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Assessment of the Effect of an Extract of Arnica (*Arnica montana*) on the Radiolabeling of Sanguineous Elements

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Abstract: Medicinal herbs are widely used for the human beings, however, there are many reports about their undesirable toxicological effects. Preparations from Arnica (*Arnica montana*) flowers have been used in traditional medicine since a long time for the treatment of inflammatory diseases. Red blood cells (RBC) and plasma proteins labeled with technetium-99m (99mTc) have several clinical applications and it has been reported that some natural products are capable of reducing the efficiency of this radiolabeling. The aim of this work was to assess the effect of an extract of Arnica (infusion) on the labeling of blood elements with 99mTc. In the preparation of the extract it was used 200mg of the leaves of Arnica in 10mL of saline solution (NaCl 0.9%). Samples (0.5mL) of blood from *Wistar* rats were incubated with 0.1 mL of the extract during 1 hour. After that, the samples were incubated with stannous chloride ($SnCl_2$) and 99mTc. The blood was centrifuged and plasma (P) and RBC were isolated. P and RBC were also precipitated with trichloroacetic acid and soluble (S) and insoluble (I) fraction (F) were determined. The results have shown that the extract has reduced the radiolabeling in IF-P (from 75. 91% ± 3. 58 to 67.26% ± 7.44). It was described that some extracts as *Fucus vesiculosus*, *Paullinia cupana*, *Mentha crispa L* and *Coffea arabica* were able to alter the radiolabeling. In the light of the results obtained we suggest that the referred extract may alter the efficiency of labeling of IF-P due to its capacity (i) to oxidize the stannous ion, (ii) to complex with stannous and pertechnetate ions to form double salts, (iii) to compete by the same binding sites to pertechnetate ion or (iv) by the generations of reactive species of Oxygen with direct action on the labeling process.

Key words: Arnica, *Arnica montana*, red blood cells, plasma proteins, technetium-99m

Introduction

Medicinal plants are widely used as food or food additives, or as a substance in popular medicine as an alternative way of treatment by humans. Phytoremedy are widely used in folk medicine for human beings and the sale of these plants has increased considerably over the last 10 years in the industrialized countries. Aqueous extracts of many plants are widely used in therapy as complementary medicines (Capasso *et al.*, 2000 and Oliveira *et al.*, 2003). Traditional Chinese herbal medicines (TCHM) are increasingly used throughout the Earth, as they are considered to be effective and to have few side-effects. Contaminants of TCHM include heavy metals and undeclared drugs. The toxicity of these contaminants and additives, and the toxic effects of the herbal ingredients have important implications during the perioperative period. The anesthetist must consider the potential for drug interactions and systemic adverse effects of these natural products (Kam and Liew, 2002). Nuclear Medicine is a tracer technique. Some tracers are both specific and sensitive and others are highly sensitive but not specific. Radiopharmacy is an essential and integrated activity in all nuclear medicine facilities. Technetium-99m (99mTc) has been the most utilized radionuclide in nuclear medicine procedures and it has also been used in basic research.99mTc-labeling of erythrocytes has come into wide use in clinical nuclear medicine for several important applications. Red blood cells labeling with 99mTc can be done *in vitro* technique methods, or by a combination of these two, called *in vitro/in vivo* labeling. The labeling of blood elements with 99mTc needs a reducing agent and the stannous ions are frequently used. Many drugs and vegetable extracts have been reported to affect the biodistribution of different radiopharmaceuticals (Early and Sodee, 1995 and Braga *et al.*, 2000). Natural and synthetic drugs can alter the labeling of red blood cells with technetium-99m (99mTc) (Braga *et al.*, 2000 and Oliveira *et al.*, 2003). When a radionuclide has its capability to bind to blood elements altered by natural and therapy drugs, the process of labeled red blood cells may be repeated, resulting in an additional radiation dose to the patient (Hesselewood and Leung, 1994 and Sampson, 1996).

