Assessment of the Biological Effects of a Natural Extract of *Equinacea Purpurea*: An *In Vitro* Analysis

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Abstract: Medicinal plants have been widely used by human beings. However, sometimes the biological effects of these plants are not fully known. It is concerned that many natural medicines 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. *Equinacea purpurea* is a plant with medicinal properties. It is indicated to treatment of the inflammation in the respiratory system and in the skin. The aim of this work was to assess the effects of *Echinacea purpurea* on the labeling of blood elements with 99mTc. A freshly extract of *E. purpurea* (300 mg/10 mL) was administered to the aliquots of blood withdraw from *Wistar* rats during 1 hour. After that, samples (0.5 mL) of blood 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 referred extract was able to reduce the radiolabeling in BC to the concentrations of: 25% (from 93.09%±3.63 to 55.17%±7.85), 12.5% (from 93.09%±3.63 to 43.22%±3.92) and to the 6,25% (from 93.09%±3.63 to 35.15%±2.36). In the light of the results the referred extract has reduced the efficiency of.radiolabeling in the blood cells. We suggest that the extract may induce the generation of reactive oxygen species with oxidant properties with direct action on the labeling process. [Nature and Science, 2004,2(1):1-5].

Key words*:* *Echinacea purpurea*, red blood cells, plasma proteins, technetium-99m

1 Introduction

Natural products are widely used as food or food additives, or as a substance in medicinal treatment for humans. Medicinal plants are widely used worldwide for the treatment of many diseases. Aqueous extracts of many plants are widely used in therapy as complementary medicines (Oliveira, 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 neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and others (Silva, 2002). The toxicity of these contaminants and additives, and the toxic effects of the herbal ingredients have important implications during the preoperative period. The anesthetist must consider the potential for drug interactions and systemic adverse effects of these natural products (Kam, 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, 1995; Braga, 2000). Natural and synthetic drugs can alter the labeling of red blood cells with technetium-99m (99mTc) (Braga, 2000; Oliveira, 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, 1994; Sampson, 1996).

Preparations from *Echinacea purpurea* are among the most widely used herbal medicines. Most uses of *E. purpurea* are based on the reported immunological properties. A series of experiments have demonstrated that *E. purpurea* extracts do indeed demonstrate significant immunomodulatory activities. Among the many pharmacological properties reported, macrophage activation has been demonstrated most convincingly. Phagocytotic indices and macrophage-derived cytokine concentrations have been shown to be Echinacea-responsive in a variety of assays. Activation of polymorphonuclear leukocytes and natural killer cells has also been reasonably demonstrated. Changes in the numbers and activities of T- and B-cell leukocytes have been reported, but are less certain. Despite this cellular evidence of immunostimulation, pathways leading to enhanced resistance to infectious disease have not been described adequately. Several dozen human experiments including a number of blind randomized trials have reported health benefits. The most robust data come from trials testing *E. purpurea* extracts in the treatment for acute upper respiratory infection. Although suggestive of modest benefit, these trials are limited both in size and in methodological quality. Hence, while there is a great deal of moderately good-quality scientific data regarding *E. purpurea,* effectiveness in treating illness or in enhancing human health has not yet been proven beyond a reasonable doubt (Barret, 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 (Srivastva, 1990; Bernardo-Filho, 1994; Early, 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 *E. purpurea* extract on the labeling of RBC and plasma proteins with 99mTc using *in vivo* and *in vitro* studies and the effect of this extract on the labeling of blood elements with 99mTc.

2 Material and Methods

To prepare the extract it was used 360 mg of *E. purpurea* dilute in 10 mL of saline solution 0.9%. It was used the natural product from Herbarium botanical laboratory (Brazil, Rio de Janeiro, Lot10932-01/01). The solution of the referred extract was centrifuged during 5 min (1,500 rpm) and after that the aqueous phase was separated and dilutions of 50% were performed to obtain five concentrations (100%; 50%; 25%; 12.5% and 6.25), which were used in this experimental.

Samples of 0.5 mL of blood from *Wistar* rats were incubated with 0.1 mL of the referred extract, after that these samples were incubated with 0.5 mL of stannous chloride (1.2 μg/mL), as $SnCl_2.2H_2O$ for 1 hour 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 fraction (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, 1994). A statistical analysis (Mann Whitneyt test, n=5) was utilized to compare the experimental data.

