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Artigo

An aqueous extract of *Pfaffia* sp. does not alter the labeling of blood constituents with technetium-99m and the morphology of the red blood cells

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ABSTRACT: Plants have been used for the human beings as food, as additives and/or as medicines. There are controversies about the biological effects of several natural products and, it is worthwhile to try to develop experimental assays to evaluate properties of extracts of plants. *Pfaffia* sp. is utilized in popular medicine and various properties have been attributed to its extract. Red blood cells (RBC) and plasma proteins are labeled with technetium-99m (Tc-99m) and this labeling procedure depends on a reducing agent and stannous ion is usually used. There are reports that drugs can alter the labeling of blood elements with Tc-99m. We have evaluated the influence of a *Pfaffia* sp. extract on the labeling of blood constituents with Tc-99m and on the morphology of RBC. Blood was incubated with an aqueous extract of *Pfaffia* sp., stannous chloride and Tc-99m. Samples were centrifuged and plasma and blood cells were separated and also precipitated with trichloroacetic acid. Soluble and insoluble fractions were separated. The results did not show alteration in the uptake of radioactivity and no modifications on the shape of the RBC in presence of *Pfaffia* sp. Once this labeling process depends on a reducing agent, probably, this extract has compounds with anti-oxidant properties as already described elsewhere, that could protect the stannous ions against the oxidation process. This fact would aid the labeling process of blood elements with Tc-99m.

Keywords: *Pfaffia*, red blood cells, anti-oxidant properties, plasma proteins, technetium-99m.

INTRODUCTION

The use of natural products, as the medicinal plants, has increased in all over the world. Medicinal plants are used for the human being however several biological effects and the consequences for the health have not been well established yet. Many plants contain active substances that can induce biological effects and their frequent use has been correlated with a high incidence of diseases or undesired biological effect in the population (Nguyen et al., 1989; Subiza et al., 1991; Fonseca et al., 1994). However, various active compounds derived from medicinal plants have been assessed for their efficacy and tolerability in the treatment of breast cancer and have been evaluated in clinical trials (Mantle et al., 2000). Protective effect on radiation-induced deoxyribonucleic acid (DNA) double strand breaks and anti-oxidant activities have also attributed to the medicinal plants (Kim et al., 1996; Keum et al., 2000).

In nuclear medicine, examinations using radiopharmaceuticals provide a different kind of information that is supplied by other medical procedure, as X-ray radiography or sectional imaging techniques (computer tomography and magnetic resonance imaging). For diagnostic purposes, radionuclides that emit gamma radiation, such as technetium-99m (99mTc) are widely used. 99mTc has been the most utilized radionuclide in diagnosis nuclear medicine procedures to label compounds and cellular structures used as radiopharmaceuticals (Hladik III et al., 1987; Early & Sodee, 1995; Saha, 1998; Nigri et al., 2004). It has also been used to label biological structures (Plotkowski et al., 1993; Bernardo-Filho et al., 1992; Bernardo-Filho et al., 1993; Marques et al., 2004) in basic scientific research. This wide use of this radionuclide is due to its optimal physical characteristics, availability from 99Mo/99mTc generator and negligible environmental impact (Hladik III et al., 1987; Early; Sodee, 1995; Saha, 1998).

There are many applications of 99mTc-labeled red blood cells (RBC) (Hladik III et al., 1987; Early;

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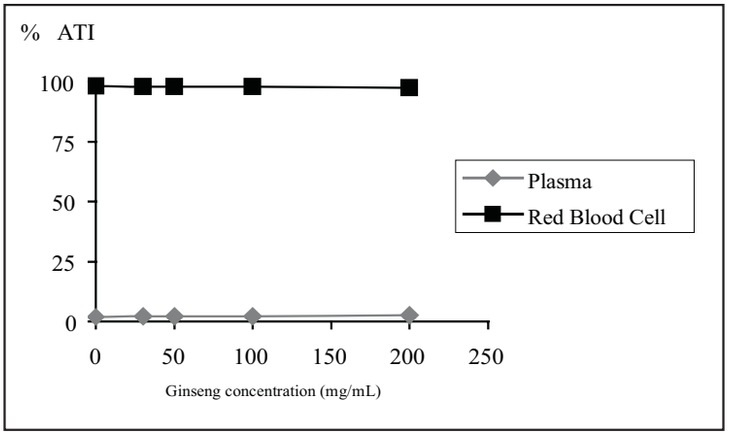

Figure 1. Effect of *Pfaffia* sp. extract on the labeling of red blood cells and plasma with 99mTc.
Samples of heparinized blood were incubated with different concentrations of *Pfaffia* sp. extract (20.0; 30.0; 50.0; 100.0 and 200.0 mg/mL). A sample of whole blood was incubated with saline solution (NaCl 0.9%) as control. Then, stannous chloride (1.2 μg/mL) and 99mTc, as sodium pertechnetate were added. The radioactivity in plasma and red blood cells was determined in a well counter and the % of radioactivity (%ATI) was calculated. A statistical analysis (ANOVA test, n= 10) was used to compare the values found.

