Effect of barbatimão [*Stryphnodendron adstringens* (Mart.) Coville] infusion on the labling of blood elements with technetium–99m.

Thadeu E. M. Maramaldo Costa[1,2]; Aline P. Muzy Dias[1,2]; Priscila V. S. Zabala Capriles[1,2]; Márcia Betânia N. Oliveira[1]; Elba Lúcia Cavalcanti de Amorim[3]; Cláudia Sampaio de Andrade Lima[3]; Mario Bernardo-Filho[1]*

[1]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, Rio de Janeiro, RJ, Brasil
[2]Universidade Federal do Rio de Janeiro
[3]Universidade Federal de Pernambuco, Brasil
bernardo@uerj.br

Abstract

The Barbatimão is a tree which bark is rich in tannin. It is used on popular medicine as a wound healing agent, in the treatment of gastric lesions, as anti-leishmanial agent and as anti-inflammatory. Red blood cells (RBC) are labeled with technetium-99m (Tc-99m) and are utilized in many procedures in nuclear medicine. Some authors have reported that drugs (natural and synthetic) can alter the labeling of RBC with Tc-99m. This study evaluates the effect of barbatimão infusion on the labeling of red blood cells (RBC) and plasma (P) proteins with Tc-99m. Heparinized blood from *Wistar* rats was incubated with NaCl 0.9% as control and different concentrations of barbatimão infusion. Following the addition of stannous chloride ($SnCl_2$), as reducing agent, and Tc-99m, as sodium pertechnetate, the blood samples were centrifuged. P and RBC were separated and were also precipitated with trichloroacetic acid 5%. Insoluble (IF-P and IF-RBC) fractions were isolated. The percentage of radioactivity in all the samples was determined. All the barbatimão infusion concentrations decreased the labeling of RBC, IF-P and IF-RBC. We can speculate that the barbatimão infusion interfered on the labeling of RBC due to the redox properties and/or it can also act as a chelator of the stannous ion.

The use of natural products, as medicinal plants, is very frequent in all the world. *Stryphnodendron adstringens* (Mart.) Coville, popularly known as barbatimão, is a native Brazilian tree widely used in folk medicine as a wound healing agent in the treatment of gastric lesions[1], as anti-leishmanial agent[2] and as an anti-inflammatory agent[3]. The stem bark of barbatimão contains at least 20% of tannins[4]. Tannins is a group of polyphenols found in the majority of plants and some of them are able to scavenge hydroxyl radicals (˙OH), preventing the peroxidation of the lipids and nucleotide degradation[5, 6].

In clinical nuclear medicine procedures[7, 8, 9], technetium-99m (Tc-99m) has been the most utilized radionuclide and it has also been used in basic research[10, 11, 12, 13, 14]. It is widely use in nuclear medicine due to its optimal physical, chemical and biological characteristics[15].

Tc-99m labeling of red blood cells has come into wide use in clinical nuclear medicine for several important applications, including imaging of the cardiovascular system, detection and localization of gastrointestinal hemorrhage, measurement of red cell volume and spleen imaging. Erythrocyte labeling with Tc-99m pertechnetate can be done either by a completely *in vitro* technique, by *in vivo* methods or by a combination of both. This labeling process depends on the presence of a reducing agent and stannous chloride ($SnCl_2$) is utilized with this purpose.

The influence of drugs (natural or synthetic drugs) on the labeling of red blood cells has been well described 15,16,17.

The aim of this study is to evaluate the influence of different concentrations of *Stryphnodendron adstringens* (Mart.) Coville infusion on the labeling of red blood cells with Tc-99m.

A therapeutic drug can also modify the nature/amount of the Tc-99m-radiopharmaceutical bound to blood elements and this may result in unexpected behavior of the radiopharmaceutical. In our study, we observed the effect of barbatimão infusion on the labeling of red blood cells with Tc-99m and on the fixation of this radionuclide in the soluble and insoluble fractions of plasma and blood cells.

