Journal of Food Technology 3 (3): 417-422, 2005

Interaction of the Product *Mulher ativa*® on the Labeling of Red Blood Cells and Plasma Proteins with Techenetium -99 M

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Abstract: Medicinal plants originate natural products that are biologically active and widely employed as an alternative source in health care. *Mulher ativa*® is a compound which exhibits estrogen properties in the reproductive tract. Hence, the purposes of this work were to determine the antioxidant properties of natural supplement *Mulher ativa*® on the labeling of blood cells and plasmatic protein with ^{99m}Tc *in vitro*. Incubating heparin with blood carried out the experiments. Different concentrations of *Mulher ativa*® were chosen as (200, 100, 50, 25, 12.5 mg mL^{-1}). A stannous chloride solution was also added and incubation was kept for 60 min. After this, ^{99m}Tc was added and the incubation was continued for 10 min. The mixture was centrifuged, precipitated with thichloroacetic acid 5% and soluble (SF) and insoluble fractions (IF) were separated. The radioactivities in P, BC, IF-P, SF-P, IF- BC, SF-BC were determined in a well counter The analysis of radioactivity in the samples of P and BC isolated from samples of whole blood treated with *Mulher ativa*®, showed decrease significant ($p<0.05$) uptake of the radioactivity by blood cells in presence of *Mulher ativa*®, whereas there was increase in the amount of ^{99m}Tc in the TCA-insoluble fraction of plasma. It is also concluded that present s antioxidant properties.

Key words: Phytoestrogen, antioxidant, red blood cells, plasma proteins, ^{99m}Tc

INTRODUCTION

The use of medicinal plants or natural products has increased in the last decades all over world. Medicinal plants originate natural products that are biologically active and widely employed as an alternative in health. *Mulher ativa*® is a compound, which exhibits weak estrogen properties in the reproductive tract. Phytoestrogens are estrogenic compounds found in plant foods or derived from plants precursors. The main motivation for phytoestrogen by peri- and postmenopausal women is reduction of diseases. Estrogen replacement therapy (ERT) is reported to lower the incidence of cardiovascular disease in postmenopausal women. ERT also lowers the levels of oxidatively modified low-density lipoprotein (LDL). Plant sources of estrogenic compounds have been used as alternatives for ERT because they avoid a number of negative health effects produced by estrogen[1]. Phytoestrogens are a group of naturally occurring diphenolic compounds present in legumes, whole grains, fruits and vegetables. These dietary flavonoids have drawn much attention because there are suggestions that they might benefit human health. High consumption of phytoestrogen-rich food has been linked to a reduced incidence of cancers at different sites including breast, prostate and colon [2-5] Flavonoids in regularly consumed foods may also reduce the risk of death from coronary heart disease[6,7].

Several *in vitro* and animal studies were undertaken to clarify the biological and physiologic processes that account for the cancer chemoprotective effects of flavonoids. The estrogenic activities of the flavonoids may play an important role in their health-enhancing properties. Flavonoids have been reported to bind to estrogen receptors (ER) 3 and prevent cell growth in hormone-dependent cancer cells[2-6]. Another proposed mechanism for cellular protection is the induction of phase II detoxification enzymes, leading to detoxification of proximate carcinogens generated by phase I metabolism. Indeed, some phytoestrogens were found to induce the phase II enzyme NADPH quinone reductase [7-8]. In addition to these different modes of action, the protective effects of flavonoids have been attributed mainly to their antioxidant properties. Flavonoids scavenge free radicals, chelate redox-active metal ions and

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increase metallothionein expression[9,10].

Metallothionein can protect cells from heavy-metal toxicity, but also exerts antioxidant activity. In the defense against oxidative stress, the enzyme (AOE) system of cells plays an important role. The antioxidant enzymes include the superoxide dismutase (SOD); catalase and gluthione peroxidases (GPx)[11,12]. Catalase and GPx convert H_2O_2 to H_2O and the SOD catalyzes the dismutation of the superoxide radical anion. There are three forms of SOD in mammals, MnSOD located in mitochondria, CuZnSOD found mainly in the cytosol and extracellular fluid. The expression of AOE can be regulated by oxidative stress [13-16]. Induction of catalase mRNA expression could be elicited by H_2O_2 or hyperoxia in different mammalian cells [14-17]. As well as oxidative stress provoked either by H_2O_2[13,17,18,19,20] or the redox-cycling compound [15]. We previously demonstrated that the antioxidant activity of bioactive phytoestrogen constituents might involve direct activity, such as scavenging free radicals, or indirect activity, which may involved in chelating transition elements[21-23]. ^{99m}Tc have been the most utilized radionuclide both in diagnosis nuclear medicine procedures and basic scientific research[24-27] This wide use in nuclear medicine is due to its optimal physical characteristics, convenient availability from Mo-99/Tc-99 m generator a negligible environmental impact[13,17].

