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Full Length Research Paper

Assessment of a fruit extract (*Sechium edule*) on the labeling of blood elements with technetium-99m

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Natural products have been widely used by human beings. However, sometimes the biological effects of these products are not fully known. Chayotte (*Sechium edule*) is a vegetable used in the folk medicine. Red blood cells (RBC) labeled with technetium-99m (99mTc) have several clinical applications. The aim of this work was to evaluate the influence of an extract of chayotte on the labeling of blood elements with 99mTc using stannous chloride ($SnCl_2$) in the concentrations like to 1.2, 0.006, 0.0005 and 0.0006 μg/ml. The extract of chayote was incubated in various concentrations for 1hour with blood which was withdrawn from *Wistar* rats. After that $SnCl_2$ was added and the incubation continued for more 1 h. Elapsed this time 99mTc as sodium pertechnetate ($NaTcO_4$) was toted. The blood was centrifuged and plasma (P) and RBC were isolated, also precipitated with trichloroacetic acid (TCA, 5%) and soluble (S) and insoluble (I) fractions (F) of plasma and cells (C) were determined. The radioactivity (ATI%) was rated in RBC, IF-P and IF-C. The results have showed that extract was able to reduce the radiolabeling using $SnCl_2$ (0.006, 0.0005 and 0.0006μg/ml). We can speculate that this effect may be on account of the products with oxidant proprieties.

Keywords: chayote, red blood cells, plasma proteins, technetium-99m, radiopharmacy.

INTRODUCTION

The use of natural products as medicine has been spread out the world. The real knowing of the composition of the herbs which are used as medicine can be a complicated process because of the large and diverse group of compounds present in each herb, and is further complicated by the mixtures of herbs used. Furthermore, there is no standardization in the manufacture of several preparations based on plants (Kam and Liew, 2002). There is considerable evidence that the biodistribution or kinetics of radiopharmaceutical may be altered during a disease state as well as due to the consumption of many drugs and vegetable extracts which are used as remedy in folk medicine (Diré et al., 2001; Capriles et al., 2002; Moreno et al., 2002; Gomes et al., 2002; Nigri et al., 2002). Natural and synthetic drugs can alter the labeling of red blood cells with Technetium-99m (99mTc) (Oliveira et al., 1997; Vidal et al., 1998; Braga et al., 2000; Oliveira et al., 2000; Santos-Filho, 2002; Oliveira et al., 2002; Oliveira et al., 2003). 99mTc has been the most utilized radionuclide in nuclear medicine procedures and it has also been used recently in basic research (Bernardo-Filho et al., 1992; Gutfilen et al., 1993). 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

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been labeled with 99mTc for *in vitro, in vivo* or *in vivo*/*in vitro* techniques. The labeling process depends on a reducing agent. Stannous ion (Sn) is usually used for this purpose. The band-3 anion transport system (Callahan and Rabito, 1990) and calcium channels (Gutfilen et al., 1992) may be the means by which 99mTc, as pertechnetate (Callahan and Rabito, 1990), and Sn, as stannous ions (Gutfilen et al., 1992), reach the interior of the RBC, where the radionuclide is mainly housed in the B-chain of hemoglobin (Rehani and Sharma, 1980). Nevertheless, there is not a well established *in vitro* model to study the interaction of therapeutic drugs with radiopharmaceuticals (Srivastava and Straub, 1992; Bernardo-Filho et al., 1994; Early and Sodee, 1995). The chayotte (*Sechium edule*), a subtropical vegetable with potent diuretic action, is a cucurbitaceus species which is used as food or as medication in popular medicine (Flores, 1989). It was reported a case of severe hypokalemia pregnancy and that a chayotte preparation was implicated, as the potassium level returned to normal, without recurrence of hypokalemia, once the ingestion of this vegetable stopped. The medicinal use of chayotte enclose the relief of diseases related to the kidneys, circulatory system, intestinal and coetaneous inflammation and to the cauterize the sores. The infusion of the leaves and of the skin bear a substance with cardiovascular properties indicated to the pulmonary ailment and intestinal inflammation (Jensen and Lai, 1986). Gordon (2000), described the effect of chayotte on the decrease of diastolic pressure. Rodriguez et al., 1984 recounted the diuretic effect of chayotte juice. In a *in vivo* study Diré et al., 2001, described that a chayotte extract (macerated) was capable of altering the biodistribution of. sodium pertechnetate on *Wistar* rats. In other *in vivo* analysis, Diré et al (2002), eyed that the chayotte extracts (macerated and decoct) were able to alter radiolabeling of blood elements with 99mTc. In many *in vitro* works some authors have associated the altering on the radiolabeling process due to the action of the extracts involving morphological alterations of the RBC (Braga et al., 2000; Oliveira et al., 2000; Diré et al., 2001; Diré et al., 2002, Santos-Filho, 2002; Oliveira et al., 2002; 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 (RBC) may be repeated, resulting in an additional radiation dose to the patient (Hesslewood and Leung, 1994; Sampson, 1996). Then, we have evaluated the influence of a chayote extract (macerated) on the labeling of RBC and plasma proteins with 99mTc.

