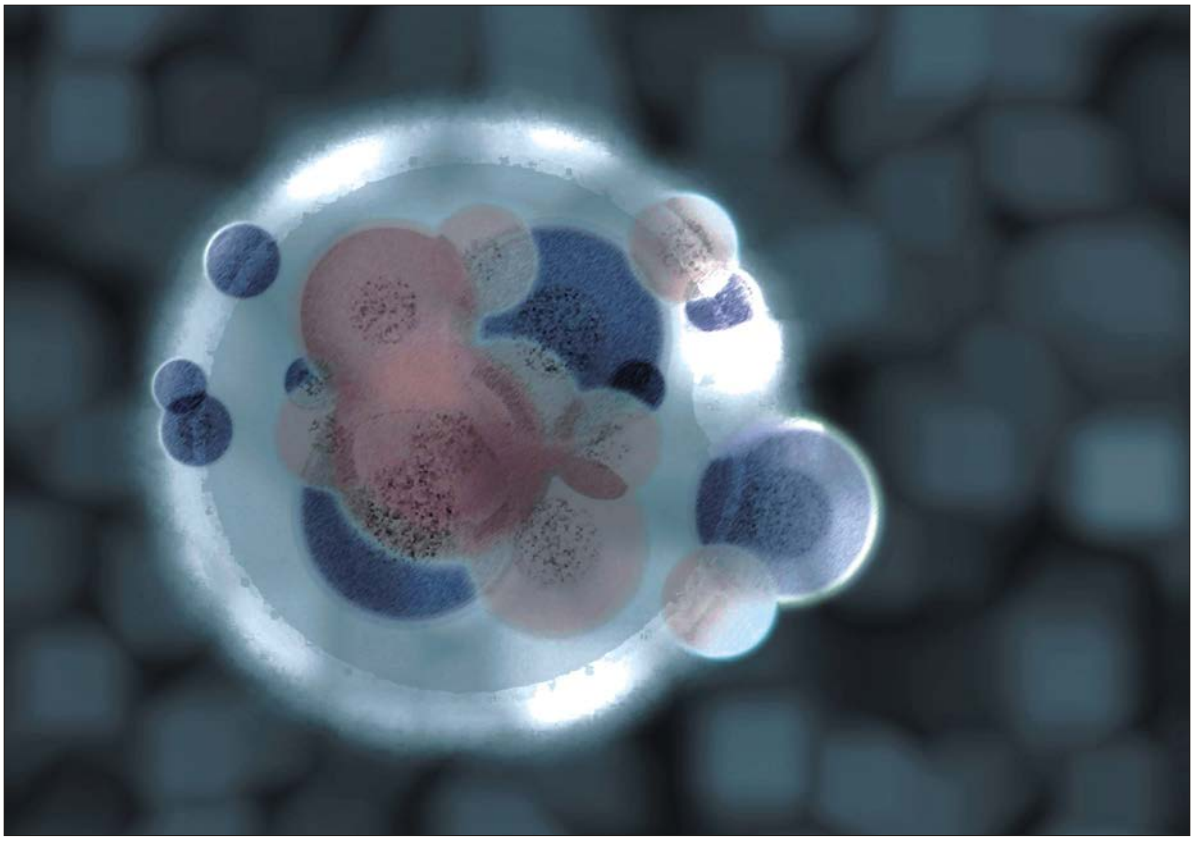

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Effect of *Mentha crispa* (mint) Extract on the Labeling of Blood Elements with Technetium-99m: A Possible Evaluation of Free Radicals