Arnica (*Arnica montana*) is also commonly called leopard's bane. The arnica plant has a bright yellow, daisy-like

flower that blooms around July. Preparations made from the flowering heads have been used in homeopathic medicine for hundreds of years. It is popular in Germany and over 100 drug preparations are made from the plant. Arnica is a perennial that is protected in parts of Europe. The active components in Arnica are sesquiterpene lactones, which are known to reduce inflammation and decrease pain. Other active principals are thymol (an essential oil), flavonoids, inulin, carotenoids and tannins. Arnica works by stimulating the activity of white blood cells that perform much of the digestion of congested blood, and by dispersing trapped, disorganized fluids from bumped and bruised tissue, joints and muscles. Arnica is known to stimulate blood circulation and can raise blood pressure, especially in the coronary arteries. The plant is used externally for arthritis, burns, ulcers, eczema and acne. It has anti-bacterial and anti-inflammatory qualities that can reduce pain and swelling, improving wound healing. In homeopathy Arnica is widely used as a woundhealing medication and for the treatment of hematomas. It has been reported the efficacy of Arnica in varicose vein surgery (Wolf *et al.*, 2003). Previously, it was shown that natural products present in Arnica attack inflammatory processes at a very central point by inhibiting the transcription factors NF-kappa B and NF-AT at micromolar concentrations. Both transcription factors regulate the transcription of genes encoding for many inflammatory mediators. Thus, these new insights on their molecular mode of action are an important contribution for a better understanding of the anti-inflammatory activity of preparations from Arnica. First clinical studies show that they can support the treatment of rheumatic diseases. The agreed use is important to avoid undesirable side effects (Merfort, 2003).

There are many applications of 99mTc-labeled red blood cells, in cardiovascular nuclear medicine, in the detection of gastrointestinal bleeding, and in the determination of the RBC mass in patients. Nevertheless, there is not a well established *in vitro* model to study the interaction of therapeutic drugs with radiopharmaceuticals. Then, we have evaluated the influence of a *A. montana* on the labeling of sanguineous cells and proteins with 99mTc by an *in vitro* study.

Materials and Methods

In this experimental it was used the extract bought of Estrela da Terra Produtos Naturais LTDA [Arnica (*Arnica montana*) which was produced in 05/03 validly to 05/06, lot: 00.547.944/001-83]. To prearrange the extract (100%v/v) it was prepared an infusion solution with 200mg of leaves of *A. montana* in 10mL of saline solution (NaCl 0.9%). The extract was diluted (50%) in three different concentrations (50%; 25% and 12.5%v/v).

Samples of 0.5 mL of blood withdraw from *Wistar* rats were incubated with 0.1 mL of the extract during 1 hour. In the control group saline solution was used. Elapsed this period of time it was added 0.5 mL of stannous chloride (1.2 $\mu g\ mL^{-1}$), as $SnCl_2.2H_2O$ for 1hr at room temperature. After this period of time, 99mTc (0.1 mL), as sodium pertechnetate, was added and the incubation continued for another 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, as previously reported (Bernardo-Filho *et al.*, 1994). A statistical analysis (Tukey-Kramer Multiple Comparisons Test, $n = 5$) was utilized to compare the experimental data.

Results

The Fig. 1 has shown the effect of *Arnica montana* extract on the labeling of blood elements with 99mTc. The analysis of the results revealed that the refereed was not capable of altering the on the labeling of BC.

The Fig. 2 has shown the effect of *Arnica montana* extract on the labeling of blood elements with 99mTc. The analysis of the results revealed that the was capable of altering the efficiency of radiolabeling on the IF-P (from 75.91 ± 3.58 to 67.26 ± 7.44).

The Fig. 3 has shown the effect of *Arnica montana* extract on the labeling of blood elements with 99mTc. The analysis of the results revealed that there was not an alteration on the efficiency of radiolabeling on the IF-C in the presence of the extract.

Discussion

The evidence that drugs can effect either radiolabeling or biodistribution of red blood cells in the context of nuclear medicine clinic has come to light only comparatively recently, and a number of workers have turned their attention to *in vitro* testing of the drug with labeled cells (Hesselewood and Leug, 1994). Several authors have studied the effect of different drugs (natural and synthetic) on the labeling of blood elements with radionuclides and have reported important findings. Extracts of medicinal can also alter the labeling of blood elements with 99mTc (Sampson *et al.*, 1996). We agree with Hesselewood and Leung (1994), that many reports on drug interactions with radiopharmaceuticals are anecdotal and in some instances a direct cause and effect relationship has not been unequivocally established. This fact could be diminished with the development of *in vitro* tests to evaluate the drug/radiopharmaceuticals interactions and the consequence for the bioavailability of the radiopharmaceuticals and the labeling of blood constituents. There are concerns that some natural medicines may contain potentially toxic