MATERIALS AND METHOD

Characterization of the chayotte sample

The presence of toxic compounds was evaluated and we did not find them in the extracts of chayotte used in our experiments. The method to verify the presence of these toxic products is based on inhibition of acetylcholinesterase in the presence of the pesticides (Cunha Bastos et al., 1991). In this method, brain acethyl-cholinestarase is utilized as an *in vitro* detector of organo-phosphorus and carbamate insectides. Briefly, a preparation of acetylcholinesterase was obtained after extraction of a rat brain microsomal fraction with Triton X-100 and was incubated with the extract of chayotte. Enzyme assay was performed by a potentiometric method based on the formation of acetic acid in the incubation mixture (preparation of acetylcholinesterase and extract of chayotte) (Diré et al., 2003).

Labeling of blood constituents

Samples of 0.5 ml of heparinized blood were incubated with 100 μl of a preparation (macerated) of *Sechium edule* which was diluted in different concentrations. The extract was prepared in the concentration of 0.1 g/ml (considered as the concentration like 100%), it was used the skin (50g) of the chayote which were triturated in a liquidizer with 500 ml of saline solution (0.9% NaCl). The experiments were performed with the administration of chayote extract in an *in vitro* studies after the withdrawn of the blood of animals by cardiac punching. Samples of 0.5 ml of blood were incubated with the extract in the concentrations of (100%; 50%; 25%; 12.5% and 6.25%) during 1h in room temperature. In the control, the samples were incubated with saline solution. After this period of time the samples were incubated with 0.5 ml of stannous chloride ($SnCl_2.2H_2O$) (Reagen, Quimibrás Indústrias Químicas SA, Brazil) in the concentration like to 1.2μg/ml for 1 h at room temperature. The extract in the concentration of 100% was also incubated with 0.5 ml of $SnCl_2$ in the concentrations of 0.006; 0.0005 and 0.0006 μg/ml during the same time. Elapsed this period of incubation, 99mTc (0.1 ml), as sodium pertechnetate recently milked from a 99Mo/99mTc generator (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, Brazil), 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 percentage of radioactivity (%ATI) was calculated. A statistical analysis (Kruskal Wallis and Unpaired tests) was used to compare the experimental data.

RESULTS

Table 1 has shown the effect of a chayotte extract on the distribution of the radioactivity on the red blood cells and in the plasma. The analysis of the results indicates that there is not an alteration ($p>0.05$) in the uptake of 99 mTc by the RBC in the plasma.

Table 2 has shown the effect of a chayotte extract on the distribution of the radioactivity in the plasma proteins. The analysis of the results indicates that there is not an alteration ($p>0.05$) in the liaison of 99mTc in the plasma proteins.

Table 3 has shown the effect of a chayotte extract on the distribution of the radioactivity in the blood cells proteins. The analysis of the results indicates that there is not an alteration ($p>0.05$) in the fixation of 99mTc in the blood proteins.