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Abstract: The present study was carried out to investigate the possibility of *Mentha crispa* (Mint; *M. crispa*) extract being capable to alter the labeling of blood elements with Technetium-99m (99mTc). Blood was incubated with *M. crispa* extract. Stannous chloride solution and 99mTc, as sodium pertechnetate, were added. Blood was centrifuged and plasma (P) and blood cells (BC) were isolated. Samples of P and BC were also precipitated, centrifuged and insoluble (IF) and soluble (SF) separated. The percentage of radioactivity (%ATI) in BC, IF-P and IF-BC was calculated. Histological evaluations were performed and the morphology of the red blood cells was observed under optical microscopy showing important morphological alterations on the shape of the RBC treated with *M. crispa* extract. The %ATI decreased: on BC from 97.3±1.92 to 60.0±2.44; on IF-P from 74.8±3.78 to 9.99±3.61; on IF-BC from 88.6±5.41 to 58.4±11.55. The substances of the *M. crispa* extract could increase the valence of the tin ion from stannous (+2) to stannic (+4). This fact would decrease the %ATI on blood elements and would indicate the presence of oxidant agents in the *M. crispa* extract with possible generation of free radicals.

Key words: *Mentha crispa,* oxidant agent, reducing agent, technetium-99m, blood elements, free radicals, microscopy, stannous chloride, labeling

INTRODUCTION

The determination of the redox properties of the usual and xenobiotic agents is very worthwhile for the biological systems. We are trying to develop a model to evaluate the action and/or production of free radicals and/or the redoxi capability of crude extracts of medicinal plants. In this model it is used a reducing agent and the labeling of blood elements with technetium-99m (99mTc) is determined.

The use of natural products, as medicinal plants, is very frequent in the world. *Mentha crispa* (mint; *M. crispa*) is utilized in herbal medicine. Mint is part of a genus of the *Labiatae* family, which comprises a wide number of species, varieties and hybrids. They are herbaceous; rhizome plants (underground stems) that emit quadrangular green or purple stalks. Their leaves are oval, with serrate edges, rich in aromatic oil-bags. This plant is originally from Europe[1,2]. Mint flowers form a spike shape and leaves are used for tea infusions, which have digestive, calming, tonic, antiseptic and anti-asthmatic properties. They are used against biliary disorders, jaundice, constipation and catarrh[2]. The refreshing summery flavor and the cool tasting leaves make for a delicious tea, jelly or garnish for meats and deserts. Taste is at first warm, aromatic, bitter and the after taste provides a cooling feel. Its oil scents of citronella and is used in aromatic soaps, perfumery, detergents, repellants and pesticides for various insects and as a strewing herb. Infusions are used as medicine against spasms, crams, or colds. They are also used in treating fainting, flatulence, gall ailments, gout, hepatitis and nervous disorders[3].

Chemical and physical agents can interact with the medium and energy may be directly transferred to DNA, modifying its structure, or to an intermediate molecule, like water, with the generation of free radicals (FR), such as, hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\bullet -}$). Humans are exposed to these

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chemical and physicals agents that can be carcinogens, but the most significant may be the reactive species derived from metabolism of oxygen and nitrogen[4]. The role of FR in the biological systems is a subject of intense study motivated, in part, by the growth of the number of associations between pathological conditions and changes in the oxidative balance, in the redox status and in the oxidative injury. H_2O_2 and $O_2^{\bullet-}$ toxicity is thought to result from the conversion of these FR in presence of transition metal ions into $OH^{\bullet}$ (the Fenton-like reactions and Haber-Weiss cycle) which reacts with components of the cell. Metals, such as, Fe^{+2}, Sn^{+2} and Cu^{+1}, act as reducing agents in the formation of FR[5]. DNA is a sensible target to the FR. When these DNA damages are not repaired, proteins and other macromolecules accumulate with age and this has been postulated to be a major, but not singular, type of endogenous damage leading to aging, infection, apoptosis, carcinogenesis, mutagenesis and teratogenesis[5-10]. Studies on the biological effects of $SnCl_2$ revealed that it can generate FR[11], induce lethality in *Escherichia coli*[12] and promote damages in plasmid deoxyribonucleic (DNA)[13]. The effect of stannous ion has been abolished or reduced by extracts of some medicinal plants[14,15].

Blood elements labeled with technetium-99m (99mTc) are used in nuclear medicine procedures. Red blood cells labeled with 99mTc (99mTc-RBC) can be prepared with *in vivo*, *in vitro* or in a combination of these two techniques[16-21]. This procedure depends on the present of a reducing agent and tin is widely utilized, mainly as stannous chloride. The *in vitro* technique is easily carried out and produces a better and well controlled product[19-21].

