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An *In vitro* Evaluation of an *Ageratum conyzoides Linn.* Extract on the Labeling of Sanguineous Cells and Proteins with ^{99m} Technetium

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Abstract: *Ageratum conysoides* is a plant with analgesic, antibacterial, anti-inflammatory, depurative, febrifuge, stimulant and vulnerary properties. Blood cells (BC) labeling with ^{99m}Tc(technetium-99m) has progressed around the world. The influence of drugs (natural and synthetic) on the labeling of blood elements with ^{99m}Tc has been reported. The purpose of this study was to examine the effect of an *A. conysoides* extract on the radiolabeling of blood elements with ^{99m}Tc. Extract (0.1g/mL) was prepared with NaCl (0.9%) and diluted in five different concentrations 100%; 50%; 25%; 12.5% and 6.25% v/v. Blood was withdrawn and it was incubated with the referred extract for 1hr. Elapsed this period of time stannous chloride was added for more 1 hr followed by the addition of ^{99m}Tc. Plasma (P) and (BC) were separated P and C were precipitated with5% trichloroacetic acid (TCA) and soluble (SF) and insoluble (IF) fractions were obtained. The percentage of radioactivity (%ATI) was determined. Related to the results obtained it was noticed that the extract was capable of altering the efficiency of radiolabeling in Cells to the concentrations of 100% (from 96.86 ± 1.80 to 52.84 ± 2.26); 50% (from 96.86 ± 1.80 to 58.05 ± 11.63); 25% (from 96.86 ± 1.80 to 81.98 ± 8.57) and 12.5% (from 96.86 ± 1.80 to 88.83 ± 8.57); in the IF-C to the concentrations of 100% (79.97 ± 9.87 to 15.85 ± 7.06); 50% (from 79.97 ± 9.87 to 17.54 ± 8.00); 25% (from 79.97 ± 9.87 to 31.73 ± 9.04) and 12.5% (79.97 ± 9.87 to 48.74 ± 9.90) and in the IF-P to the concentrations of 100% (from 89.04 ± 5.41 to 41.04 ± 8.67) and 50% (from 89.04 ± 5.41 to 35.40 ± 7.38). Due to the results found we can suggest that refereed extract was capable of altering the radiolabeling of blood elements with ^{99m}Tc probably could be explained by the generation of reactive Oxygen species with oxidant properties, which might be altering the stabilizing of the red blood cell membrane as the radiolabeling of blood elements.

key words: *Ageratum conysoides*, red blood cells, plasma proteins, technetium-99m

Introduction

Many plant species are used medicinally. In Brazil there are many vegetables, which is traditionally used in folk medicine. *Ageratum conysoides* is known in Brazil as *Catinga de Bode*. It is a plant from the Central and Meridian America. *A. conysoides* is a commonly used medicinal plant for a variety of indications due to its analgesic, antibacterial, anti-inflammatory maybe due to the presence of alkaloids with vase constrictor vase action, depurative, febrifuge, stimulant and vulnerary properties. Shirwaikar *et al.* (2003) have been demonstrated the gastroprotection effect of the referred extract in rats. have immunostimulant, antioxidant, and more recently antimutagenic properties. The findings suggested that the significant gastroprotective activity could be mediated by its antioxidant activity, Ca^{2+} channel blocking and antiserotogenic properties. Jagetia *et al.* (2003) described that the radioprotection afforded by *A. conysoides* may be in part due to the scavenging of reactive oxygen species induced by ionizing radiation. The biodistribution of the radiopharmaceuticals can be altered by natural and synthetic drugs as well as the radiolabeling of blood elements with technetium-99m (^{99m}Tc) (Dire, *et al.*, 2002; Oliveria *et al.*, 2002; Oliveria *et al.*, 2000; Oliveira *et al.*, 1997 and Vidal *et al.*, 1998). When a radionuclide have its capability to bind to blood elements altered by drugs therapy, the process of labeled red blood cells may be repeated, resulting in an additional radiation dose to the patient (Early and Sodee, 1995 and Hesselewwod and Leung, 1994).

^{99m}Tc has been the most utilized radionuclide in nuclear medicine procedures and it has also been used in basic research (Early and Sodee, 1995; Gutfilen and Bernardo, 1996; Mattos *et al.*, 2001 and Saha, 1998). The wide utilized in nuclear medicine is due to its optimal physical characteristics (half-life of 6h, gamma rays energy of 140 keV and minimal dose to the patients), convenient availability from ^{99}Mo/^{99m}Tc generator and negligible environmental impact. Nearly almost all scanning devices currently in use are optimized for detecting the eletromagnetic emission from this radionuclide (Early and Sodee, 1995 and Hesselewwod and Leung, 1994).