Figure 1 shows the distribution of the radioactivity in RBC and P from blood, treated with different concentrations of *Stryphnodendron adstringens* (Mart). Coville infusion. The analysis of the results indicates that there is a significant ($P < 0.05$) decrease in radioactivity in the RBC in the presence of barbatimão infusion from 96.67 ± 1.48 to 55.40 ± 8.07.

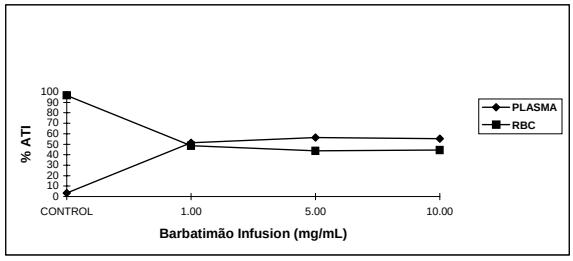

Figure 1. Effect of *Stryphnodendron adstringens* (Mart). Coville infusion on the labeling of RBC and P with Tc-99m. Heparinized blood was incubated with different concentrations of barbatimão extract infusion (0; 1; 5 and 10 mg/ml). Following, all samples were incubated with stannous chloride (1.2 mg/ml) and Tc-99m. The radioactivity was determined in a well counter and the percent of radioactivity (%ATI) was calculated.

Figure 2 shows the distribution of the radioactivity in insoluble fractions of RBC and P from blood treated with different concentration of *Stryphnodendron adstringens* (Mart.)

Coville infusion. The analysis of results indicates a significant ($p < 0.05$) alteration in the radioactivity fixation in IF-P (from 80.78 ± 7.30 to 26.55 ± 5.98) and IF-RBC (from 85.61 ± 2.25 to 39.25 ± 11.11) in all concentrations of the barbatimão solution studied.

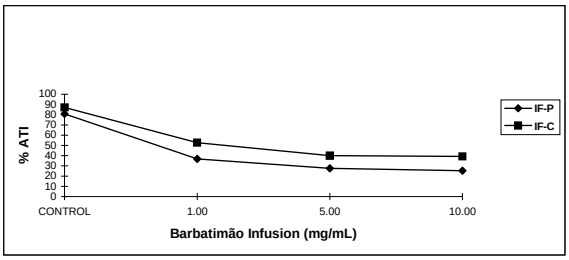

Figure 2. Effect of *Stryphnodendron adstringens* (Mart). Coville infusion on the labeling of IF-RBC and IF-P with Tc-99m. Heparinized blood was incubated with different concentrations of barbatimão extract infusion (0; 1; 5 and 10 mg/ml). Following, all samples were incubated with stannous chloride (1.2 mg/ml) and Tc-99m. The blood was centrifuged in order to separate the RBC from P. Samples of plasma were precipitated with TCA-5% and its insoluble fractions (IF-RBC and IF-P) were isolated. The radioactivity was determined in a well counter and the percent of radioactivity (%ATI) was calculated.

In the labeling process of red blood cells with technetium-99m, the stannous and pertechnetate ions pass through the plasma membrane[16,17]. As in presence of infusion of barbatimão, there was a decrease on the labeling of red blood cells and insoluble fractions of the blood elements, we can speculate that barbatimão interfered on the labeling of RBC due to the redox properties and/or as a chelator of the stannous ion.

