Global Journal of Pharmacy and Pharmacology

ISSN: xxxx-xxxx Vol. 1 (1), pp. 098-104, November, 2013. © Global Science Research Journals

Full Length Research Paper

Evaluation of biologic effects of an *Ilex paraguariensis* aqueous extract on the labeling of blood constituents with technetium-99m and on the morphology of red blood cells

Ana Cristina S. Braga[1], Maria Luisa Gomes[2], Josefina da Silva Santos[1], Joelma Fonseca de Oliveira[1], Lúcia de Fátima Amorim[3], Gláucio Diré Feliciano[4], Sebastião David Santos-Filho[1]* and Mario Bernardo- Filho[1]

[1]Department of Biophysics and Biometry, Institute of Biology Roberto Alcantara Gomes, State University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.
[2]Instituto National Cancer, Rio de Janeiro, RJ, Brazil.
[3]Department of Biophysics and Pharmacology, Biosciences Center, Federal University of Rio Grande do Norte, Natal, RN, Brazil.
[4]Centro State University West Zone, Rio de Janeiro, RJ, Brazil.

Accepted 30 October, 2013

***Ilex paraguariensis* (mate-tea) has been used to treat type-2 diabetes and emphysema. This tea presents antioxidant activity and promotes satiety, body weight lowering and improves bone mineral density. Blood constituents have been labeled with technetium-99m (^{99m}Tc) and this labeling depends on the presence of a reducing agent. Stannous chloride has been widely utilized with this purpose. The influence of natural and synthetic drugs in this procedure has been reported. It was evaluated with effect of an aqueous mate-tea extract on the labeling of blood constituents with ^{99m}Tc and on the morphology of erythrocyte. Blood samples of Wistar rats were incubated with mate-tea extract, stannous chloride and ^{99m}Tc-sodium pertechnetate. Blood cells (BC) and plasma (P) were isolated. BC and P were also precipitated and soluble (SF) and insoluble (IF) fractions separated. The radioactivity was counted and percentage of incorporated radioactivity (%ATI) determined. Blood smears were performed for morphological evaluation. The data show a significant ($p < 0.05$) alteration of %ATI in BC, IF-C and IF-P as well as on the erythrocyte morphology. These findings could be related to the redox properties of the substances of the mate-tea extract, as well as due to the interference in the transport of stannous and/pertechnetate into the BC due to the morphological alterations of the erythrocytes.**

Key words: *Ilex paraguariensis*, blood constituents, radiolabeling, morphology, technetium-99m, stannous ion.

INTRODUCTION

Ilex paraguariensis (mate-tea) is an herb whose leaves are utilized to prepare infusion. This infusion is a popular drink known as mate-tea. It has been consumed under different forms. Various properties of this plant in the

*Corresponding author. E-mail: santos-filho@ueri.br, sdavidsfilho@gmail.com. Tel/Fax: (5521)2868-8332.

form of tea have been already described and it has been popularly used to treat type-2 diabetes (Hussein et al., 2011) and emphysema (Lanzetti et al., 2011). In addition, data have demonstrated that this tea possesses antioxidant activity (Coentão et al., 2011) and promotes satiety, body weight lowering (Hussein et al., 2011) and improves bone mineral density (Confort et al., 2012). Consumption of mate powder would significantly contribute to antioxidant and stimulant intake, providing high amounts of phenolic acids, tannins and methylxanthines with biological effects potentially beneficial for human health (Vieira et al., 2010).

Blood constituents have been labeled with technetium-99m (99mTc) and used in several procedures in nuclear medicine. These radiopharmaceuticals can be used for important applications, including imaging of cardiovascular system (Niemeyer et al, 1995; Crandall et al, 2008; Javadi et al, 2011), peripheral arterial blood flow (Blond and Madsen, 2000; Harel et al, 2005; Hsieh et al, 2009), evaluation of gastrointestinal bleeding (Zaman et al, 2004; Wong et al, 2004; Olds et al, 2005; Schillaci et al, 2009; Dolezal et al, 2011), measurement of red cell volume (Hladik III et al, 1987), hepatic hemangiomas (Artiko et al, 2004; Verdu et al, 2005; Yarlagadda et al, 2008), renal carcinoma (Cortes et al, 2003), splenic reticuloendothelial system (Jin et al, 2004; Slart et al, 2004) and imaging infection (Stoeckli et al, 1996)..