There are many applications of ^{99m}Tc, red blood cells (RBC), the most important being cardiovascular nuclear medicine. Some other applications include imaging the blood pool of other organs, detection of gastrointestinal bleeding sites and determination of RBC mass in-patients. RBC has been labeled with for ^{99m}Tc, *in vitro* [24,28,29] techniques. The labeling process depends on a reducing agent. Stannous ions (Sn) are usually used for this purpose. The band-3 anion transport system (24) and calcium channels[24] maybe the means by which ^{99m}Tc, as pertechnetate[30] and Sn, as stannous ions, reach the interior of the RBC, where the radionuclide is mainly housed β-chain of hemoglobin[31,32].

Unexpected patterns of radiopharmaceutical biodistribution can be associated with diseases. However, many factors, including dietary conditions besides pathological processes could affect the biodistribution of the radiopharmaceutical. Any chemical, physical or biological agent which alters the chemical identity of the tracer or modifies the physiological status of the organ of interest or its binding capability to plasma protein or other blood element could be expected to alter the radiopharmacokinetics and the disposition of the raiopharmaceutical in the specific target[33-35] The labeling of red blood cells with ^{99m}Tc has been also influenced by patient medications[35] Hence, the purposes of this work were to determine the antioxidants property of *Mulher ativa*® to labeling red blood cells and plasmatic proteins with ^{99m}Tc, using *in vitro*[28,26] technique.

MATERIALS AND METHODS

Supplement natural *Mulher ativa*®: A commercial supplement natural *Mulher ativa*® (agoniada *plumeria lancifolia*-peel, algodoeira *Gossypium herbaceum*, jequitibá *Cariniana brasiliensis*-peel, Cana do brejo *Costus spicatus*-stump and leaf, flor de paschoa *Euphorbia pulcherrima* stump, abatua *Cissampelos pareira*-vine, João da Costa *Echites peltata*-stem, Cabreuva *Myrocarpus frandosus-peel*) was obtained from the laboratory Oito ervas Industry e Comerica de predates natures Ltda.,Marataízes-ES, Brazil, lot 290 (January, 2003 and validity january, 2005). As indicated by manufacturer, seeds of *Mulher ativa*® were used to prepare this dried powder.

Preparation of the supplement natural *Mulhe ativa*®: Different concentrations 200, 100, 50, 25, 12.5 mg mL^{-1} *Mulher ativa*® were prepared in NaCl 0.9% solution(w/w).

Animals: Experiments were carried out on freely moving female *Wistar* rats weighing 200-250 g from Federal University of Pernambuco Department de Biophysics and Radiobiology were used. The animals received a standard pelleted rat diet and water ad *libitium and* were maintained under constant environmental conditions (22±5°C, 12 h of light/dark cycle). All experiments were conducted according to the guidelines of the principles outlined by the Brazilian College for Animal Experimentation (COBEA). Every effort was made to minimize animal suffering and number of animals used.

Study protocol: An *in vitro* technique employed to label RBC[33,26] technique, described elsewhere was used with minor modification. Heparinized whole blood was withdrawn from *wistar* rats. Samples of 0.5 mL were incubated with 100 μL of different concentrations of *Mulher ativa*® preparation were (200, 100, 50, 25, 12.5 mg mL^{-1}) for a stannous chloride solution was added to the incubation of was applied. Then 0.5 mL of stannous chloride (1.2 μg mL^{-1}) Isofar, Brazil and lot. 001426 were added and the incubation continued for another 1 h. After this period of time, ^{99m}Tc (3.7 MBq/mL-0.1mL), as sodium pertechnetate, [recently milked from a 99 Molybdenum/99m technetium generator (Institute de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, São Paulo, Brazil), was added and the

incubation continued for another 10 min. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Sample (20 μL) of P and BC were precipitated with 1mL of trichloroacetic acid (TCA) 5% and soluble (SF) and insoluble fractions (IF) were separated. The radioactivity in P, BC, FI-P.FS-P, FI-B and FS-BC were determined in a well counter (DPC Gambyt Cr). Thus, the percentage of radioactivity (ATI%) was calculated, as previously described. A statistical analysis (ANOVA test, n=10) was utilized to compare the values found.