3 Results

Table 1 has shown the fixation of the radioactivity on blood elements isolated from samples of whole blood treated with *E. purpurea* extract. The analysis of the results indicates that there is a decrease (P<0.05) on the labeling of red blood cells. Samples of heparinized blood from Wistar Rats were incubated during 1 hour with the extract of *E. purpurea*, after that these samples were incubated for 1 hour 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, BC, SF-BC , IF-BC, SF-P and IF-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 ± SDs.

Table 1 Effect of *E. purpurea* 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 99mTc.

Echinacea purpurea	BC	IF-C	IF-P
Control	93.09 ± 3.63	75.88 ± 1.81	69.33 ± 7.46
100%	91.81 ± 2.46	78.80 ± 2.42	71.63 ± 5.89
50 %	85.94 ± 7.51	77.73 ± 2.85	73.03 ± 4.48
25%	55.17 ± 7.85	77.15 ± 8.56	74.09 ± 4.03
12.5%	43.23 ± 3.92	61.97 ± 2.17	77.36 ± 2.85
6.25%	35.15 ± 2.36	74. 76 ± 1.59	72.58 ± 5.74

4 Discussion

Extracts of medicinal can also alter the labeling of blood elements with 99mTc. We agree with Hesslewood & 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, 2002). Some substances may alter the labeling of blood constituents with 99mTc (Oliveira, 2003). In this study it was verified that in the samples of *Echinacea purpurea* extract on the radiolabeling of blood elements. 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, 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, 1997), *Nicotiana tabacum* (Vidal, 1998), *Maytenus ilicifolia* (Oliveira, 2000), *Syzygium jambolanum* (Santos, 2002), *Stryphnodendron adstringens (Mart.) Coville* (Costa, 2002) and *Ginkgo biloba* (Moreno, 2002*),* possibly, would have oxidants compounds, and the labeling of blood elements decrease in the presence of these extracts. In a research was verified that *Paullinia cupana* extract was capable of altering the radiolabeling of blood (Oliveira, 2002). 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, 2003). In an *in vivo* studies in this study it has demonstrated that the chayotte extracts were capable of altering the radiolabeling of blood elements. Similar results were observed with an extract of *Solanun melongena* (eggplant), which was capable of altering radiolabeling (Capriles, 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 an *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 an *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 an *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, 1992). Then, as reported to the tobacco (Vidal, 1998) *Maytenus ilicifolia* (Oliveira, 2000), *Sechium edule* (Diré, 2001), *Mentha crispa L*. (Santos-Filho, 2002), *Paullinia cupana* (Oliveira, 2002), *Gingko biloba* (Moreno, 2002) and *Fucus vesiculosus* (Oliveira,

2003) extracts, histological alterations of red blood cells could be responsible for the modifications on the labeling of RBC with 99mTc. In this study, we observed that the extract of *E. purpurea* has been capable of altering the labeling of red blood cells to the concentrations of 25%; 12.5% and 6.25%, this results may be due to the fact that in these concentrations, the active principles may be capable of interfering strongly in the homeostasis of cell membrane. Like described by Oliveira *et al*., 2003 to the study of *F. vesiculosus*, the extract of *E. purpurea* could be acting in the oxireduction system or in the transport of ions through the membrane decreasing the radiolabeling process in the cells. Furthermore, we can speculate that if the chemical compounds present in these extracts could complex with these ions as a chelating agent, this fact could explain the decrease in the fixation of radioactivity on the blood elements. 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 together with alteration on the radiolabeling process. In this *in vitro* study although the morphology of the cells has not been analyzed similar to the studies which have focused the stabilizing of red blood cell membrane as well as the inducing of the generation of reactive oxygen species (ROS) as already reported to other natural product such as the *Maytenus icilifolia* (Oliveira, 2000) and *Fucus vesiculosus* (Oliveira, 2003) extracts we may suggest that a similar pattern could be observed to the effects of *E. purpurea* extract.

5 Conclusion

We may suggest that the *Echinacea purpurea* extract could be capable of generating of the reactive oxygen species with oxidant properties that could probably be responsible for the decreasing of the efficiency of radiolabeling of the blood cells.

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