The influence of drugs on the labeling of red blood cells and plasma proteins with 99mTc has been reported[16,22-25] and also that of the labeling conditions[16-19,21]. Thus, the presence of the disease may be missed and/or underestimated[16,21,22].

In the model that we are trying to develop to assess redox properties and the possible generation of free radicals for the crude extracts of medicinal plants, we are using an *in vitro* technique to label RBC and plasma proteins with 99mTc. In addition, this finding could also be important to the nuclear medicine evaluations. In this study, we have investigated the possibility of mint extract being capable to alter the labeling of blood elements with 99mTc.

MATERIALS AND METHODS

The labeling of RBC with 99mTc was in accordance with some work previously described by us[26,14,12,25,29]. A crude extract with 4 g of crushed leaves of *Mentha crispa* (Herbarium, Brazil) was prepared with 100 mL of 0.9% NaCl. The preparation was centrifuged (clinical centrifuge, 3000 rpm, 10 min) and filtered by paper filter and a solution 40 mg mL^{-1} (100% extract) was obtained. Saline (0.9% NaCl) was used in all tested dilutions. Heparinized whole blood was withdrawn by cardiac puncture from *Wistar* rats. Samples of 0.5 mL were gently mixed and incubated with 100 μL of 6.25, 12.5, 25, 50 and 100% solutions of the *Mentha crispa* extract for 60 min. After that, 500 μL of a stannous chloride solution of 1.2 μg mL^{-1} were added and the incubation continued for 60 min. We have prepared the solution of stannous chloride just in the moment of the experiment, because it is easily oxidized. Then, 100 μL of 99mTc (3.7 MBq), as sodium pertechnetate, recently milked from a 99 Molybdenum/99mTechnetium generator (IPEN, National Commission of Nuclear Energy, SP, Brazil) were added and the incubation was continued for another 10 min. This generator is used in the routine of the Nuclear Medicine Department of the Pedro Ernesto University Hospital. These samples were centrifuged in a clinical centrifuge and plasma (P) and blood cells (BC) were isolated. Samples of 20 μL of P and BC were also precipitated with 1.0 mL of trichloroacetic acid (TCA) 5%, centrifuged and insoluble (IF) and soluble (SF) separated. The percentage of radioactivity (%ATI) in BC, IF-P and IF-BC was calculated in a well counter (gamma counter).

The %ATI was determined, as previously reported[7,17]. The experiments were repeated five times and the means and standard deviations (SD) were determined. The experiments were performed with various batches of the dried leaves. Statistical analysis was performed (Variance analysis, $P<0.05$).

Histological evaluations were performed with blood samples treated with various concentrations of *Mentha crispa* for 60 min at room temperature. Blood smears were prepared, dried, fixed and staining. After that, the morphology of the red blood cells was observed under optical microscope (Eclipse E400, 100x).

RESULTS

Table 1 shows the distribution of the radioactivity in blood cells (BC) and plasma (P) from blood treated with different concentrations of *Mentha crispa* extract. The analysis of the results indicates that there is a significant decrease ($p<0.05$) in radioactivity uptake by BC in presence of *Mentha crispa* extract from 97.3±1.92 to 60.0±2.44.

The analysis of the results indicates that there is a significant ($P<0.05$) alteration in the radioactivity fixation in IF-BC (from 88.6±5.41 to 58.4±11.55) and IF-P (from 74.8±3.78 to 9.99±3.61) with the tested *Mentha crispa* concentrations.