RESULTS

Figure 1 shows the distribution of the radioactivity in plasma and blood cells from blood treated with different concentrations of *Mulher ativa*®. The analysis of the results indicates that there is a significant ($p<0.05$) increase in the uptake of ^{99m}Tc by the red blood cells up to the concentrations up 50 mg mL^{-1} (from 95.22-52.51%).

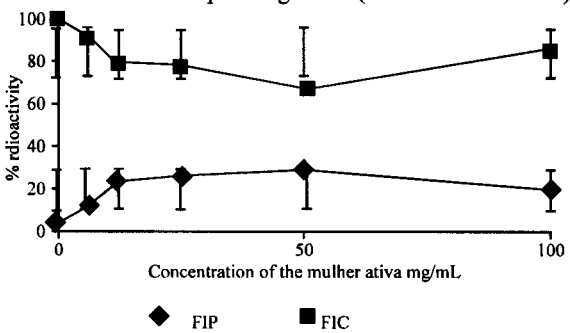

Fig. 1: Effects of *Mulher ativa*® in the uptake of ^{99m}Tc by blood cells and plasma, The radioactivity in P and BC was determined in a well counter and the percentage of radioactivity (%ATI) was calculated. A statistical analysis (ANOVA test, n=10) was used.

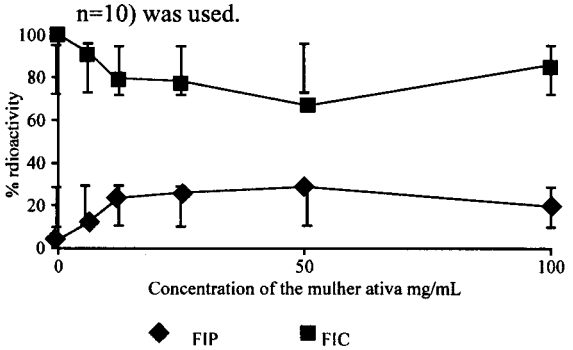

Fig. 2: Effects *Mulher ativa*® in the uptake of ^{99m}Tc by blood cells and plasma. The radioactivity in the insoluble fractions (IF) were separated. The radioactivity in BC-IF and P-IF was determined in a well counter and the percentage of radioactivity (%ATI) was calculated. A statistical analysis (ANOVA test, n=10) was used.

Figure 2 shows the distribution of the radioactivity in plasma and blood cells from blood treated with different concentrations of *Mulher ativa*®. The analysis of the results indicates that there is a significant ($p=0.05$) decrease in radioactivity uptake ^{99m}Tc by IF-BC (from 96.10-64.91%) up to the *Mulher ativa*® when the concentrations of 50%. IF-P threshold concentration of 12.5 to 200 mg mL^{-1} and there is a significant increases the uptake ^{99m}Tc in IF-P (from 3.84-18.14%).

DISCUSSION

Contrary to the studies of pharmacologically active agents used, the data concerning the interaction of diagnostic agents as radiopharmaceuticals are relatively scarce. The used can also modify uptake of ^{99m}Tc radiopharmaceutical to blood cells.

We have studied the effect treated with different concentrations of *Mulher ativa*® on the labeling of RBC with ^{99m}Tc and fixation of this radionuclide to insoluble fractions of plasma proteins and blood cells. Other biological. Effects they seem to be due to the generation of reactive oxygen species (ROS). The mechanism of the supplement effect on the labeling of RBC with ^{99m} Tc is not completely elucidated. As in this labeling process, the stannous and pertechnetate ions pass through the plasma membrane[26,33,36] suggest that effect might be explained by an inhibition of the transport of these ions, or oxidation of the stannous to stannic on or by damages induced in plasma membrane or by generating of ROS. The *Mulher ativa*® on some properties of the red blood cellular membrane, probably, due to the metabolization of cellular that could be capable to generation the active metabolites. In summary, it is well understood that the generation of ROS beyond the capacity of a biological system to eliminate them gives rise to oxidative stress. This stress may play a role in several diseases, such as heart disease, degenerative neuronal and cancer[21]. Our study demonstrated that has *Mulher ativa*®. Excellent antioxidant activities. Hence, the biological activity of these compounds of this natural supplement is more likely to involve their estrogenic properties[37].

Much discussion has centered on the fact that many reports are individual case studies and are rarely written up in the nuclear medicine literature. In order to make an accurate assessment of the impact of and other factors on cell labeling to nuclear medicine procedures[33,35,38].

ACKNOWLEDGMENTS

We greatly acknowledgments the CAPES-Brazil

for supporting this work.

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