, it is possible to speculate that an alteration would be not found on the biodistribution of the 99mTc-pertechnetate in the person that is undergoing 3TB extract. However, this would be found with the 99mTc-MDP.

INTRODUCTION

Radiopharmaceuticals are used in nuclear medicine that as a diagnostic modality has grown to such an extent that it is practiced in almost all hospitals nationwide (Saha, 2010). Several radiopharmaceuticals labeled with technetium-99m (99mTc) are employed in different static and dynamic procedures in nuclear medicine to evaluate various organs and systems. Two of them are sodium pertechnetate ($Na99mTcO_4$) and sodium methylene diphosphonate (99mTc-MDP). Sodium pertechnetate ($Na99mTcO_4$) is the tracer of choice for imaging and determination of size and location of thyroid (Ito *et al.*, 2009) and Meckel's diverticulum detection (Kiratli *et al.*, 2009). Phosphonate and phosphate compounds localize avidly in bone and, therefore, are suitable for bone imaging (Saha, 2010). Sodium methylene diphosphonate (MDP), and hydroxymethylene diphosphonate (HMDP or HDP) are the most commonly used in nuclear medicine for the bone evaluation. The interaction of a radiopharmaceutical with plasma proteins is an important characteristic influencing both its biodistribution over the whole body and its pharmacokinetics (Tavaré *et al.*, 2009; Hsie *et al.*, 2010). In consequence, an important step to understand the mechanism of localization of radiopharmaceuticals in specific target organs, as well as how these

** Corresponding Author*
Sebastião David Santos-Filho, Universidade do Estado do Rio de Janeiro, Instituto de Biologia Roberto Alcantara Gomes, Departamento de Biofísica e Biometria, Laboratório de Radiofarmácia Experimental, Av 28 de setembro, 87, 20551-030, Vila Isabel, Rio de Janeiro, RJ, Brazil. Ph: 55-21-8715-1371, E-mail: nspvet@gmail.com,

radiopharmaceuticals are cleared from blood or eliminated from the body or the rate at which their excretions occur, is the determination of their binding to the proteins present in the blood.

To investigate the binding of radiopharmaceuticals on blood proteins, the complexes of protein-bound-radiopharmaceutical must be separated from the free radiopharmaceutical. This has been accomplished by precipitation of the proteins with ammonium sulphate (AS) or trichloroacetic acid (TCA), gel chromatography, dialysis or ultrafiltration (Schumichen *et al.,* 1980; Vanlic-Razumenic *et al.,* 1984; Domenech *et al.,* 1989; Freitas *et al.,* 2001; Gurudutta *et al.,* 2001; Glaudemans *et al.,* 2010).

Some authors have described that synthetic or natural products, as well as the labeling conditions can affect the labeling of blood constituents (Hesslewood and Leung, 1994; Fonseca *et al.,* 2005).

The use of natural products has increased in the last decades all over the world (Rotblatt and Ziment, 2002; Monbaliu *et al.,* 2010) and, there is a growing interest in the studies about various properties of medicinal and dietary plants (Bahramikia and Yazdanparast, 2010; Ameer *et al.,* 2010). Different experimental models have been used with this purpose (Bahramikia and Yazdanparast, 2010; Ameer *et al.,* 2010; Santos-Filho and Bernardo-Filho, 2005; Benarroz *et al.,* 2007).

The prevalence of obesity is growing worldwide and in the United States, it is estimated that this prevalence has reached almost 30% (Flegal *et al.,* 2010). Due to the lack and limitation of weight loss medicines, herbal teas and functional food ingredients have become important tools in improving obesity-related parameters. Tea has been consumed as a popular beverage world-wide for the last thousands of years because of its health benefits and pleasant aroma (Katiyar, 2011). Green tea is one of the most extensively studied plants for the prevention of metabolic syndrome by stimulating fat oxidation and increasing energy expenditure (Dulloo *et al.,* 1999; Wolfram *et al.,* 2006; Koh *et al.,* 2011). “3 Ballerina” Tea Dieters' Drink is blended with the premium natural herbs. Following the instructions of the manufacturer (Truong Giang Corp.), “this special formula Dieters’ Drink is all natural tea, soothing and relaxing especially delightful for those desiring to adjust weight”, although ‘this statement has not been evaluated by the Food and Drug Administration”.

