Analysis of the biological effects of Sechium *edule* fruit extract: a morphological and radiobiological study

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Abstract
Scientists have reported that synthetic or natural drugs can interfere with the labeling of blood constituents with 99mTc. Drugs can alter the morphology of red blood cells. An increasing number of people in the world are using natural products. In this study it was evaluated the influence of a chayotte extract on the morphology of red blood cells and on the radiolabeling of blood elements with technetium-99m (^{99m} Tc). Blood was withdrew from *Wistar* rats and treated with chayotte, and then it was incubated with stannous chloride and 99mTc. The blood smears were prepared. It was observed that the extract was capable of altering the morphology of red blood cells. The effect of the extract could be explained by its stabilizing activity in the red blood cell membrane as well as its antioxidant effect due to the radiolabeling process which has not been altered. [Life Science Journal. 2009; 6(3): 80– 82] (ISSN: 1097 – 8135).

Key words: chayotte, plasma proteins, red blood cells, technetium-99m.

1 Introduction

Natural products are widely used as food, food additives or a substance in medicinal treatment for humans. Medicinal plants are widely used worldwide for the treatment of many diseases. Sometimes the toxic and/or genotoxic effects of these products are not fully known. Practically all countries utilize radioisotopes in medicine, industry, agriculture and research. Technetium-99m (99mTc) has been the most utilized radionuclide in nuclear medicine procedures and it has also been used in basic research. Natural drugs can alter the labeling of red blood cells with technetium-99m (^{99m}Tc) [1-3]. When a radionuclide has its capability to bind to blood elements altered by natural and therapeutic drugs, the process of labeled red blood cells may be repeated, resulting in an additional radiation dose to the patient [4,5]. The chayotte, a subtropical vegetable with potent diuretic action, is a cucurbitaceus species which is used as food or as medication in popular medicine[6]. Siciliano et al isolated eight flavonoids, including three C-glycosyl and five O-glycosyl flavones, were detected, characterized by nuclear magnetic resonance spectroscopic and quantified in roots, leaves, stems, and fruits of the plant by LC-photodiode array-MS[7]. The aglycone moieties are represented by apigenin and luteolin, while the sugar units are glucose, apiose, and rhamnose. Diré et al in an experimental study of induced diabetes in *Wistar* rats described that chayotte extract may induce the generation of activity metabolites with direct action on the radiolabeling process which may probably acting in the cell membrane and in the binding protein sites together with an oxidative stress present in the pathology of diabetes[8]. In another study Diré et al described that chayotte extract was capable of reduce the plasma level of glucose and globulin as well as reducing the lethal effect induced by stannous chloride on the survival of the *Eschecrichia coli* culture in the presence of chayotte extract[9].

There are many applications of ^{99m}Tc-labeled red blood cells (^{99m}Tc-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 ^{99m}Tc for *in vitro, in vivo* or *in vivo*/*in vitro* techniques [1, 10, 11]. Then, we have evaluated the influence of a chayotte extract (decoct) on the labeling of RBC and plasma proteins with ^{99m}Tc using *in vitro* study and on the morphology of red blood cells.

2 Materials and Methods

2.1 Radiolabeling process.

Samples of heparinized blood (0.5 mL) withdraw from *Wistar* rats were incubated with 100 µL of a preparation (decoct) (100%v/v) of *Sechium edule* extract (0.1g.mL^{-1}) during 1h at room temperature. After that, it was added 0.5 mL of stannous chloride (1.2 µg. mL^{-1}), as $SnCl_2.2H_2O$, for 1h at room temperature. After this

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period of time, ^{99m}Tc (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 (SF) and insoluble fractions (IF) were separated. The radioactivity were determined in a well counter. After that, the % of radioactivity (%ATI) was calculated, as previously reported [11].

2.2 Morphometric analysis. For the morphology analysis, samples of the blood were collected and smears were prepared. The blood smears were dried, fixed and stained. The analysis was done by video optical microscope using image pro-plus program.

2.3 Enzymatic activity (AchE activity) examination. To the watery phase 0.5mL of the enzymatic preparation of the Kit had been added and the residue of the total evaporation of the solvent was dissolved in 0.25 mL of the same enzymatic preparation diluted 2 times. After incubation of 120 min 37°C, 50μL had been removed of the incubation mixture and it was added 0.5 mL of reagent of color and 0.5 mL of substratum. The reaction of formation of the product was mediated in 412 nm during 5 min. The enzymatic activity was express in average of addition of absorvance per minute. This value determined for the control (distilled water extract) corresponds the 100% of the enzymatic activity. The results of percentage of inhibition of the samples had been interpolated in the express curve metil paration standard and results in ppm of metil paration equivalents. The limit of detention of the method is of 0.2 ppm in metil paration equivalents.