Table 1. Effect of a chayote extract on the labeling of blood cells and plasma with 99mTc.

Sechium edule	P	BC
	Percentage of radioactivity	
control	92.62 ± 4.88	7.38 ± 4.88
6.25%	90.96 ± 5.79	9.04 ± 5.79
12.5%	86.44 ± 5.40	13.56 ± 5.40
25%	89.43 ± 4.76	10.57 ± 4.76
50%	84.89 ± 2.62	15.11 ± 2.62
100%	89.14 ± 3.56	10.86 ± 3.56

Sample of blood were incubated with different concentrations of chayotte extract. In the control blood was treated with saline solution (NaCl 0.9%). After that, stannous chloride and 99mTc were added. The ATI% was calculated. A statistical analysis (Kruskal Wallis test, n=5) was used to compare the results.

Table 2. Effect of a chayotte extract on the labeling of plasma proteins with 99mTc.

Sechium edule	IF	SF
	Percentage of radioactivity	
control	71.43 ± 9.37	28.57 ± 9.37
6.25%	81.56 ± 3.36	18.44 ± 3.36
12.5%	70.29 ± 9.45	29.71 ± 9.45
25%	74.52 ± 7.66	25.48 ± 7.66
50%	70.55 ± 29.45	25.97 ± 29.45
100%	72.70 ± 10.43	27.30 ± 10.43

Sample of blood were incubated with different concentrations of chayotte extract. In the control blood was treated with saline solution (NaCl 0.9%). After that, stannous chloride and 99mTc were added. The ATI% was calculated. A statistical analysis (Kruskal Wallis test, n=5) was used to compare the results.

Table 3. Effect of a chayotte extract on the labeling of cell proteins with 99mTc.

Sechium edule	IF	SF
	Percentage of radioactivity	
control	87.10 ± 2.74	12.90 ± 2.74
6.25%	81.56 ± 3.36	18.44 ± 3.36
12.5%	70.29 ± 9.45	29.71 ± 9.45
25%	74.52 ± 7.66	25.48 ± 7.66
50%	70.55 ± 10.43	29.45 ± 10.43
100%	72.70 ± 10.43	27.30 ± 10.43

Sample of blood were incubated with different concentrations of chayotte extract. In the control blood was treated with saline solution (NaCl 0.9%). After that, stannous chloride and 99mTc were added. The ATI% was calculated. A statistical analysis (Kruskal Wallis test, n=5) was used to compare the results.

Table 4. Effect of a chayotte extract on the labeling of blood elements with 99mTc.

Sechium edule	BC	FIP	FIC
	Percentage of radioactivity		
control	81.01 ± 5.20	66.74 ± 6.36	81.85 ± 3.13
100%	65.90 ± 1.89	6.92 ± 1.17	71.01 ± 3.22

Sample of blood were incubated with different concentrations of chayotte extract. In the control blood was treated with saline solution (NaCl 0.9%). After that, stannous chloride and 99mTc were added. The ATI% was calculated. A statistical analysis (Unpaired t test, n=5) was used to compare the results.

Table 5. Effect of a chayotte extract on the labeling of blood elements with 99mTc.

Sechium edule	BC	FIP	FIC
	Percentage of radioactivity		
control	72.89 ± 1.13	8.4 ± 1.12	73.16 ± 5.17
100%	56.97 ± 1.04	6.7 ± 0.82	58.43 ± 10.23

Sample of blood were incubated with different concentrations of chayotte extract. In the control blood was treated with saline solution (NaCl 0.9%). After that, stannous chloride and 99mTc were added. The ATI% was calculated. A statistical analysis (Unpaired t test, n=5) was used to compare the results.

Table 6. Effect of a chayotte extract on the labeling of blood elements with 99mTc.

Sechium edule	BC	FIP	FIC
	Percentage of radioactivity		
control	86.49 ± 2.20	10.05 ± 2.06	78.10 ± 5.34
100%	57.61 ± 4.01	10.92 ± 4.90	50.67 ± 6.46

Sample of blood were incubated with different concentrations of chayotte extract. In the control blood was treated with saline solution(NaCl 0.9%). After that, stannous chloride and 99mTc were added. The ATI% was calculated. A statistical analysis (Unpaired t test, n=5) was used to compare the results.