Table 1: Distribution of the radioactivity in blood cells and plasma from blood treated with different concentrations of *Mentha crispa* L. solution

Concentrations of *Mentha crispa* Solution (%)	BC	P
Control	97.36±1.92	2.63±1.92
100	60.02±2.44	39.97±2.44
50	66.21±8.90	34.09±8.63
25	78.42±12.60	21.57±12.60
12.5	81.12±13.16	18.87±13.16
6.25	91.53± 8.53	8.65±8.56

Samples of heparinized blood, obtained by cardiac puncture of *Wistar* rats, were incubated with *Mentha crispa* L. solution (6.25, 12.5, 25, 50 and 100%). Then stannous chloride and 99mTc were added. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. The %ATI was calculated. The results are averages (SD of five isolated experiments, P<0.05)

Table 2: Distribution of the radioactivity in insoluble fractions of blood cells (IF-BC) and plasma (IF-P) from blood treated with different concentrations of *Mentha crispa* L. solution

Concentrations of *Mentha crispa* Solution (%)	FI-P	FI-BC
Control	74.84±3.78	88.62±5.41
100	9.99±3.61	58.45±11.55
50	14.70±7.43	75.06±16.54
25	28.50±14.95	91.55±7.03
12.5	20.04±8.31	92.47±1.69
6.25	30.64±15.05	89.93±4.95

Samples of heparinized blood, obtained by cardiac puncture of *Wistar* rats, were incubated with *Mentha crispa* L. solution (6.25, 12.5, 25, 50 and 100%). Then stannous chloride and 99mTc were added. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Aliquots of P and BC were precipitated with trichloroacetic acid (TCA 5%) and soluble and insoluble fractions were separated. The %ATI was calculated. The results are averages (SD of five isolated experiments, P<0.05)

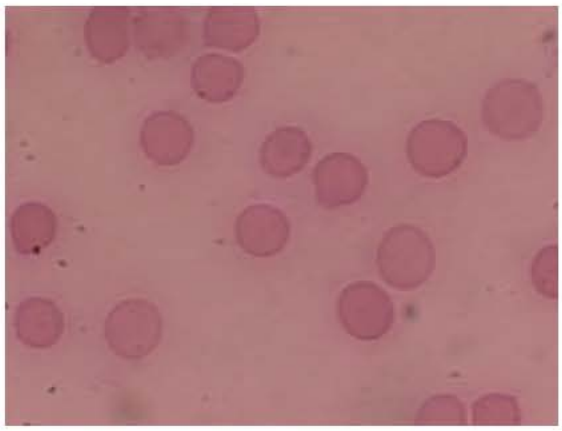

Fig. 1: Samples of whole blood were incubated with NaCl 0.9% solution for 60 min. After that, stannous chloride solution was added and the incubation continued for 60 min. Then, 99mTc, as sodium pertechnetate was added. Blood smears were prepared, dried, fixed and staining. After that, the morphology of the red blood cells was evaluated under optical microscope (x 1000)

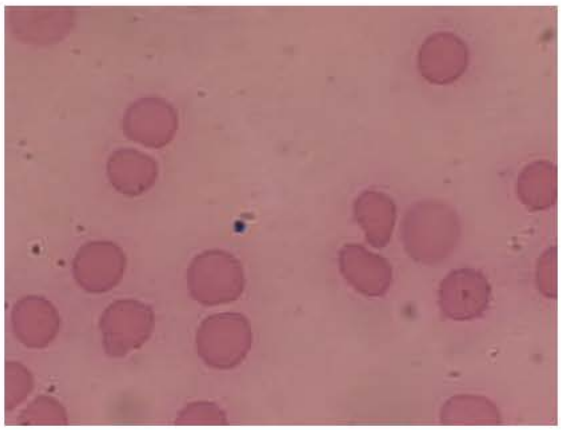

Fig. 2: Samples of whole blood were incubated with *Mentha crispa* extract (6.25%) for 60 min. After that, stannous chloride solution was added and the incubation continued for 60 min. Then, 99mTc, as sodium pertechnetate was added. Blood smears were prepared, dried, fixed and staining. After that, the morphology of the red blood cells was evaluated under optical microscope (x 1000)

