Effect of Chayote Extract on the Biochemical Determinations and on Molecular and Cellular Levels

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Abstract: The use of natural products occurs around the world. The labeling of blood constituents with technetium-99m (99mTc) has been influenced by natural extracts. We evaluated the influence of a chayote (Sechium edule) extract on the labeling of blood elements with 99mTc, in the structural conformation of DNA, in the biochemistry of blood and in the measurement of blood pressure. The animals were treated with chayote during 15 days and samples of blood were withdrawn. The samples were incubated with stannous chloride and with 99mTc. Plasma (P) and blood cells (BC) were isolated, also precipitated with trichloroacetic acid and soluble (SF) and insoluble fractions (IF) separated. There was a decrease in the radioactivity in IF-P (from 83.96±4.28 to 53.26±6.69). Samples of blood from the treated group were carried out with specific biochemistry kits and the blood biochemistry analysis compounds was done. It was analyzed the level of uric acid, albumin, cholesterol, creatinine, glucose, high density lipoprotein (HDL), globulin and triglycerides. The gauging of the blood pressure of the animals was taken. Our results showed a reduction on the level of glucose (from 118.40±10.69 to 97.20±4.32) and globulin (3.52±0.13 to 3.08±0.19) as well as in the diastolic pressure (from 123.80±9.12 to 84.40±3.85). It was observed that the referred extract has induced lesions on the DNA molecule. The effect of chayote extract probably, could be explained by the metabolism of the chayote that could be capable to induce the generation of active metabolites with oxidant properties.

Key words: Chayote, technetium-99m, biochemistry, blood elements

INTRODUCTION

It has been reported that natural and synthetic drugs are able to alter the biodistribution of different radiopharmaceuticals[1,2]. Sechium edule (chayote), a subtropical vegetable with potent diuretic action, is a cucurbitaceae species which is used as food or as medication in popular medicine. It was reported a case of severe hypokalemia pregnancy and that a chayote preparation was implicated, as the potassium level returned to normal, without recurrence of hypokalemia, once the ingestion of this vegetable was stopped[3]. Technetium-99m (99mTc) has been the most utilized radionuclide in nuclear medicine procedures[4] and it has also been used in basic research[5,6]. The wide use in nuclear medicine is due to its optimal physical characteristics (half-life of 6 h, gamma rays energy of 140 keV and minimal dose to the patients, convenient availability from 99Mo/99mTc generator and negligible environmental impact). Nearly almost all scanning devices currently in use are optimized for detecting the electromagnetic emission from this radionuclide[7].

It is known many applications of 99mTc-labeled red blood cells (99mTc-RBC), as in cardiovascular evaluations, in the detection of gastrointestinal bleeding and in the determination of the RBC mass in patients. RBC have been labeled with 99mTc through of in vitro, in vivo or in vitro/in vitro techniques[8,9]. In spite of that, there is not a well established model to evaluate the effects of drugs (synthetic or natural) on the radiolabeling of blood components. In this study, we have evaluated the influence of a chayote extract (i) on the labeling of blood constituents with 99mTc using an in vitro technique (ii...
on the biochemistry of the blood of the animals treated with the referred vegetable extract (iii) on the gauging blood pressure from the animals treated with chayotte extract and (iv) in the structural conformation of a plasmid pUC 9.1.

**MATERIALS AND METHODS**

Chayotte was purchased from a local market in Rio de Janeiro city, RJ, Brazil. To prepare the extract, 50 g of skin of chayotte were mixed with 500 mL of water in an electric extractor. This preparation was filtered and this extract was considered 100%.

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[9]. In this method, brain acetylcholinesterase is utilized as an in vitro detector of organophosphorus and carbamate insecticides. 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).

The animals were treated during 15 days with chayotte extract. After that, samples of 4 mL of blood of each animal were withdrawn. Assays to evaluate the level of blood compounds were performed through a biochemistry test using specific kits. The level of glucose, uric acid and creatinine and total proteins was available by Dried Chemistry Method in a Vitros machine from Johnson, U.S.A. The level of albumin and globulin was available by Bromocresol Green Method in a Mega machine from Merck, U.S.A. The level of cholesterol and triglycerides was utilized the Cholesterol oxidize Method in a Mega machine from Merck and the level from HDL was determined by the Direct Method without desproteinization in an Integra machine from Switzerland. The experiments were performed with the chayotte extract administrated to the animals. Whole blood was withdrawn from animals that received water or chayotte extract, as drinking water, for 15 days. The vegetable extract was prepared in the concentration of 0.1 g/mL and it was used the skin of the chayotte. Then, 0.5 mL of stannous chloride (1.2 µg mL⁻¹), as SnCl₂·2H₂O was added and the incubation continued for another 1 hour. After this period of time, 99mTc (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 also 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 % of radioactivity (%ATI) was calculated. Statistical analysis (Mann-Whitney test) was utilized to compare the experimental data.

Preparations of plasmids were performed using the alkaline method described by Sambrook et al.[19]. Plasmid samples were further purified from high molecular weight RNA contaminants, performing LiCl precipitation (2.5 M final concentration), while the residual RNA contaminants, were digested by RNAase (20 µg mL⁻¹) treatment for 30 min at room temperature. For incubation with SnCl₂, plasmids were diluted, dispensed into eppendorf tubes (200 ng per tube) and incubated with 200 µg mL⁻¹ of SnCl₂ in 10mM Tris-HCl buffer at pH 7.4. To evaluate the influence of the chayotte extract in DNA breakage induced by SnCl₂, an extract of chayotte (100%) were added before the treatment of DNA with the reducing agent. In all cases, reaction mixtures were incubated at 37°C for 60 min.