The labeling process of red blood cells (RBC) is based on the transmembrane transport of the reducing agent (Sn^{+2}) and pertecnetate ($^{99m}TcO_4^-$) ions into the RBC, reduction of $^{99m}TcO_4^-$ by Sn^{+2}, and subsequent binding of the reduced ^{99m}Tc to internal structures (Early and Soddee, 1995; Dewanjee et al., 1982). The band-3 anion transport system and calcium channels may be involved in transport of $^{99m}TcO_4^-$ and Sn^{+2}, respectively (Callahan and Rabito, 1990; Gutfilen et al., 1992; Sampson, 1996). The fixation of ^{99m}Tc on plasma proteins also depends on the presence of a reducing agent (Saha, 2010).

Data have demonstrated the effects of synthetic and natural drugs on this radiolabeling process (Mousinho et al., 2008; Holanda et al., 2009; Bustani et al., 2009; Rocha et al., 2009; De et al., 2009; Cekic et al., 2011; Souza et al., 2011). In consequence, the labeling of blood constituents with ^{99m}Tc has been used as an *in vitro* assay to screening effects of synthetic or natural products that could have actions related to the band-3 and calcium channels or antioxidant/oxidant properties. Moreover, qualitative and quantitative morphological analysis of RBC has been used as a method to evaluate if the effects of drugs on this radiolabeling process could be related to changes on shape of RBC (Rocha et al., 2009; De et al., 2009).

The aim of this study was to evaluate biologic effects of an aqueous extract of mate-tea on the labeling of the blood constituents with ^{99m}Tc and on the morphology of RBC.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (3 to 4 months of age, body weight 250 to 350 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 of the Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro that has approved the protocols used with the number CEA/213/2007.

Preparation of mate-tea extract

As the *I. paraguariensis* extract can be used in different forms of beverage, it is difficult to determine a specific concentration to be studied (Bastos et al., 2007; Miranda et al., 2008; Burris et al., 2012). In our investigation, to prepare the extracts, 10 g of dry leaves of commercial *I. paraguariensis* (Shapira, Brasil) were vortexed in 50 ml NaCl 0.9%. The crude extract was filtered, centrifuged (clinical centrifuge, 1500 rpm, 5 min) to obtain the final extract. The supernatant was considered to be in the concentration of 200 mg/ml.

In vitro labeling of blood constituents assay

Heparinized blood (500 µl), was withdrawn from Wistar rats and incubated with 100 µl of mate-tea extract at different concentrations (12.5, 25, 50, 100 and 200 mg/ml) or with a saline solution alone, as control, for 1 h (room temperature). Afterwards, 500 µl of stannous chloride (1.20 µg/ml) was added and the incubation continued for further 1 h. After this period of time, 100 µl of ^{99m}Tc (3.7 MBq) as sodium pertechnetate ($Na^{99m}TcO_4$), recently milked from a $^{99}Mo/^{99m}Tc$ generator (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, São Paulo, Brasil) were added and the incubation was continued for 10 min. These samples were centrifuged in a clinical centrifuge (1500 rpm, 5 min) and aliquots of 20 µl of plasma (P) and blood cells (BC) were isolated. Another aliquots of 20 µl of P and BC were separated and precipitated in 1.0 ml of trichloroacetic acid (5%) and centrifuged (1500 rpm, 5 min) to isolate soluble (SF) and insoluble fractions (IF). The radioactivity in P, BC, SF-P, IF-P, SF-BC and IF-BC were determined in a well counter (Packard, model C5002, Illinois, USA) and the percentage of incorporated radioactivity (%ATI) was calculated as described elsewhere (Oliveira et al., 2003).