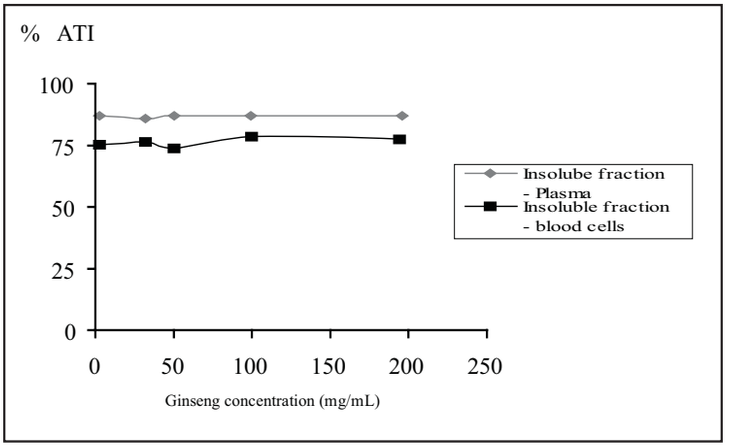

Figure 2. Effect of *Pfaffia* sp. extract on the labeling of the insoluble fractions from red blood cells and plasma with 99mTc.
Samples of heparinized blood were incubated with different concentrations of *Pfaffia* sp extract (20.0; 30.0; 50.0; 100.0 and 200.0 mg/mL). A sample of whole blood was incubated with saline solution (NaCl 0.9%) as control. Then, stannous chloride (1.2 μg/mL) and 99mTc, as sodium pertechnetate were added. These samples were centrifuged and plasma and blood cells were separated. Samples (20 μL) of plasma and red blood cells were precipitated with trichloroacetic acid 5% and soluble and insoluble fractions were separated. The radioactivity in soluble and insoluble fractions of plasma was determined in a well counter and the % of radioactivity was calculated. A statistical analysis (ANOVA test, n= 10) was used to compare the values found.

Sodee, 1995; Saha, 1998; Schillaci et al., 2004). RBC had been labeled with this radionuclide for *in vitro*, *in vivo* or *in vivo*/*in vitro* techniques. This labeling depends on a reducing agent and stannous ion (Sn^{2+}) is usually used. When whole blood is used in the labeling of RBC with 99mTc, radioactivity is mainly found inside of RBC (beta-chain of the hemoglobin). However, it is also bound outside of the red blood cells on plasma proteins. This labeling process depends on optimal stannous chloride concentration, and stannous and pertechnetate ions across the erythrocyte membrane. The band-3 anion transport system and calcium channels may be the ways that 99mTc and Sn^{2+} ions have, respectively, to reach the interior of the RBC (Dewanjee, 1994; Callahan; Rabito, 1990; Bernardo-Filho et al., 1992; Gutfilen et al., 1992).

Many drugs (patient medications) have been reported to affect the labeling of blood constituents with 99mTc (Hladik III et al., 1987; Hesslewood; Leung, 1994; Nigri et al., 2002; Frydman et al., 2004), or the labeling conditions (Srivastava; Straub, 1992; Bernardo-Filho et al., 1994; Sampson, 1996) or the presence of extracts of medicinal plants (Oliveira et al., 1997; Vidal et al., 1998; Oliveira et al., 2000; Oliveira et al., 2003a). Therapy with β-adrenergic blockers, calcium channel blockers or nitrate may result in normal exercise radionuclide ventrilograms even in the presence of significant coronary artery disease. Thus, the presence of the disease may be missed and/or understimated (Hesslewood; Leung, 1994).

Pfaffia sp. is a large, scrambling, shrubby ground vine which has an intricate and deep root system. It is indigenous from the Amazon basin area and other tropical parts of Brazil, Ecuador, Panama, Paraguay, Peru and Venezuela. Since its first botanical recording in 1826, it has been referred to by several botanical names including *Pfaffia paniculata*, *Hebanthe paniculata* and *Gomphrena paniculata*. The genus *Pfaffia* sp. is well known in Central and South America with over 50 species of *Pfaffia* sp. growing in the warmer tropical regions of the area. The common names *of Pfaffia* sp. are Suma, Ginseng, Pfaffia, Para Toda and Corango (Gemtchújnicov, 1976; Joly, 1987).