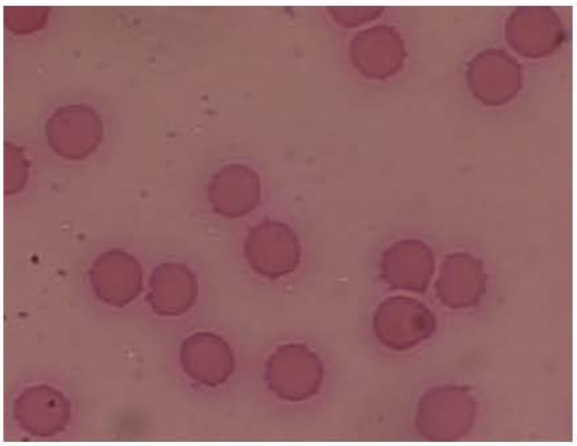

Fig. 3: Samples of whole blood were incubated with *Mentha crispa* extract (25%) for 60 min. After that, stannous chloride solution was added and the incubation continued for 60 min. Then, 99mTc, as sodium pertechnetate was added. Blood smears were prepared, dried, fixed and staining. After that, the morphology of the red blood cells was evaluated under optical microscope (x 1000)

The comparison of the shape of the RBC (no treated and treated with natural extracts) under optical microscopy has revealed only lightly morphological alterations due to the treatment of blood with 6.5 and 25% *Mentha crispa* extract. In Fig. 1 is shown the histological

preparation of a sample of blood not treated (control) and in Fig. 2 and 3 are shown the histological preparations of blood treated with 6.25 and 25% *Mentha crispa* extract.

DISCUSSION

The establishment of models to evaluate the redox properties of usual and xenobiotics products are worthwhile and can aid to understand the assessment of the biological effects associated with the use of various agents. The evidence that drugs can affect either radiolabeling of blood element with 99mTc in the context of the nuclear medicine clinic has come to light recently and workers have tuned their attention to *in vitro* testing of the drug with labeled cells[16,20,28,21,25]. Moreover, the developing of a model that permits to evaluate redox properties of medicinal products using this labeling procedure is simple and inexpensive.

As the use of natural products, as medicinal plants, is very frequent in the world and *Mentha crispa* is utilized in herbal medicine[29], we decided to evaluate the effect of this medicinal plant on the labeling of blood elements with 99mTc.

Our group has already demonstrated that the natural products *Thuya occidentalis*[24], tobacco[25], *Peumus boldus*[4] and *Maytenus ilicifolia*[27] are able to interfere with the labeling of red blood cells with 99mTc and to alter the fixation of this radionuclide to the precipitated blood proteins.

Furthermore, the results obtained with the quality comparison of the shape of the RBC (no treated and treated with natural extracts) under optical microscopy could justify the modifications in the uptake of 99mTc by red blood cells in the presence of *Mentha crispa* extract. The found results have revealed important morphological alterations due to the treatment of blood with *Mentha crispa* extract in 6.25 and 25% of the original solution.

We can speculate that, if the products present in *Mentha crispa* could complex with these ions as a chelating agent, this fact could explain the decrease in the fixation of radioactivity on RBC, IF-P and IF-BC. The substances of the *M. crispa* extract could increase the valence of the tin ion from stannous (+2) to stannic (+4). This fact would decrease the %ATI on blood elements and would indicate the presence of oxidant agents in the *M. crispa.* Another possibility to understand the decrease of the fixation of radioactivity on the blood elements in presence of *Mentha crispa* extract would be the generation of FR by herbal product, as already reported to other natural product *Maytenus ilicifolia*[27]. These FR could oxidize the stannous ion, indispensable to reduce the 99mTc, as pertechentate, to get radiopharmaceuticals labeled with this radionuclide.

Much discussion has centered on the fact that many reports are individual case studies and the rarely written up in the nuclear medicine literature. In order to make an accurate assessment of the impact of drugs and other factors o cell labeling to nuclear medicine procedures, additional data are required[21,27].

In conclusion, our experimental data show that 99mTc-RBC, 99mTc-IFP and 99mTc-IFBC can be decreased in the presence of *Mentha crispa* solution and we can suggest that this effect may be due to the products presents in this natural product solution that may: complex, with these ions; have a direct or an indirect effect on intracellular stannous ion concentration with the generation of FR. The use of this methodology could be used as a possible model to verify the redox properties of the chemical agents as the medicinal plants.

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Herbarium; FAPERJ.

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