3 Results

The Table 1 has shown the effect of the chayotte extract on the labeling of blood elements with 99mTc. Related to the results obtained the extract was not capable of altering the pattern of radiolabeling of blood elements. The Table 2 has shown the effect of the chayotte extract on the morphology of red blood cells. It was verified that the extract was capable of altering the morphology of red blood cells from 0.72 ± 0.07 to 0.91 ± 0.08). The presence of toxic compounds was tested and we did not find them in the preparations of chayotte used in our experiments (Table 3).

Table 1. Effect of a chayotte extract on the labeling of blood elements with ^{99m}Tc

Sechium edule	BC	IF-BC	IF-P
Control	94.81 ± 2.57	91.26 ± 3.57	77.67± 7.44
100 %	93.04 ± 4.97	91.36 ± 2.29	72.69 ± 9.55

Samples blood were incubated with the extract (100%v/v). Saline solution (NaCl 0.9%) was used as control. Then, stannous chloride (1.2 μg. mL^{-1}) and 99mTc, as sodium pertechnetate were added. These samples were centrifuged and (P) and (BC) were separated. Blood samples were precipitated with TCA and SF and 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 (Kruskal Wallis test, n= 5) was used to compare the results.

Table 2. Effect of a chayotte extract on the morphometry of red blood cells

Concentration %	Perimeter/ Area (μm/ μm^2)
Control	0.72 ± 0.07
100	0.91 ± 0.08

The blood smears were observed under optical microscope. In the treated group blood was incubated with chayotte extract (100%v/v) during 1 hour. In the control group blood was incubated with saline solution (NaCl 0.9%). The morphometric results were compared employing the ANOVA and Dunnet tests.

Table 3. Detection of pesticide in the samples of chayotte

Samples	% Absorvance	AchE activity	equivalent of metal paration
Control			
Watery	0.086	100	0
Dicloro	0.075	100	0
Organic Chayotte			
Watery	0.084	98	<0.2
Dicloro	0.075	100	0
Commercial Chayotte			
Watery	0.073	85	<0.2
Dicloro	0.073	97	<0.2

The values were obtained through the pattern curve of metil paration described by Moura, 1998. The concentration 0.2 ppm correspond to the limit of detection of the method.

4 Discussion

The developing of models that permit evaluation of the biologic properties of natural products is worthwhile. The pharmacokinetics of radiopharmaceuticals may be altered by variety of drugs, disease states and surgical procedures. The evidence that natural and synthetic drugs can affect radiolabeling or bioavailability of radiopharmaceuticals in setting of nuclear medicine clinic is already known. It was noticed that the extracts of *Thuya occidentalis a*nd *Nicotiana tabacum*[2], *Maytenus ilicifolia* [12], *Mentha crispa L* [13] and *Fucus vesiculosus* [14] have induced the decrease of radiolabeling as well as qualitative alteration on the shape of red blood cells. In this study through a quantitative analysis it was noticed that the chayotte extract in spite of altering the morphology of red blood cells was not capable of modifying the pattern of radiolabeling of blood elements. Due to the analysis of the results obtained in the molecular examination it was verified that there are not toxic compounds in the extract and that the effect of the referred extract is probably related to the presence of natural constituents as flavonoids which may establish a phytocomplex with antioxidant properties. A similar result was observed with the *Peumus boldus* extract [2] which has not altered the efficiency of labeling of blood elements with ^{99m}Tc. Diré et al described that the extract of chayotte (macerated) has been capable of inducing qualitative alterations on the shape of red blood cells as well as the bioavailability of ^{99m}Tc-radiopharmaceutical as sodium pertechnetate[15].

Different results had been described by Jesus et al who related that an extract propolis at high concentration extract could alter the labeling of plasma proteins probably by competing with same binding sites of the 99mTc on the plasma proteins or acting as antioxidant compounds[16]. Although Abreu et al suggested that an aqueous guava extract could present antioxidant action and/or alters the membrane structures involved in ion transport into cells, thus decreasing the radiolabelling of BC with ^{99m} Tc[17].

5 Conclusion

Concerning to the results obtained we can suggest that chayotte extract has antioxidant compounds which could probably be responsible to alter the morphology of red blood cells without altering the radiolabeling of blood elements.

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