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Comparative Analysis of the Biological Effects Related to a Natural Extract Processed from the Bark of Chayote (Sechium Edule)

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Abstract: Several people on the planet make use of natural therapies exploiting the power of herbs in the cure of various diseases at the level of folk medicine. Drugs can alter the radiolabeling morphology of red blood cells due to its oxidant properties. In this study, the influence of a chayotte extract was evalued on (i) the morphology of red blood cells, (ii) on the radiolabeling of blood elements with technetium-99m (99mTc) and on (iii) the electrophoretic mobility of plasmid pUC 9.1 DNA. Blood was withdraw from *Wistar* rats and treated with chayotte, after that, it was incubated with stannous chloride and 99mTc. The blood smears were prepared and analyzed under light microscope. Plasmid deoxyribonucleic acid (DNA) was exposed to chayotte extracts (macerated) (0.1g. mL^{-1}) in presence of stannous chloride ($SnCl_2$). Samples of the plasmid DNA were analyzed through agarose gel electrophoresis. It was observed that the extract was capable of altering the morphology of red blood cells. The chayotte extract was capable of damaging the DNA in the presence and in the absent of $SnCl_2$.. In conclusion, the effect of the chayote extract could be explained by the presence of flavonoids as well as different classes of proteinases and caspase-like proteases sensitive related to the effects of radiolabeling, the lesions of DNA molecules and to the alteration of cell membrane.

Key words: Chayotte · Blood proteins · Red blood cells · Technetium-99m · DNA · Natural extract

INTRODUCTION

Natural products are widely exploited and used both in terms of food and medicine 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 in 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. It was recorded that 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 therapy 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. The chayote (*Sechium edule*) is a vegetable or fruit, a vegetable category of fruits, also known as chayote or machuchar (Azores).

Although it is a vegetable that is capable of being cultivated in the vegetable garden, is considered a fruit,

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such as tomatoes (because its seeds are inside, surrounded by edible part). Its origin is attributed to Central America countries like Costa Rica and Panama.

According to some historians this vegetable fruit has been cultivated in the Caribbean at the time of the discovery of America. Herbaceous vine of Cucurbitaceae family.

It was well known in antiquity by the Aztecs and had great prominence among the other vegetables grown at the time, because of its flavor and smoothness enough to be consumed throughout the year. easy to digest, rich in fiber and low in calories, good for a diet. Stands out as a good source of potassium and provide vitamins A and C. The chayote is a cucurbits, such as cucumbers, pumpkins, melons and watermelons [6]. Siciliano *et al.* [14] indicated the highest total amount of flavonoids was in the leaves, roots and finally by stems.

The nucellus is a maternal tissue that embeds and feeds the developing embryo and secondary endosperm. During seed development, the cells of the nucellus suffer a degenerative process soon after fertilization as the cellular endosperm expands and accumulates reserves. Nucellar cell degeneration has been considered to be a form of developmentally programmed cell death (Apoptosis). Evidence showed that cell death is mostly localized in the border region of the tissue adjacent to the expanding endosperm. Cell death is accompanied by profound changes in the morphology of the nuclei and by a huge degradation of nuclear DNA. Moreover, an increase of activity of different classes of proteinases is reported and the induction of caspase-like proteases sensitive to specific inhibitors was detected. Nucellar caspase-like proteases are characterized by an acid pH optimum suggesting a possible localization in the vacuole in the cells of *Sechim edule* [17].

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. Kumar *et al.* [13] suggested that that the (99m)Tc-5-FU possesses selectivity towards solid tumor tissue. In other study Thompson *et al*. [14] related that intraoperative subareolar injection of Tc-99 localizes the sentinel lymph nodes and avoids the pain, vessel vagal events, delays and cost associated with preoperative procedure. RBC have been labeled with ^{99m}Tc for *in vitro, in vivo* or *in vivo/in vitro* techniques [1, 7, 8].

In this work, 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 was evaluated.

MATERIALS AND METHODS

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, 0.5 mL of stannous chloride (1.2 µg. mL^{-1}) was added as $SnCl_2.2H_2O$, for 1h at room temperature. After this 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 [8].