Morphological evaluation of red blood cells

Histological preparations were carried out with blood samples *in vitro* treated with mate-tea extract at different concentrations during 60 min at room temperature, or with saline solution as control group. Blood smears were prepared, dried, fixed and stained by May-Grünwald-Giemsa method (Bancroft and Gamble, 2007). After that, the images of the red blood cells were acquired (Optronics, USA) from blood smears to qualitative morphology analysis under optical microscopy (×1000, Olympus, BX model, Japan).

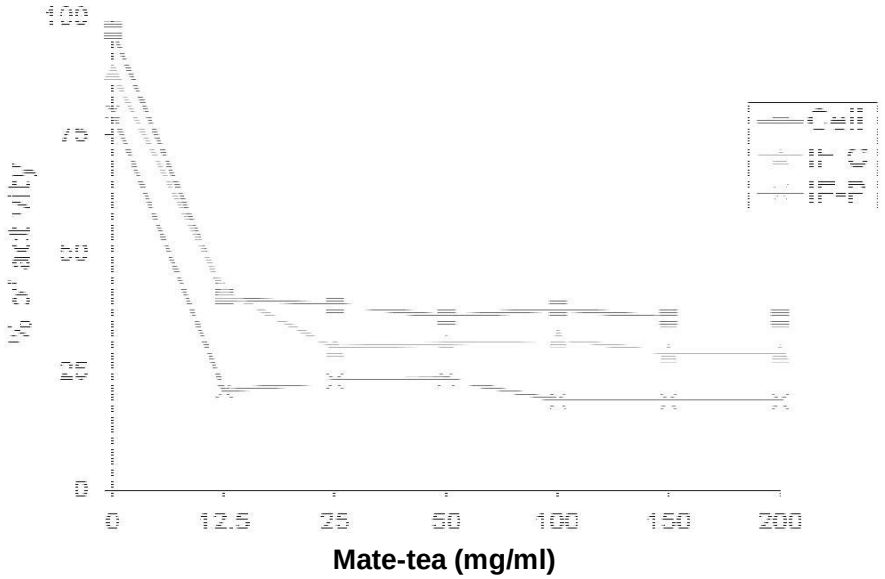

Figure 1. Effect of mate-tea extract on the uptake of the ^{99m}tc in the blood cells (Cell), insoluble (IF-C) fractions of blood cells and insoluble (IF-P) fractions of plasma, in the radiolabeling procedure of blood constituents. Heparinized blood samples of w*istar* rats were incubated with different concentrations of mate-tea extract (1 h) and after with $SnCl_2$ (1.20 ∝g/ml, 1 h) and in sequence with $Na^{99m}TcO_4$ (3.7 MBq, 10 min). Insoluble and soluble fractions of plasma were obtained by precipitation with trichloroacetic acid (5%) and centrifugation (1500 rpm, 5 min). the radioactivity in these fractions was counted and the %ATI were calculated.

Statistical analysis

Data are reported as (means ± SD) of %ATI. The comparison of the obtained data of the treated (n = 10 for each extract concentration) and control groups (n = 10) by one way analysis of variance – (ANOVA), followed by Tukey post test, with a $p < 0.05$ as significant level was performed. InStat Graphpad software was used in the statistical analysis (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California, USA).

RESULTS

Figure 1 shows the distribution of the radioactivity in the cells (cellular compartment) when the blood was treated with different concentrations of the mate-tea extract (12.5, 25.5, 100 and 200 mg/ml). The results show an inhibition in the process of labeling of the cells where the efficiency of labeling was reduced significantly ($p < 0.05$) for all the concentrations of the mate-tea extract tested.