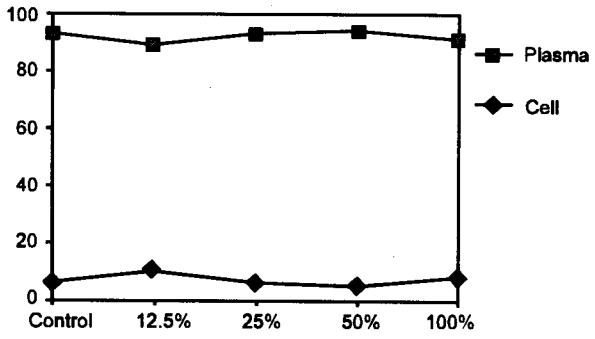

Fig. 1: Effect of *Arnica montana* extract on the labeling on the labeling of BC with 99mTc Samples of heparinized blood from *Wistar* Rats (treated or not with the extract) were incubated for 1 hour with stannous chloride (1.2 μg mL^{-1}) and 99mTc, as sodium pertechnetate were added. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Samples (20 μL) of BC were precipitated with trichloroacetic acid (TCA) 5% and soluble (SF) and insoluble fractions (IF) were separated. The radioactivity in P and BC was determined in a well counter and the % of radioactivity (% ATI) was calculated. A statistical analysis (Tukey-Kramer Multiple Comparisons Test, n=5) was used to compare the results. The values are averages.

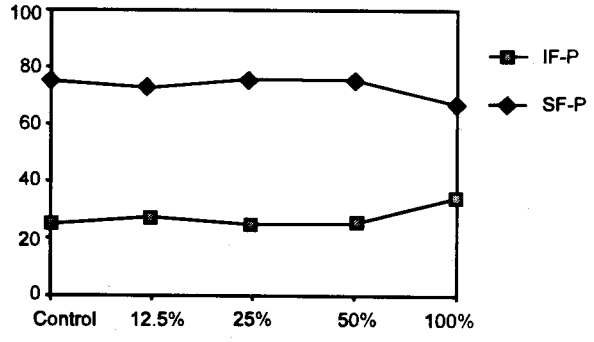

Fig. 2: Effect of *Arnica montana* extract on the labeling on the labeling of IF-P with 99mTc. Samples of heparinized blood from *Wistar* Rats (treated or not with the extract) were incubated for 1 hour with stannous chloride (1.2 μg mL^{-1}) and 99mTc, as sodium pertechnetate were added. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Samples (20 μL) of BC were precipitated with trichloroacetic acid (TCA) 5% and soluble (SF) and insoluble fractions (IF) were separated. The radioactivity in IF-P and SF-P was determined in a well counter and the % of radioactivity (% ATI) was calculated. A statistical analysis (Tukey-Kramer Multiple Comparisons Test, n=5) was used to compare the results. The values are averages

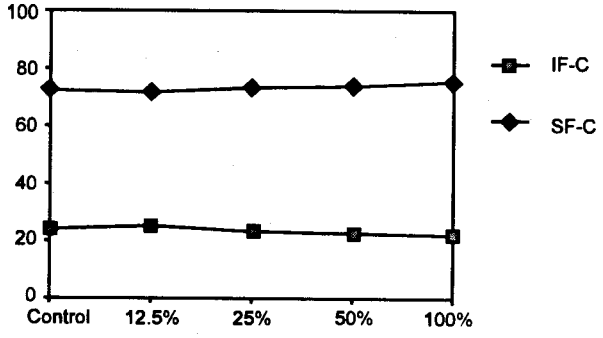

Fig. 3: Effect of *Arnica montana* extract on the labeling on the labeling of IF-C with 99mTc. Samples of heparinized blood from *Wistar* Rats (treated or not with the extract) were incubated for 1 hour with stannous chloride (1.2 μg mL^{-1}) and 99mTc, as sodium pertechnetate were added. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Samples (20 μL) of BC were precipitated with trichloroacetic acid (TCA) 5% and soluble (SF) and insoluble fractions (IF) were separated. The radioactivity in IF-C and SF-C was determined in a well counter and the % of radioactivity (% ATI) was calculated. A statistical analysis (Tukey-Kramer Multiple Comparisons Test, n=5) was used to compare the results. The values are averages.