The analysis of SSB formation was performed using 0.8% agarose gel electrophoresis in order to separate different conformations of plasmid DNA, form I supercoiled (SC) native conformation, form II open circle (OC) resulting form single strand breaks. Aliquots from each sample (100 ng) were mixed to 2 µL of 6 x concentrated loading buffer (0.25% xylene cyanol FF, 0.25% bromophenol blue, 30% glycerol in water) 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 µg mL⁻¹) and the DNA bands were visualized by fluorescence in an ultraviolet (UV) transiluminator system. Permanent records were performed using a polaroid MP-3 system.

It was analyzed the blood pressure in the animals treated with the referred extract during 15 days. The tails of the rats were warmed under glowing light during 10 min to hit the swelling of tail artery. The gauging of tail pressure was done by the use of a special apparatus of gauging of tail pressure in rats (LE 5002 Storage Pressure Meter, E.U.A). To each animal (n=10) it was taken twice the BPM (beating per minute), systolic, diastolic and the mean were analyzed to the end of the procedure to obtain the relative means due to the systolic and diastolic pressures (mm/Hg).
RESULTS

Table 1 has shown the level of the blood compounds of Wistar rats treated with chayotte extract and treated with water during 15 days. The analysis of the results indicates that there is a significant decrease (p<0.05) in the level of glucose and globulin.

Table 2 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 to the macerated extract indicates that there is a slight decrease alteration (p<0.05) in the uptake of 99mTc by the RBC (from 98.16±1.57 to 90.35±5.04).

Fig. 1: Electrophoresis in 0.8% agarose gel of pUC 9.1 plasmid treated with SnCl₂ and an extract of chayotte, Lane 1-Control-DNA+ water, Lane 2-Chayotte 100%, Lane 3-Chayotte 100%+Sn200 μg ml⁻¹, Lane 4-Sn 200 μg ml⁻¹

Table 3 has shown the effect of a chayotte extract on the distribution of the radioactivity in the plasma proteins. The analysis to the macerated effects indicated a strong decrease (p<0.05) in the fixation of radioactivity in the insoluble fraction of plasma proteins (from 83.96±4.28 to 53.26±4.28).

Fig. 1 has shown the electrophoresis in 0.8% agarose gel pUC 9.1 plasmid treated with SnCl₂ and an extract of chayotte. Due to the results obtained, it was observed that the extract has induced lesions in the DNA molecule.

Table 4 has shown the effect of a chayotte extract on the distribution of the radioactivity in the blood cells proteins. The analysis of the results to the macerated study indicates that there is not an alteration (p>0.05) in the fixation of 99mTc in the blood proteins.

Table 5 has shown the effect of a chayotte extract on the gauging blood pressure. In this study it was observed a decreased on the diastolic pressure.

In Table 5, it is evident that the pressure systolic and diastolic decreased in rats treated with the extract compared to the control group.

DISCUSSION

A therapeutic drug is capable to modify the nature/amount of the 99mTc-radiopharmaceutical bound to the blood elements and this may result in unexpected behavior of the radiopharmaceutical. Therapeutic drugs and extracts of medicinal can also alter the labeling of...
blood elements with technetium-99m[12,13]. We agree with Hesselwood and Leung that many reports on drug interactions with radiopharmaceuticals are anecdotal and in some instances a direct cause and effect relationship has not been unequivocally established. This fact could be diminished with the development of in vitro tests to evaluate the drug/radiopharmaceuticals interactions and the consequence for the bioavailability of the radiopharmaceuticals and the labeling of blood constituents[9].

In an in vivo study Dire et al.[14] described that chayote extracts (macerated and decoct) were capable of altering the labeling of blood elements with 99mTc. Lima et al.[15] described that a leaf extract isolated from cauliflower which was administrated to the animals during the same time was not capable to alter the radiolabeling of blood elements. In the labeling process of blood elements with 99mTc needs a reducing agent and probably the stannous ion would be oxidized. In in vitro studies were verified that extracts of Thuya occidentalis[16], Nicotiana tabacum[17] and Maytenus ilicifolia[18] possibly, would have oxidants compounds and the labeling of blood elements decrease in the presence of these extracts.

The decrease of diastolic pressure observed by Gordon et al.[19] could be due to the action of metabolites which were produced by the metabolism of chayote. The diuretic effect described by Jensen et al.[20] have encouraged us to the tail pressure of the animals treated during 15 days with chayote extract. The results obtained are according with the ones described by Gordon et al.[19]. We observed a decrease in the diastolic pressure in the animals treated with the referred extract.

The genotoxic effect of Paulinia cupana[21] and Brassica oleracea (cauliflower)[22] natural products, could be associated to the generation of reactive oxygen species (ROS) that are oxidant agents. It was reported that Sechium edule extract was capable to alter the biodistribution of 99m-Tc-radiopharmaceutical[13]. In this study it was observed that the referred extract have induced lesions on the DNA molecule. This result would probably be due to the generation of ROS, which could be oxidizing the stannous to stannic ion. Then, we can speculate that this fact could be associated with the decrease on the labeling of blood elements with 99mTc and with the results observed in the molecular and blood biochemistry analysis. Alterations on the shape of the red blood cells were found with blood treated with tobacco[13], Sechium edule[13] and Maytenus ilicifolia[14].

There is not a well established model to study the interaction of therapeutic drugs (natural or synthetise) with radiopharmaceuticals. However, care must be taken when attempting for extrapolate experimental data to the clinical situation, once the observed effects may depend on the amounts of the drug[14].

In general, we can conclude that Sechium edule extract is capable to alter the labeling of blood elements with 99mTc as well as to induce lesions in the DNA molecule and to alter the diastolic blood pressure. In this case, we suggest that these effects can be due to the generation of active metabolites in vivo and by the presence of antioxidant properties in the extract.

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REFERENCES