Material and Methods

The infusion was prepared by adding 60 mg of the stem bark from "barbatimão" to 6 ml of NaCl 0.9% at room temperature. The preparation was centrifuged in clinical centrifuge (1000 rpm, 10 min) and the supernatant isolated. Heparinized blood from *Wistar* rats (0.5 ml) was incubated with different concentrations of barbatimão infusion (10, 5 e 1mg/ml) or NaCl 0.9% for 60 min at room temperature. After this period of time, 0.5 ml of $SnCl_2.2H_2O$ (1.2 mg/ml) was added and it was incubated for more 60 min. In addition, 0.1 ml of Tc-99m (3.7 MBq), as sodium pertechnetate, freshly milked from a 99Mo/99mTc generator (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, São Paulo, Brazil), was added and the incubation continued for more 10 min. The samples were centrifuged and plasma (P) and red blood cells were separated. Aliquots (20 ml) of P and RBC were precipitated with 1 ml of trichloroacetic acid (TCA) 5% and soluble (SF) and insoluble (IF) fractions were isolated. The radioactivity in all samples collected were determined in a well counter (Clinigamma, gamma counter LKB, Wallac, Finland). The percentage of radioactivity (%ATI) was calculated. A statistical analysis (ANOVA test) was used to compare the experimental data.

References

[1] Audi EA, Toledo DP, Peres PG, Kimura E, Pereira WK, de Mello JC, Nakamura C, Alves-do-Prado W, Cuman RK, Bersani-Amado CA. Gastric antiulcerogenic effects of *Stryphnodendron adstringens* in rats. Phytotherapy Research 1999; 3:264-6

[2] Jorge AS, Silveira TGV, Arraes SMAA, Mello JCP, Bertolini DA. Anti-leishmanial "barbatimão" (*Stryphnodendron adstringens* (Martius) Coville, Mimosaceae) extract against promastigotes forms of Leishmania (Leishmania) amazonensis. Memórias do Instituto Oswaldo Cruz 1996 (Supplement); 91: 116-20

[3] Lima JCS, Martins DTO, Souza PT. Experimental evaluation of stem bark of *Stryphnodendron adstringens* (Mart.) Coville for anti-inflammatory activity. Phytotherapy Research 1998; 12: 218-20

[4] Mello JCP, Petereit F, Nahrstedt A. A dimeric proanthocyanidin from *Stryphnodendron adstringens*. Phytochemistry 1999; 51: 1105-07

[5] Lopes GKB, Schuman HM, Hermes-Lima M. Polyphenol tannic acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions. Biochemica et Biophysica Acta 1999; 1472: 142-52

[6] Pietta PG. Flavonoids as antioxidants. Journal of Natural Products 2000; 63: 1035-42

[7] Early PJ, Sodee DB. Principles and Practice of Nuclear Medicine. Mosby -Year Book Inc, Toronto, 1995, 877pp.

[8] Saha GB. Fundamentals of Nuclear Pharmacy. Springer-Verlag, New York, 1998, 331pp

[9] Gutfilen B, Pellini M, Roure-Neder J, Amarante JLM, Evangelista MG, Fernandes SR, Bernardo-Filho, M. 99mTc labeling white blood cells with a simple technique: clinical application. Annals of Nuclear Medicine. 1994; 8: 85-9

[10] Gutfilen B, Marinho JCA, Rozemblum S, Bernardo-Filho M. 99mTc-labeled leucocytes obtained with a suitable technique: the biodistribution study in rabbits. Acta Medica et Biologica. 1993; 41: 193-6

[11] Plotkowsky MC, Bernardo-Filho M, Meireles MN, Tournier JM, Puchelle E. *Pseudomonas aeruginosa* binds to soluble cellular fibronectin. Current Microbiology 1993; 26: 91-5.

[12] Bernardo-Filho M, Pires ET, Boasquevisque EM, Hassón-Voloch A. Studies on the incorporation of 99m-Technetium to the platyhelminth *Dugesia tigrina*. Revista di Parassitologia 1993; XLIV: 7-11

[13] Bernardo-Filho M, Silva JRM, Reis RJN, Boasquevisque EM, Hassón-Voloch. Conditions for labeling of *Schistosoma mansoni* cercaria with Technetium-99m. The Journal of Nuclear Biology and Medicine 1992; 36: 56-9