As in the PubMed databank was not found scientific publications about “3 Ballerina” (3TB), we have determined some physicochemical parameters of an aqueous extract of 3TB and its influence on the binding of the radiopharmaceuticals $Na^{99m}TcO_4$ and 99mTc-MDP on blood constituents.

MATERIAL AND METHODS

Animals

Adult male *Wistar* rats (6 animals, 3–4 months, 200–250 g) were maintained in a controlled environment. The animals had free access to water and food and ambient temperature was kept at 25±2 °C. Experiments were conducted in accordance with the Institutional Committee of Animal Care (Protocol CEA/024/2009).

PubMed and Google strategies

A search was performed in February 14th 2011 in the pubMed (http://www.ncbi.nlm.nih.gov/ pubmed) using the keywords “3 Ballerina” or “3 Ballerina” and tea or “three ballerina” or “three Ballerina” and tea. A search was performed in February 14th 2011 in the Google (http://www.google.com.br/) using the keywords “3 Ballerina” or “3 Ballerina” and tea or “three ballerina” or “three Ballerina” and tea.

Characterization of the 3 *Ballerina* extract

Manufacturer information about the 3 Ballerina

The commercial extract of 3 *Ballerina* (Truong Giang Corp. South El Monte, USA, Lot 1563, validity up to March 2012) has the ingredients *Malva verticellata* and *Cassia angustifolia*, without caffeine and with no chemical additives.

- Preparation of the aqueous extract of 3 Ballerina

A solution with 2.34 g of *Three Ballerina* (3TB) extract was prepared with 100 ml of a hot 0.9% NaCl (saline). The preparation was centrifuged (clinical centrifuge, 2000 rpm, 15 min) and the supernatant was isolated. Then, the obtained solution was considered 23.4 mg/ml or 100% solution of the extract. Saline was used in all the dilutions.

- Physicochemical parameters

Spectrophotometry of 3TB extract

The absorbance spectrum was determined in a spectrophotometer (*Analyser Comércio e Indústria Ltda., São Paulo, Brazil*) with the 3TB extract (23.4 mg/ml) in the range of 400–700 nm. Saline solution was used as the blank. The absorbance was measured at each interval of 10 nm. This value was considered as the marker of the reproducibility of the conditions used to prepare all the extracts utilized in the assays.

Viscosimetry of TB extract

The viscosimetry (centiStokes) of the TB extract was performed with a viscosimeter (*Copo Ford - Hipperquimica Suprimentos para Laboratório LTDA, Rio de Janeiro*). Saline solution was used as the control. This value was considered as the second marker of the reproducibility of the conditions used to prepare all the extracts utilized in the assays.

pHmetry of TB extract

The pHmetry of the TB extract was performed with a pHmeter (PHTEK modelo pHS-3B, *Labiocenter produtos para laboratório, Rio de Janeiro*). Buffer saline solution was used to calibrate the pHmeter. This value was considered as the third marker of the reproducibility of the conditions used to prepare all the extracts utilized in the assays.

***In vitro* assay**

Blood samples were withdrawn from *Wistar* rats, anaesthetized with sodium thiopental (0.67%), by cardiac puncture.

The 99mTc, as sodium pertechnetate, was freshly eluted from a 99Mo/99mTc generator (*Instituto de Pesquisas Energéticas e Nucleares-IPEN, Comissão Nacional de Energia Nuclear-CNEN*, Brazil) in 0.9% NaCl solution.

The MDP was labeled with 99mTc and the radiochemical purity was 87.84±0.34%.

Blood samples (0.5ml) were incubated with 3TB extract (0.1ml) at different concentrations for one hour with gently mixed. A solution of 0.9% NaCl was used as a control. After that 0.1ml of the radiopharmaceutical was added and incubation continued (10 min). Samples of blood were centrifuged and plasma (P) and blood cells (BC) were separated. Aliquots (20 µl) of P and BC were also precipitated with 1 ml of trichloroacetic acid (TCA) and soluble (SF) and insoluble fractions (IF) were separated. The radioactivity in P, BC, IF-P, SF-P, IF-BC and SF-BC was determined in a well counter. After that, the percentage of radioactivity (%ATI) was calculated.

Statistical analysis

Data are reported as mean ± SD values of the absorbance, viscosimetry (centiStokes/cm), pHmetry and percentage of radioactivity (%ATI) from radiolabeling assay. Oneway analysis of variance was performed to verify possible statistical differences. After that, a rigorous statistical posttest (Bonferroni) was chosen to identify the *P* value ($P < 0.05$ was considered significant) and to compare each treated set with the control set. InStat Graphpad software was used to perform statistical analysis (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego, CA).