In addition, Figure 1 also shows the fixation of the radioactivity in the insoluble fractions of the plasma (IF-P) and the insoluble fractions of the cells (IF-C) when treated with different concentrations of the mate-tea extract (12.5, 25.5, 100 and 200 mg/ml). The results obtained after the treatment of the blood with the different concentrations of the mate-tea extract, show that it produced significant reduction ($p < 0.05$) in the efficiency of labeling of the IF-C, from 88.0 ± 3.7 to 41.8 ± 3.7, until the concentration of 12.5 mg/ml with this reduction more accentuated for the concentration of 200 mg/rnl when the %ATI was reduced from 88.0 ± 3.7 to 29.0 ± 1.3. An important consideration is that these effects are not related to a dose-response relationship. Moreover, with a strong dilution of the extract, its effect was observed.

Figures 2, 3, 4 and 5 represented photomicrographs of the blood smears from samples of whole blood treated with saline solution (control) or with an aqueous mate-tea extract with different concentrations (12.5, 50 and 200 mg/ml), respectively. The qualitative morphological analysis by the comparison between these figures suggests the treatment with mate-tea extract could induce important changes on shape of red blood cells observed under optical microscopy. Furthermore, these qualitative morphological effects seem to be independent on the dose of the extract and they appear with strong dilutions of the extract.

DISCUSSION

The knowledge about properties and effects of a natural product is worthwhile due to the consumption of these

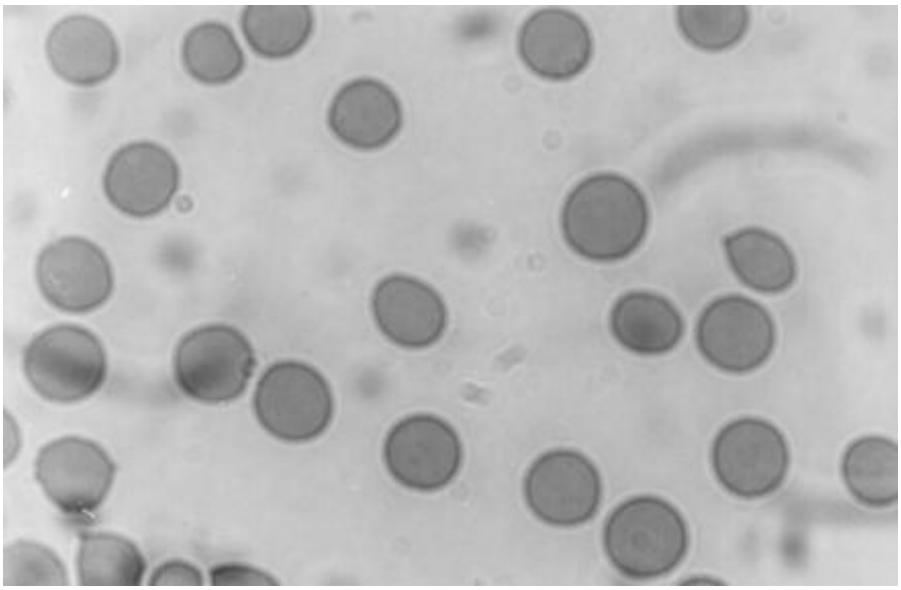

Figure 2. Photomicrography of blood smears from blood samples treated with NaCl 0.9% solution (control). Samples of whole blood from Wistar rats were treated with NaCl 0.9% solution during 60 minutes. Blood smears were prepared, dried, fixed and staining by May-Grünwald-Giensa method. The morphology of red blood cells was evaluated under optical microscopy after image capture.