In herbal medicine, many important properties and actions have been attributed to *Pfaffia* sp., as anabolic, analgesic, anti-inflammatory, antimutagenic, aphrodisiac, estrogenic, hypocholesterolemic, immunostimulant, nutritive, sedative, steroidal and tonic. Moreover, in Ecuador, it is considered a tonic for the cardiovascular system, the central nervous system, the reproductive system, and the digestive system and is used to treat hormonal disorders, sexual dysfunction and sterility, arteriosclerosis, diabetes, circulatory and digestive disorders, rheumatism, and bronchitis. In European herbal medicine it is used as to restore nerve and glandular functions, to balance the endocrine system, to strengthen the immune system, for infertility, menopausal and menstrual symptoms, for high cholesterol, to neutralize toxins and as a general restorative tonic after illness. In North and South American herbal medicine Suma root is used as an adaptogenic and regenerative tonic regulating many systems of the body, as an immunostimulant, and it is also used to treat exhaustion resulting from Epstein-Barr disease and Chronic Fatigue Syndrome, hypoglycemia, arthritis, anemia, tumors, high blood pressure and many types of stress (Gemtchújnicov, 1976; Joly, 1987; Mantle et al., 2000).

In spite of these applications of *Pfaffia* sp., undesired effect of this medicinal plant has also been reported. Subiza et al. (1991) described that a patient

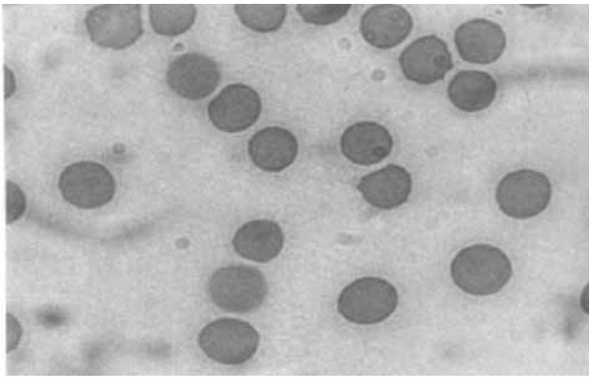

Figure 3. Photomicrography of blood smears prepared with samples of whole blood used to label RBC with 99mTc (control).
Samples of whole blood were incubated with NaCl 0.9% solution for 60 min. After that, stannous chloride solution was added and the incubation continued for 1 hour. Then, 99mTc, as sodium pertechnetate was added. Blood smears were prepared, dried, fixed and staining. After that, the morphology of the red blood cells was evaluated under optical microscope (x1000).

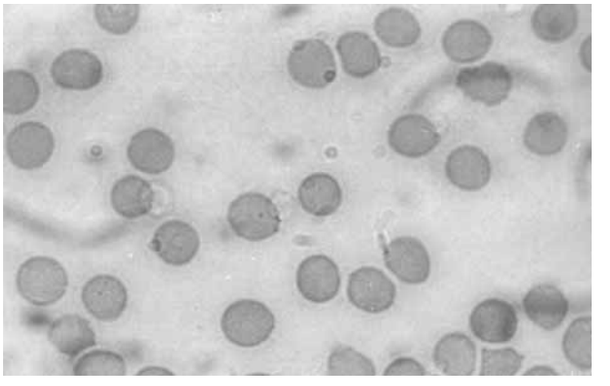

Figure 4. Photomicrography of blood smears prepared with samples of whole blood used to label RBC with 99mTc (blood samples were previously treated *Pfaffia* sp. extract 30 mg/mL).
Samples of whole blood were incubated with *Pfaffia* sp. extract (30 mg/mL) for 1 hour. After that, stannous chloride solution was added and the incubation continued for 1 hour. Then, 99mTc, as sodium pertechnetate was added. Blood smears were prepared, dried, fixed and staining. After that, the morphology of the red blood cells was evaluated under optical microscope (x1000).