Table 4 has shown the effect of a chayotte extract on the distribution of the radioactivity on the red blood cells to the concentrations of 0.006μg/ml of stannous chloride. The analysis of the results indicates that there is an alteration ($p<0.05$) in the uptake of 99mTc by the RBC (from 81.01 ± 5.20 to 65.90 ± 1.89), in the fixation of radioactivity in the FIP (from 66.74 ± 6.36 to 6.92 ± 1.17) and in the FIC (from 81.85 ± 3.13 to 71.01 ± 3.22).

Table 5 has shown the effect of a chayotte extract on the distribution of the radioactivity on the red blood cells to the concentrations of 0.0005μg/ml of stannous chloride. The analysis of the results indicates that there is an alteration ($p<0.05$) in the uptake of 99mTc by the RBC (from 72.89 ± 1.13 to 56.97 ± 1.04), in the fixation of radioactivity in the FIP (from 8.4 ± 1.12 to 6.7 ± 0.82) and in the FIC (from 73.16 ± 5.17 to 58.43 ± 10.23).

Table 6 has shown the effect of a chayotte extract on

the distribution of the radioactivity on the red blood cells to the concentrations of 0.0006μg/ml of stannous chloride. The analysis of the results indicates that there is an alteration ($p<0.05$) in the uptake of 99mTc by the RBC (from 86.49 ± 2.20 to 57.61 ± 4.01) and in the fixation of radioactivity in the FIC (from 78.10 ± 5.34 to 50.67 ± 6.46).

DISCUSSION

Extracts of medicinal plants can alter the labeling of blood constituents with 99mTc. According to Hesslewood and Leung (1994), the drug interactions with radio-pharmaceuticals are anecdotal and in some instances a direct cause and effect relationship has not been unequivocally established. This could be revolved with the development of *in vitro* tests to evaluate the drug/radiopharmaceuticals interactions and the consequence for the bioavailability of the radio-pharmaceuticals and the labeling of blood constituents. Similar results have been reported by other authors. Oliveira et al (2002), observed that an extract of *Paullinia cupana* was capable of altering the labeling 99 mTc. Also, Costa et al (2002), observed that an extract of *Stryphnodendron adstringens (Mart.) Coville* was able to alter the radiolabeling of blood elements. Moreno et al (2002), described that the *Ginkgo biloba* extract can alter the labelling of red blood cells, while Santos-Filho et al (2002), have shown that the extract of *Mentha crispa L.* can alter the labeling of blood elements with 99 mTc. Santos et al (2002) and Oliveira et al (2002), have demonstrated that the extracts of *Syzygium jambolanum* and *Ficus vessicutosis*, respectively, are capable of altering the binding of 99mTc to the blood elements. We have previously shown in an *in vivo* study that the extracts of *Sechium edule* were capable of altering the binding of 99mTc to the blood elements, Diré et al (2002). The *Sechium edule* extract has altered the bioavailability of 99mTc (Dire et al., 2001). Similar studies have found that extracts of eggplant has altered the uptake of 99 mTc in the blood compartments (Capriles et al., 2002). Braga et al, (2002), have also verified that extracts of *Thuya occidentalis* and *Nicotiana tabacum* affect the radiolabeling of blood elements 99mTc the extract of *Peumus boldus* has no effection the binding of 99mTc to the blood elements. In this study, we found that the extracts of chayotte has not promote alterations on the labeling of blood elements with 99mTc using stannous chloride as a reducing agent in the concentration like to 1.2 μg/ml although it was observed that but lower concentrations at 0.006, 0.0005 and 0.0006μg/ml the extract does promote alteration on the radiolabeling of blood elements. We suggest a possible oxidant effect of the chayotte extract as responsible for the labelling alteration but such effect cannot be observed using high concentrations of stannous chloride.

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