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 (Eclipse E 400 ™) using image pro-plus program.

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 37EC, 50µL had been removed of the incubation mixture and 0.5 mL of reagent of color and 0.5 mL of substratum were aded. 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 absorbance 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.

Plasmids were diluted, dispensed into Eppendorff tubes (200ng per tube) and incubated with 200µg.mL^{-1} of

$SnCl_2$. To evaluate the influence of the extract of the chayotte in DNA breakage, a concentration on a par with $0.1g.mL^{-1}$ was used. In all cases, reaction mixtures were incubated at 37EC for 40 min. The analysis of the single breaks (SSB) formation was performed using 0.8% agarose gel electrophoresis in order to separate the conformations of plasmid DNA: form I supercoiled native conformation and form II open circle resulting from SSB. Aliquots from each sample (10µL) were mixed to 2µL of 6x concentrated loading buffer (0.25% xylene cyanol FF; 0.25% bromofenol blue; 30% glycerol) and applied in a horizontal gel electrophoresis chamber in Tris acetate-EDTA buffer at pH 8.0. After electrophoresis, the gel was stained with ethidium bromide ($0.5\mu g.mL^{-1}$) and the DNA bands were visualized by fluorescence in an ultraviolet (UV) transiluminator system. Permanent records were performed using a polaroid MP-4^+system.

The morph metric results were compared employing the ANOVA and Dunnet tests.

RESULTS

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. 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 it was not found in the preparations of chayotte used in these experiments (Table 3).

In the Figure 1 is shown the electrophoresis in agarose gel of pUC. 9.1 plasmid with $SnCl_2$ and/or the extract of macerated extract. In general it was observed that the extract of chayotte was capable of inducing damages in pUC. 9.1 DNA molecules.

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

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 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 metil 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

Fig. 1: Shown the electrophoretic mobility of plasmidial DNA in various experimental conditions (macerated extract). Column 1: (Control: DNA + water), Column 2: (Chayotte100%), Column 3: ($SnCl_2$ 200µg.mL^{-1}), Column 4: ($SnCl_2$- 200µg.mL^{-1} + Chayotte 100%), Column 5: (oxidized Chayotte-10min), Column 6: (oxidized $SnCl_2$- 10min), Column 7 (oxidized Chayotte+ $SnCl_2$- 10min) and Column 8 (marker ë hind III). Photos of the gels were scanned.

DISCUSSION

The developing of models that permit evaluation of the biologic properties of natural products was 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 an*d *Nicotiana tabacum* [3]. *Maytenus ilicifolia* [9], *Mentha crispa L* [10] and *Fucus vesiculosus* [11] 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. Ordoñez *et al*. [15] had shown an antimicrobial activity of hydrogel containing *S. edule* extract on a large range of gram negative and gram positive multi-resistant bacteria and fungi. They suggested that this topical formulation may be used as antimycotic and as antibacterial in cutaneous infections. Due to the analysis of the results obtained in the molecular examination, it was verified that there is a not toxic compound in the extract and that the effect of the referred extracts is probably related to the presence of natural constituents which may establish a phytocomplex maybe related to the presence of flavonoids. A similar result was observed with the *Peumus boldus* extract [3] which has not altered the efficiency of labeling of blood elements with ^{99m}Tc. Diré *et al.* [12] 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. Maiworm *et al.* [18] had suggested that substances present on the extract of *Lantana camara* should have redoxi action decreasing the concentration of t he stannous ion and this condition could justify the effect on the decrease of efficiency of radiolabeling in the plasma proteins. Results found about the BC-%ATI-M should indicate a possible effect on the transport of ions through the erythrocyte membrane. Moreover, an increase of activity of different classes of proteinases reported and the induction of caspase-like proteases sensitive to specific inhibitors would be related to the lesions observed in the lesions of DNA in this work as well as to the alterations in the morphology of RBC membrane.

In conclusion, it can be suggested that the chayotte extract has oxidant properties due to the presence of flavonoids which could probably protect the efficiency of radiolabeling although the presence of proteinases and caspase sensitive could be responsible to alter the morphology of cells as well as inducing damages in pUC 9.1 DNA.

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