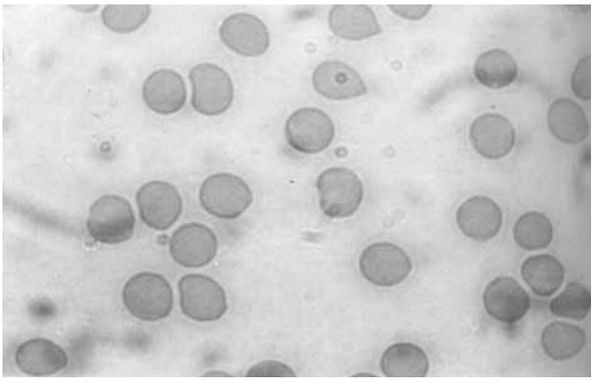

Figure 5. Photomicrography of blood smears prepared with samples of whole blood used to label RBC with 99mTc (blood samples were previously treated *Pfaffia* sp. extract 200 mg/mL).
Samples of whole blood were incubated with *Pfaffia* sp extract (200 mg/mL) for 1 hour. After that, stannous chloride solution was added and the incubation continued for 1 hour. Then, 99mTc, as sodium pertechnetate was added. Blood smears were prepared, dried, fixed and staining. After that, the morphology of the red blood cells was evaluated under optical microscope (x1000).

has developed symptoms of asthma after exposure to *Pfaffia paniculata* root powder used in the manufacturing of Brazil ginseng capsules. Airway hyperreactivity was confirmed by a positive bronchial challenge to methacholine. Sensitivity to this dust was confirmed by immediate skin test reactivity and a positive bronchial challenge (immediate response). The same study performed with Korean ginseng (*Panax ginseng*) elicited negative results.

The labeling of blood constituents with 99mTc has also used successfully to study the effect of extract of medicinal plants and we are trying to combine these results to improve an experimental model to evaluate properties of the plants used as food, additives or medicines. Authors have reported that tobacco (Vidal et al., 1998), *Maytenus ilicifolia* (Oliveira et al., 2000), *Fucus vesiculosus* (Oliveira et al., 2003a), *Coffea arabica* (Oliveira et al., 2003b), phytic acid (Lima-Filho et al., 2003), *Mentha crispa* (Santos-Filho et al., 2004) and *Gingko biloba* (Moreno et al., 2004) decrease the referred labeled process. *Peumus boldus* (Reineger et al., 1999) and *Sechium edule* do not decrease this labeling process (Diré et al., 2004). In this work, we have evaluated the influence of a *Pfaffia* sp. extract on the labeling of RBC and plasma proteins with 99mTc using an *in vitro* (Bernardo-Filho et al., 1983; Bernardo-Filho et al., 1990; Oliveira et al., 2000) technique.

MATERIAL AND METHODS

An *in vitro* technique used to label blood constituents (Bernardo-Filho et al., 1983; Oliveira et al., 2000) is described elsewhere and in these experiments was slightly modified. These experiments were performed without sacrificing the animals. Heparinized whole blood was withdrawn from *Wistar* rats. Samples (0.5 mL) were incubated with different concentrations of a commercial *Pfaffia* sp. (Herbarium, Laboratório Botânico, LTDA, Brazil) preparation (20, 30, 50, 100 and 200 mg/mL) (100 μL) for 1 hour at room temperature. A sample of heparinized whole blood was incubated with saline solution (NaCl 0.9%) as control. Then, 0.5 mL of stannous chloride solution (1.2 μg/mL) was added and the incubation continued for another 1 hour. After this period of time, 99mTc (0.1 mL), as sodium pertechnetate, recently milked from a 99Molibdenium/99mTechnetium generator (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, Brasil), was added and the incubation continued for another 10 min. These samples were then centrifuged and plasma (P) and blood cells (BC) were separated. Samples (20 μL) of P and BC were also 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 (Automatic Gamma Counter, C5002, Packard, Canada). After that, the % of radioactivity (% ATI) was calculated, as previously described. A statistical analysis (ANOVA test, with significance level p=0.05, n=10) was utilized to compare the results obtained.

Histological preparations were carried out with blood samples treated with the *Pfaffia* sp. preparation for 60 min (room temperature). Blood smears were prepared, dried, fixed and staining. After that, the morphology of the red blood cells was evaluated under optical microscope.

RESULTS

Figure 1 shows the distribution of the radioactivity in plasma and red blood cells from blood treated with different concentrations of *Pfaffia* sp. solutions. Furthermore, the radioactivity is mainly found in the cells. The analysis of the results indicates that there is no significant decrease in the uptake of 99mTc by the red blood cells, independent on the concentration of the extract.

Figure 2 shows the fixation of the radioactivity in the insoluble fraction of plasma and red blood cells obtained from whole blood treated with different concentrations of *Pfaffia* sp. Moreover, the radioactivity is mainly found in the insoluble fractions of the plasma and cells. The analysis of the results indicates that there is no significant decrease in the fixation of 99mTc in plasma proteins independent on the concentration of the medicinal plant.