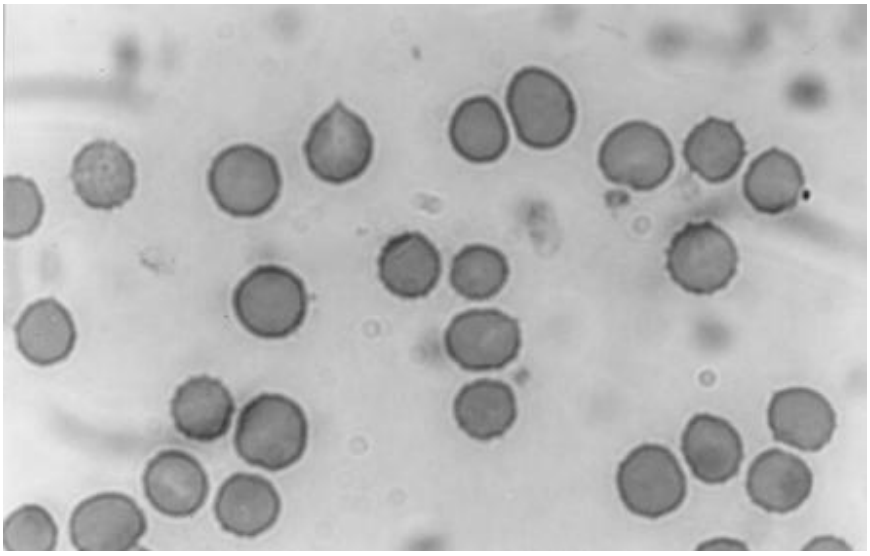

Figure 3. Photomicrography of blood smears from blood samples treated with mate-tea extract. Samples of whole blood from Wistar rats were treated with aqueous mate-tea extract (12.5 mg/ml) during 60 min. Blood smears were prepared, dried, fixed and staining by May-Grünwald-Giensa method. The morphology of red blood cells was evaluated under optical microscopy (× 1000) after image capture. The arrows indicate the alterations on the erythrocyte membrane.

products which are increasing in the entire world (Rotblatt and Ziment, 2002). *I. paraguariensis*, as tea, was capable in altering the distribution of ^{99m}Tc on plasma and cellular compartments, as well as the fixation of this radionuclide

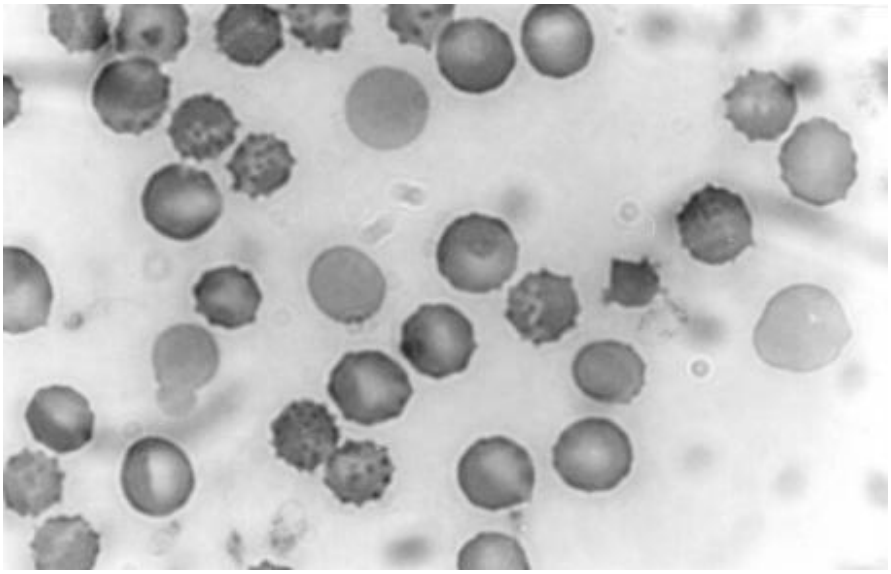

Figure 4. Photomicrography of blood smears from blood samples treated with mate-tea extract. Samples of whole blood from Wistar rats were treated with aqueous mate-tea extract (50 mg/ml) during 60 min. Blood smears were prepared, dried, fixed and staining by May-Grünwald-Giensa method. The morphology of red blood cells was evaluated under optical microscopy (× 1000) after image capture. The arrows indicate the alterations on the erythrocyte membrane.