ingredients and contaminants such heavy metals (Kam and Liew, 2002). Some substances may alter the labeling of blood constituents with 99mTc (Oliveira *et al.*, 2003). Previous studies have demonstrated that natural extracts may alter the labeling of blood elements with 99mTc (Braga *et al.*, 2000). In the labeling process of blood constituents with 99mTc is needed a reducing agent, and probably the stannous ion would be oxidized. In *in vitro* studies was verified that the extracts of *Thuya ocidentallis* (Oliveira *et al.*, 1997), *Nicotiana tabacum* (Vidal *et al.*, 1998), *Maytenus ilicifolia* (Oliveira *et al.*, 2000), *Syzygium jambolanum* (Santos *et al.*, 2002), *Stryphnodendron adstringens (Mart.) Coville* (Costa *et al.*, 2002) and *Ginkgo biloba* (Moreno *et al.*, 2002), possibly, would have oxidants compounds, and the labeling of blood elements decrease in the presence of these extracts. (Oliveira *et al.*, 2002) verified that the *Paullinia cupana* extract was capable of altering the radiolabeling of blood. In other *in vitro* study with *Fucus vesiculosus* extract was noticed that the referred extract has induced alterations on the

labeling of blood elements with 99mTc (Oliveira *et al.*, 2003). Oliveira *et al.* (2003) have related that an extract of *Coffea arabica* has altered the radiolabeling of blood elements with 99mTc. Arnica (*Arnica montana*) works by stimulating the activity of white blood cells that perform much of the digestion of congested blood, and by dispersing trapped, disorganized fluids from bumped and bruised tissue, joints and muscles. The active components in arnica are sesquiterpene lactones, which are known to reduce inflammation and decrease pain. Other active principals are thymol (an essential oil), flavonoids, inulin, carotenoids and tannins (Wolf *et al.*, 2003). In the procedure of labeling RBC with 99mTc, the stannous and pertechnetate ions pass through the plasma membrane (Gutfilen *et al.*, 1992). We can speculate, if the products present in Arnica extract could complex with these ions, this fact could explain the decreasing on the fixation of the radioactivity on IF-P.

In this study it was demonstrated that the extract was capable of altering the radiolabeling of the insoluble fraction of plasma. Similar results were observed with an extract of *Solanun melongena* (eggplant) which was capable of altering radiolabeling (Capriles *et al.*, 2002). Moreno *et al.* (2002), eyed that in a *in vitro* study the extract of *Ginkgo biloba* altered the radiolabeling of blood elements. It was reported by Santos-Filho (2002), that the extracts of *Mentha crispa L.* (mint) were capable of altering the radiolabeling process. Braga *et al.* (2000), in a *in vitro* study demonstrated that *Peumus boldus* did not alter the labeling of blood elements with 99mTc similar results were observed by Santos-Filho *et al.* (2002) with the Kava Kava (*Piper methysticum*) extract in a *in vitro* study. Lima *et al.* (2002) in a *in vivo* study have shown that an extract of cauliflower (leaf) was not capable of altering the labeling of blood elements with 99mTc. Diré *et al.* (2002), in a *in vitro* study eyed that the chayotte extracts were not capable of altering the radiolabeling of blood constituents different from the results obtained in an *in vivo* study with the referred extract once it was observed that the extract of chayotte when ingested by *Wistar* rats has promoted alteration on the efficiency on the radiolabeling of sanguineous elements. Other possibility according to the results obtained may be explained by the capacity of the extract of *A. montana* to generate reactive oxygen species (ROS) as already reported to *Maytenus icilifolia* (Oliveira *et al.*, 2000), *Fucus vesiculosus* (Oliveira *et al.*, 2003) and *Coffea arabica* (Oliveira *et al.*, 2003) extracts which possibly could be inducing alteration on the fixation of 99mTc on the insoluble fraction of plasma. Much discussion has centered on the fact that many reports are individual case studies. In order to make an accurate assessment of the impact of drugs and others factors on cell labeling, additional data are required.

Conclusion

In conclusion our experimental data has shown that the extract of *Arnica montana* was capable of altering the fixation of 99mTc on insoluble fraction of plasma. Generally speaking we may speculate that the referred extract may alter the efficiency of radiolabeling of IF-P due to its capacity (i) to oxidize the stannous ion, (ii) to complex with stannous and pertechnetate ions to form double salts, (iii) to compete by the same binding sites to pertechnetate ion or (iv) by the generations of reactive species of Oxygen with direct action on the labeling process.

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