RESULTS

The searches performed in PubMed using the keywords has shown 3 publications with “3 Ballerina”, no items found with “3 Ballerina” and tea, 2 publications with “three ballerina” and no items found with “three Ballerina” and tea. All items found were related with dancers and they were not considered. As it was expected in the Google was found various items involving the 3 Ballerina. With “3 Ballerina” 49500 items were found, with “3 Ballerina” and tea 28500 items, with “three ballerina” 2930 items and with “three Ballerina” and tea 1010 items. Our group is reporting the first scientific information about the 3TB extract. Physical chemical properties, absorbance spectrum, viscosimetry and pHmetry, of the extract of 3TB were determined. Figure 1 shows the spectrum of absorbance of the 3TB extract at highest concentration used (23.4mg/ml) in the range of 400–700 nm. The data show an absorption peak of the extract (0.93±0.01) at 490 nm. This value was considered as the marker of the reproducibility of the conditions used to prepare the extract. The value of viscosimetry (2.31±0.04 centiStokes) of the extract at higher concentration was used as a second marker of the reproducibility of the conditions use to prepare the extract. The value of the pH (5.69±0.05) of the extract at higher concentration was used as a third marker of the reproducibility of the conditions of the extract.

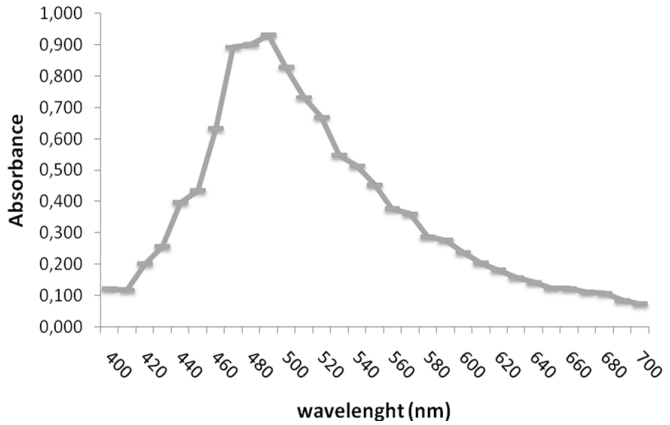

Fig. 1: Spectrum of absorbance of the 3TB extract at higher concentration used (23.4mg/ml) in the range of 400–700 nm/

Table 1 shows the distribution of radioactivity of the $Na99mTcO_4$ and of the 99mTc-MDP on the BC and P from *Wistar* rats treated with TB extract. The comparison of the %ATI in the blood compartments of the treated group with 3TB and control group (not treated) indicates that the extract at concentrations used was not capable to interfere with the distribution of the $Na99mTcO_4$ or 99mTc-MDP radioactivity between the blood compartments.

Table. 1: Distribution of radioactivity of the $Na99mTcO_4$ and MDP-99mTc on the blood cell (BC) and Plasma (P) compartments from *Wistar* rats treated with 3TB extract

Extract (%)	$Na99mTcO_4$		MDP-99mTc	
	BC	P	BC	P
0.00	52.35±7.85	47.64±7.85	73.06±6.07	26.93±6.07
6.25	55.12±3.87	44.87±3.87	80.45±7.22	19.54±7.22
12.5	51.14±6.29	48.85±6.29	82.02±3.31	17.97±3.31
50	55.35±1.85	44.64±1.85	81.85±9.30	18.14±9.30
100	51.47±0.91	48.52±0.91	82.03±3.83	17.96±3.83

Table 2 shows the distribution of radioactivity of the $Na99mTcO_4$ and of 99mTc-MDP on the IF-P and SF-P from blood of the *Wistar* rats treated with 3TB extract. The comparison of the %ATI in the IF-P of the treated group with 3TB and control group (not treated) indicates that the extract at concentrations used was not capable to interfere with the distribution of the $Na99mTcO_4$ or 99mTc-MDP radioactivity between the insoluble and soluble fractions of plasma.