[14] Oliveira JF, Braga ACS, Oliveira MBN, Ávila AS, Caldeira-de-Araújo A, Cardoso VN, Bezerra RJAC, Bernardo-Filho M. Assessment of the effect of *Maytenus ilicifolia* (espinheira santa) extract on the labeling of red blood cells and plasma proteins with Technetium-99m. Journal of Ethnopharmacology 2000; 72: 179-84

[15] Oliveira JF, Braga ACS, Ávila AS, Fonseca LMB, Gutfilen B, Bernardo-Filho M. Effect of Thuya occidentalis on the labeling of red blood cells and plasma proteins with Technetium-99m. Yale Journal of Biology and Medicine. 1996; 69: 489-94

[16] Braga ACS, Oliveira MBN, Feliciano GD, Reiniger IW, Oliveira JF, Silva CR Bernardo-Filho M. The effect of drugs on the labeling of blood elements with Technetium –99m. Current Pharmaceutical Design 2000; 6: 1179-91

[17] Reiniger IW, Oliveira JF, Caldeira-de-Araújo A, Bernardo-Filho M. Effect of *Peumus boldus* on the labeling of red blood cells and plasma proteins with Technetium-99m. Applied Radiation and Isotopes 1999; 51: 145-9

Identificação de marcadores cromatográficos de *Zollernia ilicifolia* e *Sorocea bonplandii* para o controle de qualidade de espinheira-santa

Michele Debiasi Alberton[1];Ester Santos de Souza[2]; Daniel de Barcellos Falkenberg[3]; Miriam de Barcellos Falkenberg[4]*

[1] Programa de Pós-Graduação em Farmácia
[2] Curso de Graduação em Farmácia e Bioquímica
[3] Departamento de Botânica
[4] Departamento de Ciências Farmacêuticas
Centro de Ciências da Saúde, Universidade Federal de Santa Catarina, Campus Universitário Trindade, 88040-900, Florianópolis, SC, Brasil
miriam@cos.ufsc.br

Resumo

As análises cromatográficas comparativas entre *Maytenus ilicifolia* Martius ex Reissek (Celastraceae), utilizada na medicina popular para o tratamento de problemas gástricos e *Zollernia ilicifolia* (Brongniart) Vogel (Fabaceae) e *Sorocea bonplandii* (Baillon) Burger, Lanjouw & Boer (Moraceae) revelaram a presença de uma substância marcadora para *Z. ilicifolia* e três para *S. bonplandii* , que podem ser usadas no controle de qualidade por CCD de amostras vegetais e fitoterápicos à base de espinheira-santa.

Maytenus ilicifolia Martius ex Reissek (Celastraceae), conhecida popularmente como "espinheira-santa", é uma espécie usada na medicina popular para o tratamento de problemas digestivos[1], e é bem conhecida tanto do ponto de vista químico como farmacológico[2, 3]. O extrato aquoso desta espécie apresentou efeitos antiúlcera em pacientes portadores de dispepsia alta ou úlcera péptica[3]. Seu extrato clorofórmico apresentou também atividade analgésica e antiinflamatória em camundongos[4].

Devido à similaridade morfológica, *Zollernia ilicifolia* (Brongniart) Vogel (Fabaceae) e *Sorocea bonplandii* (Baillon) Burger, Lanjouw & W. Boer (Moraceae), que também têm as margens espinhosas, são facilmente confundidas com a espinheira-santa[5,6]. Para a espécie *Maytenus ilicifolia*, considerada como espinheira-santa "verdadeira", e *Maytenus aquifolium* também considerada verdadeira por muitos pesquisadores, já existem marcadores químicos como friedelina e friedelanol, que podem ser utilizados no controle de qualidade [7]. Para *Z. ilicifolia* e *Sorocea bonplandii*, entretanto, não haviam sido descritas na literatura substâncias que servissem como marcadores destas espécies na análise por cromatografia em camada delgada, uma metodologia rápida e reprodutível[8]. Em decorrência disto, o objetivo do trabalho foi comparar extratos