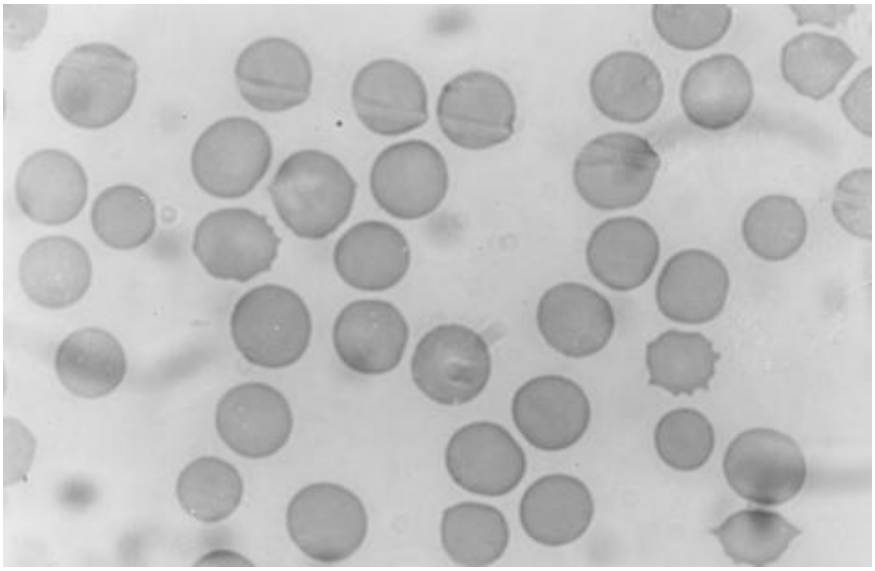

Figure 5. Photomicrography of blood smears from blood samples treated with mate-tea extract. Samples of whole blood from Wistar rats were treated with aqueous mate-tea extract (200 mg/ml) during 60 min. Blood smears were prepared, dried, fixed and staining by May-Grünwald-Giensa method. The morphology of red blood cells was evaluated under optical microscopy (× 1000) after image capture. The arrows indicate the alterations on the erythrocyte membrane.

in the insoluble fractions of plasma and blood cells (Figure 1). It is reported that mate-tea extracts contain some compounds that have redoxi properties such as phenolic acids, and its properties are attributed to

methylxanthines, such as caffeine referred in folk medicine (Vieira et al., 2010). As the labeling of blood constituents depends on a reducing agent, the redoxi properties of the substances present in the *I. paraguariensis* could be responsible by the reduction of the labeling of blood constituents with ^{99m}Tc.

In addition, in the labeling of blood cells with ^{99m}Tc, the stannous and pertechnetate ions must reach the cell compartment by channels of erythrocyte membrane. It was observed (Figures 3, 4, 5) that independently on the dose, the extract could cause qualitative and important morphological modifications on the membrane of the red blood cells and this fact can contribute to justify the alteration on the labeling of these cells with ^{99m}Tc. Our results of morphology of RBC could be related to modifications on membrane structures involved in ions transport that could alter the transport of the stannous and pertechnetate ions into cell justifying the decrease of the labeling of blood cells with ^{99m}Tc (Figure 1).

Conclusion

Our findings could be related to the redox properties of the substances of the mate-tea extract, as well as due to the interference in the transport of ions (stannous and/pertechnetate) into the blood cells due to morphological modifications of the erythrocyte membrane. Although, our experiments were performed in controlled conditions and with blood withdrawn from rats, we suggest precaution in the interpretation of the examinations done in a Nuclear Medicine Department in patients that are undertaken mate-tea.

ACKNOWLEDGEMENTS

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

REFERENCES

Artiko V, Obradovic V, Petrovic M, Perisic-Savic M, Suvajdzic N (2004). Hemangioma of the spleen confirmed by blood pool scintigraphy. Clin. Nucl. Med. 29:670-671

Bastos DHM, Oliveira DM, Matsumoto RLT, Carvalho PO, Ribeiro ML (2007). *Yerba maté*: Pharmacological properties, research and biotechnology. Med. Aromat Plant Sci. Biotechnol. 1:37-46

Bancroft JD, Gamble M (2007). Theory and Practice of Histological Techniques. 6th edition, London: Churchil Livingstone.