The qualitative comparison of the shape of the RBC (no treated and treated with the natural extracts) under optical microscopy has revealed no important morphological alterations due to the treatment of blood with *Pfaffia* sp extract in all the concentrations. In figure

3 is shown the histological preparation of a sample of blood (control- no treated) and in figure 4 and 5 are shown the histological preparations of blood treated with *Pfaffia* sp. extract in the concentrations of 30 and 200 mg/mL, respectively.

DISCUSSION

As there is not a well-established and general model to study the interaction of xenobiotic drugs (natural or synthetic) with radiopharmaceuticals, we are trying to develop a model to evaluate these phenomena. We are using two models, one based in the effect of drugs on the biodistribution of radiopharmaceuticals (Mattos et al., 1999) and other, concerning to the influence of drugs, mainly extracts of medicinal plants on the labeling of the blood constituents (Reineger et al., 1999; Oliveira et al., 2000, Santos-Filho et al., 2004). Here, we have studied the effect of the *Pfaffia* sp. extract on the labeling of red blood cells with 99mTc and in the fixation of this radionuclide to insoluble fractions of plasma (plasma proteins) and blood cells (blood cells proteins).

Although *Pfaffia* sp extracts are used in popular medicine, undesirable biological effects have also been attributed to the *Pfaffia* sp. (Subiza et al., 1991). Watanabe et al. (2000) have reported that *Pfaffia paniculata* (Brazilian ginseng) administered subcutaneously and intraperitoneally inhibits growth of allogeneic cancer cells in mice and in female AKR/J mice, oral doses of powdered roots from *P. paniculata* three times a week for 8 weeks; suppressed the enlargement of thymic lymphoma.

Kim et al. (1996) have examined the effect of ginseng on the induction and repair of gamma-ray-induced DNA double strand breaks (dsb) using neutral filter elution technique at pH 9.6 in cultured murine spleen lymphocytes and they have shown that ginseng water extract presented a protective effect against the formation of dsb when it was used for 48 hours before 100 Gy gamma-ray-irradiation. Probably this protective effect of ginseng could be explained through a scavenger action of this medicinal plant against the reactive oxygen species (ROS) generated by the gamma rays. Keum et al. (2000) have also shown an antioxidant and anti-tumor promoting activities of the methanol extract of heat-processed ginseng.

Biological effects of many medicinal plants have been attributed to the generation of ROS (Fonseca et al., 1994; Oliveira et al., 2000). Some of these effects have been abolished by the treatment of the biological system with ROS scavenger, as catalase, thiourea and superoxide dismutase, as it is the case with the genotoxic effect of guarana (Fonseca et al., 1994).

Our results show no alteration in the uptake of radioactivity for all the studied fractions isolated from the whole blood treated with *Pfaffia* sp., independently on the concentration of the extract of this medicinal plant. *Pfaffia* sp. extracts were not also capable to promote qualitative modifications on the shape of the RBC. Once this labeling process, depend on a reducing agent, probably the extract of *Pfaffia* sp. has compounds with anti-oxidant properties as already described for another authors (Kim et al., 1996; Keum et al., 2000), that could protect the stannous ions against the oxidation process. This fact would aid the labeling process of blood elements with Tc-99m.

It is always important have in mind that care must be taken when attempting to extrapolate experimental data to the clinical situation, once the observed effects may depend on the amount and/or nature of the drug. Our results permit to conclude that, the labeling of RBC with 99mTc can not be altered in presence of an extract of *Pfaffia* sp. Similarly, the fixation of radioactivity in the insoluble fraction of the RBC and PP is not modified, at least, when an *in vitro* technique to label RBC is used. Moreover, it is possible to speculate that the chemical compounds of the extract of *Pfaffia* sp could be used in the nuclear medicine as protecting agents against the stannous ion oxidations. Experiments are ongoing to try to identify the action mechanism of this extract of *Pfaffia* sp.

In conclusion, as reported to the tobacco extract (Vidal et al., 1998), *Maytenus ilicifolia* (Oliveira et al., 2000), *Fucus vesiculosus* (Oliveira et al., 2003a), *Coffea arabica* (Oliveira et al., 2003b), *Mentha crispa* (Santos-Filho et al., 2004) and *Gingko biloba* (Moreno et al., 2004) when the histological alterations of the red blood cells could be the responsible by the modifications on the labeling of the RBC with 99mTc, the results obtained with the qualitative comparison of the shape of the RBC (no treated and treated with natural extracts) under optical microscopy could justify the no modifications in the uptake of 99mTc by the red blood cells in presence of *Pfaffia* sp. extract.

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