Table. 2: Distribution of radioactivity of the $Na99mTcO_4$ and MDP-99mTc on the IF-P and SF-P from blood of the *Wistar* rats treated with 3TB extract

Extract (%)	$Na99mTcO_4$		MDP-99mTc	
	IF-P	SF-P	IF-P	SF-P
0.00	5.13±2.26	94.86±2.26	24.44±5.49	75.55±5.49
6.25	6.69±2.84	93.30±2.84	25.45±3.14	74.54±3.14
12.5	8.73±3.95	91.26±3.95	29.22±4.42	70.77±4.42
50	5.45±4.78	94.54±4.78	21.33±5.68	78.66±5.68
100	3.96±1.61	96.03±1.61	28.52±4.46	71.47±4.46

Table 3 shows the distribution of radioactivity of the $Na99mTcO_4$ or of the 99mTc-MDP on the IF-BC and SF-BC from blood of the *Wistar* rats treated with 3TB extract. The comparison of the %ATI in the IF-BC of the treated group with 3TB and control group (not treated) indicates that the extract at concentrations used was not capable to interfere with the distribution of the $Na99mTcO_4$ between the insoluble and soluble fractions of blood cells. However, the 3TB extract has increased the fixation of the 99mTc-MDP on the insoluble fraction of blood cells in the highest concentrations.

Table. 3: Distribution of radioactivity of the $Na99mTcO_4$ and MDP-99mTc on the IF-BC and SF-BC from blood of the *Wistar* rats treated with TB extract

Extract (%)	$Na99mTcO_4$		MDP-99mTc	
	IF-BC	SF-BC	IF-BC	SF-BC
0.00	14.65±3.36	85.34±3.36	44.89±9.23	55.10±9.23
6.25	10.69±4.78	89.30±4.78	48.60±5.13	51.39±5.13
12.5	12.17±3.43	87.82±3.43	39.51±7.63	60.48±7.63
50	11.85±1.01	88.14±1.01	63.92±1.97	36.07±1.97
100	12.76±0.61	87.23±0.61	59.98±6.51	40.01±6.51

DISCUSSION

The searches performed in the PubMed have shown that there are not publications in scientific journals indexed in this databank about the 3 Ballerina extract. This finding shows the high relevance of the results reported in this work. The number of items found in the Google reveals that an important number of persons in the world have access to general information about the 3 Ballerina. Moreover, it is not easy to identify in the Google publication in important scientific journals.

Physical properties of 3TB extract were not found until now through PubMed. A useful physical property to aid to characterize and to estimate the purity or to determine the concentration of a substance or solution is the viscosimetry (Ahrabi *et al.,* 1999). Phmetry (Peeters *et al.,* 2006) and the absorbance spectrum profile are other physical parameters that could be measured and used to characterize a solution of unknown composition, such as an extract of a medicinal plant.

Various authors have evaluated the binding of the radiopharmaceutical on blood constituents and have shown that these findings cannot be easily compared. The value found for the protein binding of 99mTc-radiopharmaceuticals appears to depend on the method used and the experimental conditions (Le-Cun *et al.,* 1998; Freitas *et al.,* 2000; Frydman *et al.,* 2007; Holanda *et al.,* 2009; Haudek *et al.,* 2009).

There is considerable evidence that the pharmacokinetics of radiopharmaceuticals may be altered by a variety of drugs, disease states and surgical procedures. This alteration can be due to the alteration on the binding of the radiopharmaceutical to the blood (plasma and blood cells) proteins. Unknown, such factors, may lead to poor organ visualization; a requirement to repeat the procedure resulting in unnecessary irradiation of organs or even misdiagnosis (Glaudemans *et al.,* 2010; Hojelse *et al.,* 1994; Hung *et al.,* 1996; Mattos *et al.,* 1999; Gomes *et al.,* 2001; Bernardo=Filho *et al.,* 2005). Results of the *in vitro* studies (Table 1, 2 and 3) reveal that the 3TB extract was not capable ($p>0.05$) to alter the distribution of $Na99mTcO_4$ or of 99mTc-MDP radioactivity in the compartments of plasma and blood cells. In addition, the extract was also not capable ($p>0.05$) to altering the distribution of the radiopharmaceuticals in the insoluble fractions of plasma. However, the 3TB extract has altered the fixation of the 99mTc-MDP on the insoluble fraction of the blood cells.

In conclusion, in this work, we are firstly reported physicochemical parameters (absorption spectrum, viscosimetry and pHmetry) about an aqueous 3TB extract. Moreover, it is possible to speculate that an alteration would be not found on the biodistribution of the 99mTc-pertechnetate in the person that is undergoing 3TB extract. However, this would be found with the 99mTc-MDP.

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CONFLICT OF INTEREST

'The authors report no conflicts of interest'.

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