Blond L, Madsen JL (2000). Scintigraphic method for evaluating reductions in local blood volumes in human extremities. Scand J. Clin. Lab. Invest. 60:333-339

Burris KP, Davidson PM, Stewart Jr CN, Zivanovic S, Harte FM (2012). Aqueous extract of Yerba mate (*Ilex paraguariensis*) as a natural antimicrobial against *Escherichia coli* O157:H7 in a microbiological medium and pH 6.0 apple juice. J. Food Prot. 75:753-757

Bustani H, Colavolpe C, Imbert-Joscht I, Havlik P, Pisano P, Guillet BA (2009). Chocolate Intake Associated with Failed Labeling of 99mTc Red Blood Cells. J. Nucl. Med. Technol. 37:107-110

Callahan RJ, Rabito CA (1990). Radiolabeling of erythrocytes with technetium-99m: role of band-3 protein in the transport of pertechnetate across the cell membrane. J. Nucl. Med. 31:2004-2008 Cekic B, Mutfuler FZ, Kilcar AY, Ichedef C, Unak P (2011). Effects of broccoli extract on biodistribution and labeling blood components with 99mTc-GH. Acta Cir Bras. 26:339-345

Cortes J, Alonso JI, Ruiz-Oliva F, Alvarez S, Ormijana JS, Caton B, Alcorta P (2003). Renal cell carcinoma detected on Tc-99m-labeled red blood cell imaging. Clin. Nucl. Med. 28:920-922

Crandall CG, Wilson TE, Marving J, Vogelsang TW, Kjaer A, Hesse B, Secher NH (2008). Effects of passive heating on central blood volume and ventricular dimensions in humans. J. Physiol. 586:293-301

De K, Chandra S, Misra M (2009). Assessment of the effect of *Bacopa monnieri* (L.) Wettst extract on the labeling of blood elements with technetium-99m and on the morphology of red blood cells. Rev. Bras Farmacog. 19:664-671

Dewanjee MK, Rao SA, Penniston JT (1982). 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. J. Labeled Compd. Rad. 11:1464-1466

Dolezal J, Vizda J, Kopacova M (2011). Single-photon emission computed tomography enhanced Tc-99m-pertechnetate disodium-labelled red blood cell scintigraphy in the localization of small intestine bleeding: a single-centre twelve-year study. Digestion. 84:207-211

Early PJ, Soddee DB (1995). Principles and Practice of Nuclear Medicine (Mosby Year Book, Inc, Toronto, Canada).

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

Harel F, Dupuis J, Benelfassi A, Ruel N, Gregoire J (2005). Radionuclide plethysmography for non-invasive evaluation of peripheral arterial blood flow. Am J Physiol Heart Circ Physiol. 289: H258-H262

Hladik III WB, Saha GB, Study KT (1987). Essentials of Nuclear Medicine Sciences (William and Wilkins, Sydney, Australia.

Holanda CM, Silva-Junior MF, Alves RC, Barbosa VS, Silva RP, Rocha LG, Medeiros AC (2009). The effect of the rochagan on radiolabeling with 99mTc. Cell Mol Biol (Noisy-le-grand). 55:23-28

Hsieh HJ, Lue KH, Kao CH, Hsu YH, Liu MC, Kao PF (2009). Portosystemic collateral circulation: demonstrated by Tc-99m-RBC SPECT/CT. Clin. Nucl. Med. 34:958-959

Hussein GM, Matsuda H, Nakamura S, Hamao M, Akiyama T, Tamura K, Yoshikawa M (2011). Mate tea (Ilex paraguariensis) promotes satiety and body weight lowering in mice: involvement of glucagon-like peptide-1. Biol. Pharm Bull. 34:1849-1855

Javadi H, Asli IN, Semnani S, Jallalat S, Ansari M, Amini A, Barekat M, Assadi M (2011). Comparison of technetium-99m IgG with technetium-99m red blood cells labeling in cardiac blood-pool scintigraphy: a preliminary study. Hell J. Nucl. Med. 14:30-33

Jin RB, Ma XL, Wen JL, Tang WJ (2004). Application of radionuclide imaging to hepatic impact injury in rabbits. Chin. J. Traumatol. 7:45-48

