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Evaluation of the Effect of a Natural Extract of Sacred Bark (*Rhamnus purshina*) on the Radiolabeling of Blood Constituents

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Abstract: Human beings have been widely used natural products as medicines. However, sometimes the biological effects of these products are not fully known. Sacred Bark (*Rhamnus purshina*) has been used as a folk medicine for treating constipation, inflammation, tumors and asthma. It is concerned that many natural remedies may contain potentially toxic ingredients and contaminants such as heavy metals. 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 sacred bark on the labeling of blood elements with 99mTc. In this study it was analyzed the heated and no heated extract of sacred bark. In the preparation of the extracts it was used 200mg of sacred bark diluted in 10mL of saline solution (NaCl 0.9%). Samples (0.5mL) of blood from *Wistar* rats were incubated with 0.1 mL of the extracts during 1 hr. 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 heated extract has reduced the radiolabeling in BC (from 94.76% ± 0.66 to 89.80% ± 4.23). It was described that some extracts as *Fucus vesiculosus*, *Paullinia cupana*, *Mentha crispa L* were able to alter the radiolabeling. In the light of the results obtained we suggest that the referred extract when it is heated may induce the generation of activity metabolites with oxidant properties and reactive species of Oxygen with direct action on the labeling process.

Key words: Sacred Bark, *Rhamnus purshina*, red blood cells, plasma proteins, technetium-99m.

Introduction

Products made through the herbs are widely used as food or food additives, or as a substance in folk medicine as an alternative way of treatment by humans. Aqueous extracts of many plants are widely used in therapy as complementary medicines (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. Biological effects of metals have been reported as the effect of the transition metals which catalyze free radical production that can be related to aging processes and neurodegenarative diseases such as Alzheimer's disease, Parkinson's disease and others (Silva *et al.*, 2002). 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). Technetium-99m (99mTc) has been the most utilized radionuclide in nuclear medicine procedures and it has also been used in basic research. 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 (Hesslewood and Leung, 1994; Sampson, 1996). Cascara (*Rhamnus purshina*) is a small to medium-size tree native to the provinces and states of the Pacific coast, including British Columbia, Washington, Oregon and northern California. The bark of the tree is removed, cut into small pieces and dried for one year before being used medicinally. Fresh cascara bark has an emetic or vomit-inducing property and therefore is not used. Northern California Indians introduced this herb, which they called sacred bark, to 16th century Spanish explorers. As it is much milder in its laxative action than the herb buckthorn, cascara became popular in Europe as a treatment for constipation. Cascara has been an approved treatment for

constipation in the U.S. Pharmacopoeia since 1890 (Castleman, 1991).
R. purshina (Rhamnaceae), has been used as a folk medicine in Taiwan for treating constipation, inflammation, tumors and asthma. 3-O-methylquercetin (3-MQ), a main constituent of the plant, has been reported to inhibit total cAMP- and cGMP-phosphodiesterase (PDE) of guinea pig trachealis (Ko *et al.*, 2003).
There are many applications of 99mTc-labeled red blood cells (99mTc-RBC), in cardiovascular nuclear medicine, in the detection of gastrointestinal bleeding and in the determination of the RBC mass in patients. RBC have been labeled with 99mTc for *in vitro, in vivo* or *in vivo/in vitro* techniques (Srivastava and Straub, 1990; Bernardo-Filho, 1994 and Early and Sodee, 1995). 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 *Rhamnus purshina* extract (heated and no heated) on the labeling of RBC and plasma proteins with 99mTc using an *in vitro* study.

Materials and Methods

In this experimental it was used the extract bought of Herbarium Laboratory [Cascára Sagrada (*Rhamnus purshiana*) which was produced in 02/02 validly to 02/05, lot: 602822/1.1860.0009]. It was used capsules with 200mg of *R. purshiana*. The aqueous extract was prepared by the dilution of 200mg of the extract in 10mL of saline solution (NaCl 0.9%).
We studied the influence of the heated and no heated extracts. To prepare the heated extract the aqueous solution was warmed until the boiled point during 1 minute.
Samples of 0.5 mL of blood withdraw from *Wistar* rats were incubated with 0.1 mL of the extract heated and no heated during 1 hr. Elapsed this period of time it was added 0.5 mL of stannous chloride (1.2 μg/mL), as $SnCl_2.2H_2O$ for 1 hr 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

Fig. 1 has shown the effect of *Rhamnus purshiana* extract on the labeling of blood elements with 99mTc. The analysis of the results revealed that there was a decrease on the labeling of red blood cells (BC) (from 94.76 ± 0.67 to 89.80 ± 4.23) in the treatment with the heated extract.

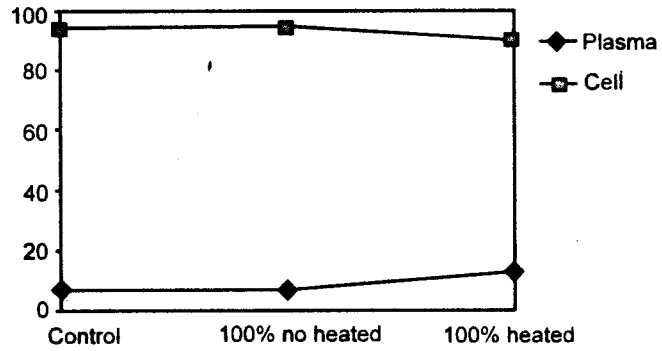

Fig. 1: Effect of *Rhamnus purshiana* extract on the labeling on the labeling of BC with 99mTc

Samples of heparinized blood from *Wistar* Rats (treated or not with the extracts) were incubated for 1 hr with stannous chloride (1.2 μg/mL) 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 has shown the effect of *Rhamnus purshiana* 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 of the insoluble fraction of plasma (IF-P) [(from 77.63 ± 0.75 to 76.43 ± 3.65 (no heated extract) and to 74.32 ± 3.65 (heated extract)] in the presence of the extracts.