There are many applications of ^{99m}Tc-labeled red blood cells (RBC). The most important is in cardiovascular nuclear

medicine, where one tries to image the heart to determine its functional status as a pump, to calculate the left ventricular function by measuring the ejection fractions and to evaluate wall motion abnormalities. Some other applications are in the detection of gastrointestinal bleeding and in the determination of the RBC mass in patients (Early and Sodee, 1995 and Hesselewwod and Leung, 1994). The labeled process with ^{99m}Tc depends on a reducing agent and stannous ion (Sn^{2+}), mainly as stannous chloride, is usually used for this purpose (Early and Sodee, 1995; Gutfilen and Bernardo, 1996; Gutfilen *et al.*, 1993; Hesselewwod and Leung, 1994; Saha, 1998 and Sampson, 1996). When whole blood is used in the labeling of RBC with ^{99m}Tc, radioactivity is found on blood cells, however it is also bound on plasma proteins. This labeling process depends on optimal stannous chloride concentration and stannous and pertechnetate ions across the RBC membrane, probably spending energy and the radionuclide is mainly bound to hemoglobin molecule (Gutfilen and Bernardo-Filho, 1996; Hesselewwod and Leung, 1994 and Hladik *et al.*, 1987). Several of the cellular labeling steps have been well characterized. The band-3 anion transport system and calcium channels may be the ways that ^{99m}Tc and Sn^{+2}, respectively, reach the interior of the RBC (Gutfilen and Bernardo, 1996 and Srivastava *et al.*, 1984). If one damages the RBC, one can do selective spleen imaging since damaged cells are rapidly sequestrated by the spleen. RBC have been labeled with ^{99m}Tc for *in vitro in vivo* or *in vivo/ in vitro* techniques (Srivastava *et al.*, 1984.).
Plasma proteins (PP) have also been labeled with the referred radionuclide. ^{99m}Tc -labeled PP has been used to locate placenta, to evaluate the cardiac function and pulmonary perfusion, to determine blood volume and to study the gastrointestinal protein loss (Early and Sodee, 1995 and Hesselewwod and Leung, 1994).
The labeling of red blood cells with ^{99m}Tc has been influenced by patient medications, by the labeling conditions (Early and Sodee, 1995; Hesselewwod and Leung, 1994 and Santos *et al.*, 1995) or by the presence of extracts of plants, as *Paullinia cupana* (Oliveria *et al.*, 2002), *Maytenus ilicifolia* (Oliveria *et al.*, 2000), *Thuya occidentalis* (Oliveira *et al.*, 1997), *Nicotiana tabacum* (Vidal *et al.*, 1998), and. Nevertheless, there is not a well established *in vitro/in vivo* model to study the interaction of therapeutic drugs with radiopharmaceuticals (Early and Sodee, 1995; Sampson, 1996 and Santos *et al.*, 1995). Then, we have evaluated the influence of an *A. conysoides* extract (i) on the labeling of blood elements with ^{99m}Tc.

Materials and Methods

Plant Material: A commercial dried powder of *A. conysoides* was obtained from the Laboratory Herbarium, Laboratório Botânico, Brazil, Lot 923661 (June, 2001 and validity June 2004). To prepare the solution, which was considered like 100% it was diluted, 10g of *A. conysoides* into 10mL of saline solution (NaCl 0.9%) obtained a solution 100% (0.1mg/mL).

Animals: Male *Wistar* rats (200-250g) from Universidade do Estado do Rio de Janeiro were used. The animals received a standard pellet rat diet and water; they were maintained under constant environmental conditions (22 ± 5°C, 12h of light/dark cycle).

Study Protocol: Samples of heparinized (0.5 mL) blood were withdrawn from animals and incubated with 0.1 mL of the extract diluted in different concentrations (100%; 50%; 25%; 12.5% and 6.25%v/v) during 1 hr. After that, 0.5 mL of stannous chloride (1.2 μg/mL), as $SnCl_2.2H_2O$ was added and the incubation carried out for more 1 hr at room temperature. After this period of time, ^{99m}Tc (0.1 mL), as sodium pertechnetate, was added and the incubation continued for 10 min. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Samples (20 μl) of P and BC were precipitated with 1 ml of trichloroacetic acid (TCA) 5% and soluble (SF) and insoluble fractions (IF) were separated. The radioactivity in P, BC, IF-P, SF-P, IF-BC and SF-BC were determined in a well counter. After that, the % of radioactivity (%ATI) was calculated. A statistical analysis (Mann Whitney and Kruskal Wallis tests) was utilized to compare the experimental data.

Results

Table 1 has shown the effect of *Ageratum conysoides* on the labeling of red blood cells (BC), insoluble fraction of the red blood cells (IF-C) and in the insoluble fraction of the plasma (IF-P) with ^{99m}Tc. The analysis of the results indicated that the referred extract was capable of altering ($p<0.05$) the labeling efficiency of blood elements with ^{99m}Tc. Related to the results obtained it was noticed that the extract was capable of altering the efficiency of radiolabeling in Cells to the concentrations of 100% (from 96.86 ± 1.80 to 52.84 ± 2.26); 50% (from 96.86 ± 1.80 to 58.05 ± 11.63); 25% (from 96.86 ± 1.80 to 81.98 ± 8.57) and 12.5% (from 96.86 ± 1.80 to 88.83 ± 8.57); in the IF-C to the concentrations of 100% (79.97 ± 9.87 to 15.85 ± 7.06); 50% (from 79.97 ± 9.87 to 17.54 ± 8.00); 25% (from 79.97 ± 9.87 to 31.73 ± 9.04) and 12.5% (79.97 ± 9.87 to 48.74 ± 9.90) and in the IF-P to the concentrations of 100% (from 89.04 ± 5.41 to 41.04 ± 8.67) and 50% (from 89.04 ± 5.41 to 35.40 ± 7.38).