Lanzetti M, Lopes AA, Ferreira TS, de Moura RS, Resende AC, Porto LC, Valenca SS (2011). Mate tea ameliorates emphysema in cigarette smoke-exposed mice. Exp. Lung Res. 37:246-257

Miranda DDC, Açari DP, Pedrazzoli Jr J, Carvalho PO, Cerutti SM, Bastos DHM, Ribeiro ML (2008). Protective effects of mate tea (*Ilex paraguariensis*) on H_2O_2-induced DNA damage and DNA repair in mice. Mutagenesis. 23:261-265

Niemeyer MG, van der Wall EE, Kuijper AF, Cleophas AT, Pauwels EK

(1995). Nuclear cardiology, current applications in clinical practice. Angiology. 46:591-602.

Olds GD, Cooper GS, Chak A, Sivak MV Jr, Chitale AA, Wong RC (2005). The yield of bleeding scans in acute lower gastrointestinal hemorrhage. J. Clin. Gastroenterol. 39:273-277.

Oliveira JF, Santos-Filho SD, Catanho MTJA, Srivastava SC, Lima-Filho GL, Bernardo-Filho M (2003). Effect of extract of medicinal plants on the labeling of blood elements with technetium-99m and on the morphology of red blood cells (RBC): toxicological actions of roast coffee beans (*Coffea arabica*). Ind. J. Nucl. Med. 18:52-56

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

Sampsom CB (1996). Complications and difficulties in radiolabeling blood cells: a review. Nucl. Med. Commun. 17:648-658.

Schillaci O, Spanu A, Tagliabue L, Filippi L, Danieli R, Palumbo B, Del Sole A, Madeddu G (2009). SPECT/CT with a hybrid imaging system in the study of lower gastrointestinal bleeding with technetium-99m red blood cells. Q J. Nucl. Med. Mol. Imaging. 53:281-289.

Slart RH, Phan TT, Talsma MD, Jager PL (2004). Different splenic uptake of Tc-99m sulfur colloid and Tc-99m heat-denatured red blood cells in an infant with complete situs inversus. Clin. Nucl. Med. 29:590-591.

Souza DE, Pereira MO, Bernardo LC, Carmo FS, Fonseca Ade S, Bernardo-Filho M (2011). An experimental model to study the effects of a senna extract on the blood constituent labeling and biodistribution of a radiopharmaceutical in rats. Clinics (Sao Paulo). 66:483-486.

Stoeckli TC, Zimmerli W, Maecke HR, Fridrich R (1996). Comparison of chemotaxis and superoxide generation of indium-111-oxine- and technetium-99m-HMPAO-labelled granulocytes. Scand J. Clin. Lab. Invest. 56:305-309.

Verdu J, Martinez A, Anton MA, Munoz JM, Riera M, Jover R, Caballero O (2005). Increased thallium-201 uptake and Tc-99m red blood cell accumulation in hemangioma. Clin. Nucl. Med. 30:25-26.

Vieira MA, Maraschin M, Pagliosa CM, Podestá R, de Simas KN, Rockenbach II, Amboni RD, Amante ER (2010). Phenolic acids and methylxanthines composition and antioxidant properties of mate (Ilex paraguariensis) residue. J Food Sci. 75:C280-C285.

Wong KT, Beauvais MM, Melchior WR, Snyder SP (2004). Enhanced liver uptake of Tc-99m-labeled RBCs during gastrointestinal bleed scintigraphy using transfused RBCs compared with autologous RBCs. Clin. Nucl. Med. 29:522-523.

Yarlagadda R, Menda Y, Graham MM (2008). Tc-99m red blood cell imaging in a patient with blue rubber bleb nevus syndrome. Clin. Nucl. Med. 33:374-376.

Zaman MU, Hussain R, Sajjad Z, Ahmad MN (2004). Localization of upper GI bleed on delayed imaging with 99mTc tagged RBC angiogram. J. Pak Med .Assoc. 54:490-491