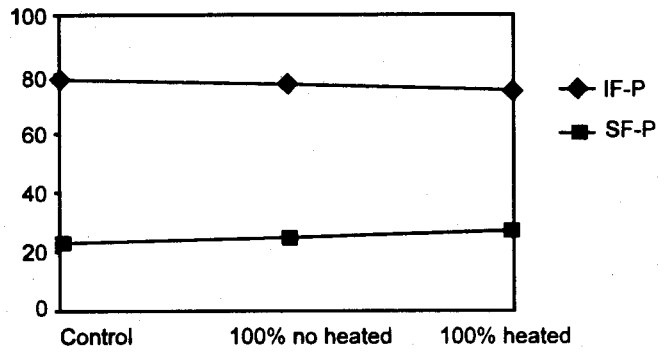

Fig. 2: Effect of *Rhamnus purshiana* extract on the labeling on the labeling of IF-P with 99mTc

Samples of heparinized blood from *Wistar* Rats (treated or not with the extracts) were incubated for 1 hr with stannous chloride (1.2 μg/mL) 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 has shown the effect of *Rhamnus purshiana* 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 of the insoluble fraction of cell (IF-C) [(from 74.55 ± 2.60 to 75. 48 ± 1.61 (no heated extract) and to 77.61 ± 1.06 (heated extract)] in the presence of the extracts.

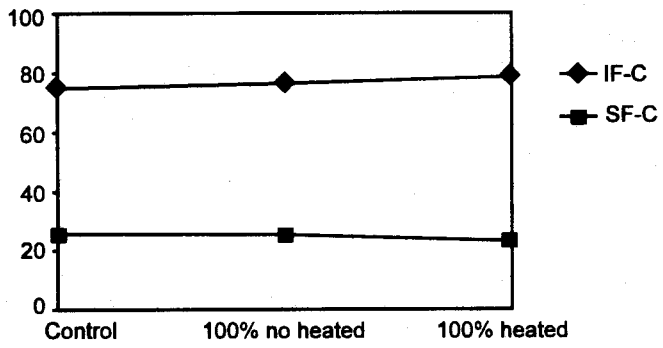

Fig. 3: The effect of *Rhamnus purshiana* extract on the labeling of blood elements with 99mTc.

Samples of heparinized blood from *Wistar* Rats (treated or not with the extracts) were incubated for 1 hr with stannous chloride (1.2 μg/mL) 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.

Discussion

Various 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 Hesslewood 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 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). Diré *et al*, 2001, have related that chayotte extract is capable of altering the biodistribution of sodium pertechnetate. Lima *et al.*, 2001, described that an extract of cauliflower (*Brassica oleracea*) was not capable of altering the biodistribution of the referred radiopharmaceutical. Some authors have related 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). In this study it has demonstrated that the *Rhamnus purshina* extract (heated) was capable of altering the radiolabeling of blood cells. 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 *et al.* (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 technetium-99m. Diré *et al.* (2002), in a *in vitro* study eyed that the chayotte extracts were not capable of altering the radiolabeling of blood constituents. In the procedure of labeling RBC with 99mTc, the stannous and pertechnetate ions pass through the plasma membrane (Gutfilen *et al.*, 1992). Then, as reported to the tobacco (Vidal *et al.*, 1998) *Maytenus ilicifolia* (Oliveira *et al.*, 2000), *Sechium edule* (Diré *et al.*, 2001), *Mentha crispa L.* (Santos-Filho *et al.*, 2002), *Paullinia cupana* (Oliveira *et al.*, 2002), *Gingko biloba* (Moreno *et al.*, 2002) and *Fucus vesiculosus* (Oliveira *et al.*, 2003) extracts, histological alterations of red blood cells could be responsible for the modifications on the labeling of RBC with 99mTc. Although in this study it was not evaluated the effect of the extract on the shape of red blood cells we may suggest that the heated extract possible could be altering the morphology of red blood cell once the efficiency in the IF of P and C has not been altered. Furthermore, we can speculate that if the chemical compounds present in these extracts influenced by the heat could complex with these ions as a chelating agent, this fact could explain the decrease in the fixation of radioactivity on the red blood cells. Diré *et al.* (2001), in a qualitative analysis *in vivo*, have eyed that a chayotte extract (macerated) has induced alteration on the shape of red blood cells. In this study the no heated extract did not alter the radiolabeling of blood elements, in question to this fact, we can suggest that the extract when it is heated is able to destabilize the active of red blood cell membrane as well as it may induce the generation of reactive Oxygen species (ROS) as already reported to other natural product *Maytenus icilifolia* (Oliveira *et al.*, 2000) and *Fucus vesiculosus* (Oliveira *et al.*, 2003) which possibly could be inducing alteration on the uptake of radioactivity by the red blood cells.

Conclusion

We can conclude that, depending on how the extract is incubated (heated or no heated) the labeling of RBC with 99mTc can be altered. We suggest that the studied natural product, when heated, could be capable of generating reactive Oxygen species as well as oxidant compounds which could be probably responsible for the decrease on the radiolabeling of red blood cells.

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