Table 1:Effect of an extract of Ageratum conysoides on the labeling of blood elements with ^{99m}Tc

Concentration of the extract (%)	C	IF-P	IF-C
Control	96.86 ± 1.80	89.04 ± 5.41	79.97 ± 9.87
6.25 %	95.47 ± 1.30	85.54 ± 7.07	75.01 ± 5.24
12.5 %	88.83 ± 8.41	89.13 ± 4.14	48.74 ± 9.90
25 %	81.98 ± 8.57	85.61 ± 5.71	31.73 ± 9.04
50 %	58.05 ± 11.63	35.40 ± 7.38	17.54 ± 8.00
100 %	52.84 ± 2.26	41.04 ± 8.67	15.85 ± 7.06

Samples of heparinized blood were incubated with different concentrations (6.25. 12.5. 50 and 100% v/v) of an extrac of *Ageratum conysoides*. A sample of heparinized whole blood was incubated with saline solution ¶NaCl.09%) as control. Them, stannous chloride and ^{99m}Tc were added. These samples were centrifuged and plasma (P) and blood cells (BC) separated. Aliquots of 20μL of P and BC were precipitated with 1.0mL of trichloroacetic acid (TCA) 5% and soluble (SF) and insoluble fractions (IF) separated. The radioactivity in P.BC.IF-P and IF-BC was determined in a well counter and the % of radioactivity (%ATI) was calculated.

Discussion

Practically all Countries utilize radioisotopes in medicine, industry, agriculture and research. The evidence that natural and synthetic drug can affect radiolabeling or bioavailability of radiopharmaceuticals in setting nuclear medicine clinic is already known. However, this drug interaction with radiopharmaceuticals is not completely understood. Many authors have described a great number of drugs, which can be due to the causes of some diseases of red cells (Filho *et al.*, 1994; Braga *et al.*, 2000 and Sampson, 1996). There are evidences that drugs can affect either radiolabeling or biodistribution of blood cells in the context of the nuclear medicine. In the literature some researches have turned their attention to *in vitro* testing of the drug with labeled cells (Filho *et al.*, 1994; Braga *et al.*, 2000; Hladik *et al.*, 1987 and Sampson, 1996). The use of natural products, as medicinal plants, is very frequent in folk medicine around the world and *Ageratum conysoides* is utilized as a therapeutic plant due to its analgesic, antibacterial, anti-inflammatory maybe due to the presence of alkaloids with vase constrictor vase action, depurative, febrifuge, stimulant and vulnerary properties (Jagetia *et al.*, 2003).

It has been described by our research group the effect of some natural products as *Thuya occidentalis* (Oliveira *et al.*, 1997), *Nicotiana tabacum*, (Vidal *et al.*, 1998), *Maytenus ilicifolia* (Oliveria *et al.*, 2000) and *Paullinia cupana* (Oliveria *et al.*, 2002) on the radiolabeling of blood elements with ^{99m}Tc. These extracts are capable to reduce this labeling procedure in *in vitro* studies.

In the present study, *Ageratum conysoides* extract was not capable of altering the radiolabeling of blood constituents with ^{99m}Tc different from the results obtained to the extract of cauliflower (leaf) (Lima *et al.*, 2002). In other study, (Diré *et al*, 2002), in an *in vivo* study have reported that chayotte extract was able to alter de radiolabling of blood elements. Thompson *et al.*, 1981 studied the labeling of intact human erythrocytes with ^{99m}Tc. The analysis of the membranes of labeled erythrocytes showed that the label bear is not due to the binding of technetium to residual haemoglobin but its binding to constituent membrane proteins demonstrating the importance of the integrity of the morphology of the cells due to radiolabeling process. Related to results found we may suggest that one of the influences of the extract could be due to the action of its in the hassling of the integrity of the shape of cells. Other hypothesis would be the action of the extract in Ca^{2+} channel blocking which could cause a decrease in the radiolabeling process. We can suggest that different *Peumus boldus* (Reiniger *et al.*, 1999), the effect of *Ageratum conysoides* could be explained by its oxidant properties which can be due to the fact that *Ageratum conysoides* may be in part related to the scavenging of reactive oxygen species induced by ionizing radiation.

Many reports about medicine plants are rarely written up in the traditional medicine literature. In order to make an accurate assessment of the impact of drugs and other factors on the biological systems additional data are required (Braga *et al.*, 2000 and Sampson, 1996).

Conclusion

We can conclude that *Ageratum conysoides* extract was capable of altering the labeling of blood elements with ^{99m}Tc. According to the findings we may suggest that the studied natural product may alter the radiolabeling due to its oxidant and Ca^{2+} channel blocking actions.

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