Evaluation of the Biological Effects of a Chayotte Extracts: an Experimental Analysis on *Wistar* Rats.

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Abstract: Vegetal products have formed the basis of traditional medicine systems for thousands of years. Modern medicine improves on this process by extracting and concentrating the active compound from the natural product. An inherent problem with the widespread use of many natural products, however, lies with harvesting and processing products that have low concentrations of active principles however even though the concentration is low some active molecules are able to induce effects in the organism. The labeling of blood constituents with technetium-99m (99mTc) has been influenced by the presence of natural products. We evaluated the influence of a chayotte (*Sechium edule*) extracts (macerated and decoct) (i) on the labeling of blood elements with 99mTc, (ii) on biochemistry of blood (iii) and on the gauging of tail pressure in *Wistar* rats. In our study, the animals were treated with chayotte macerated and decoct extracts (100% v/v), as drinking water (15 days) and samples of blood were withdrawn. The blood samples were incubated with stannous chloride and with 99mTc. Plasma (P) and blood cells (BC) were isolated, also precipitated with trichloroacetic acid (TCA 5%) and soluble (SF) and insoluble fractions (IF) separated. There was a decrease in the radioactivity on the labeling of blood elements. Samples of blood from the animals which were treated with chayotte extracts 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 trigliceridics. It was noticed that the extracts were capable of altering the levels of the non electrolytic substances in the blood. The gauging of the blood pressure of the animals was taken. Our results showed a reduction in the diastolic pressure. Concerning to the results we suggest that the effect of chayotte may be influenced by the warmness. The effect of chayotte extracts probably, could be explained by the metabolization of the chayotte that could be capable of inducing the generation of active metabolites with oxidant property. [New York Science Journal. 2009;2(6):43-48]. (ISSN: 1554-0200).

Keywords: chayotte, red blood cells, plasma proteins, technetium-99m, blood biochemistry.

Introduction

Many authors related the effect of natural and synthetic drugs concerning to the fact of them be capable of altering the labeling of blood elements with 99mTc (Early & Sodee, 1995; Hesslewood & Leug, 1994). *Sechium edule* (chayotte), a subtropical vegetable with potent diuretic action, is a cucurbitaceus specie which is used as food or as medication in popular medicine. 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 was stopped (Jensen & Lai, 1986; Flores, 1989). Gordon (2000) described the antihypertensive effect of chayotte. Diré et al (2001) have noticed that chayotte extract (macerated) was capable of altering the biodistribution of sodium

pertechnetate ($NATcO_4$). It is related by different researchers that many natural products are able to alter the labeling of blood constituents with Technetium-99m (99mTc) (Olievira et al, 1997; Vidal et al, 1998; Reiniger et al, 1999; Braga et al, 2000; Oliveira et al, 2000; Lima et al, 2002; Oliveira et al, 2002; Oliveira et al, 2003). 99mTc has been the most utilized radionuclide in nuclear medicine procedures (Oliveira et al, 1997; Vidal et al, 1998) and it has also been used in basic research (Gutfilen et al, 1996). The wide use 99mTc in nuclear medicine is due to its optimal physical characteristics (half-life of 6h, 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 eletromagnetic emission from this radionuclide (Saha, 1998). 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 vivo*/*in vitro* techniques (Srivastava et al, 1992; Early & Sodee, 1995). 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 chayotte extracts (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 and (iii) on the gauging blood pressure from the animals treated with chayotte extract.

Material and Methods:

Characterization of the chayotte sample

Chayotte was purchased from a local market in Rio de Janeiro city, RJ, Brazil. To prepare the decoct of chayotte, 50 g of skin of this fruit was put in an Erlenmeyer with 500 mL of saline solution (0.9% NaCl) and it was boiled on slow heat for tem minutes. After that, the solution was filtered and the watery extract was obtained.

To prepare the macerated of the referred fruit, 50g of the skin of the chayotte was also used with 500 mL of saline solution 0.9%. The skin was triturated with a domestic electric extractor. This macerated was filtered and a watery extract was obtained.

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 acethylcholinesterase in the presence of the pesticides (Cunha Bastos et al, 1991). In this method, brain acethylcholinestarase is utilized as an *in vitro* detector of organophosphorus and carbamate insectides. Briefly, a preparation of acethylcholinesterase 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 acethylcholinesterase and extract of chayotte)

Radiolabeling and blood biochemistry:

The animals were treated during 15 days with chayotte extracts. After that, samples of 4.0 mL of blood of each animal were withdrawn. Assays to evaluate the level of blood compounds were performed through of 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, USA. The level of albumin and globulin was available by Bromocresol Green Method in a Mega machine from Merck, USA. The level of cholesterol and trigliceridics 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 a 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 extracts, as drinking water, for 15 days. The vegetable extracts were 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), as $SnCl_2.2H_2O$ 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.

Gauging blood Pressure:

It was analyzed the blood pressure in the animals treated with the referred extracts 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, EUA). 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 (from 118.40 mg/dL ± 10.69 to 97.20 mg/dL ± 4.32) and globulin (from 3.52 g/dL ± 0.13 to 3.08 g/dL ± 0.19) to the treatment with macerated extract. The analysis of the results to the treatment with the decoct extract revealed that there was an increase in the level of albumin (from 3.30 g/dL ± 0.07 to 3.46 g/dL to 0.11), cholesterol (from 70.20 mg/dL ± 7.79 to 86.60 mg/dL ± 8.08), glucose (from 118.40 mg/dL ± 10.69 to 150 mg/dL ± 10.58) and HDL (from 25,40 UI ± 2.30 to 32.60 UI ± 4.09) and a decrease in the level of creatinina (from 0.44 mg/dL ± 0.05 to 0.29 mg/dL ± 0.01).

Figure 1 has shown the percentages of the radioactivity on the blood cells and in the insoluble fractions of plasma and blood cells isolated from whole blood withdrawn from animals that have received chayotte (15 days), as drinking water. The analysis of the results indicates that the macerated extract was capable of entailing a slight decrease ($p<0.05$) in the uptake of 99mTc by the RBC (from 98.16 %ATI ± 1.57 to 90.35 %ATI ± 5.04) and a strong decrease in the fixation of the radioactivity in the insoluble fraction of the plasma (from 83. 96 %ATI ± 4.28 to 53.26 %ATI ± 6.69). In the analysis of the effect of decoct extract it was noticed that there is only a slight, but significant decrease ($p<0.05$) in the uptake of 99mTc by the red blood cells (from 98.16 %ATI ± 1.57 to 93.98 %ATI ± 0.93) and in fixation of radioactivity in the insoluble fraction of the cell (from 89.23 %ATI ± 2.68 to 83.34 %ATI ± 1.75) and by the insoluble fraction of plasma (from 83.96 %ATI ± 4.28 to 72.21 %ATI ± 2.69).

Table 2 has shown the effect of the extracts on the gauging of blood pressure. Concerning to the results obtained it was eyed a reducing in the diastolic pressure to the both extracts studied. To the treatment with macerated it was noticed a decreased in the minimum pressure (from 123.80 mmHg ± 9.12 to 84.40 mmHg ± 3.85) as well as it was observed to the treatment with decoct extract (121.75mmHg ± 7.61 to 79.52mm Hg ± 2.33).

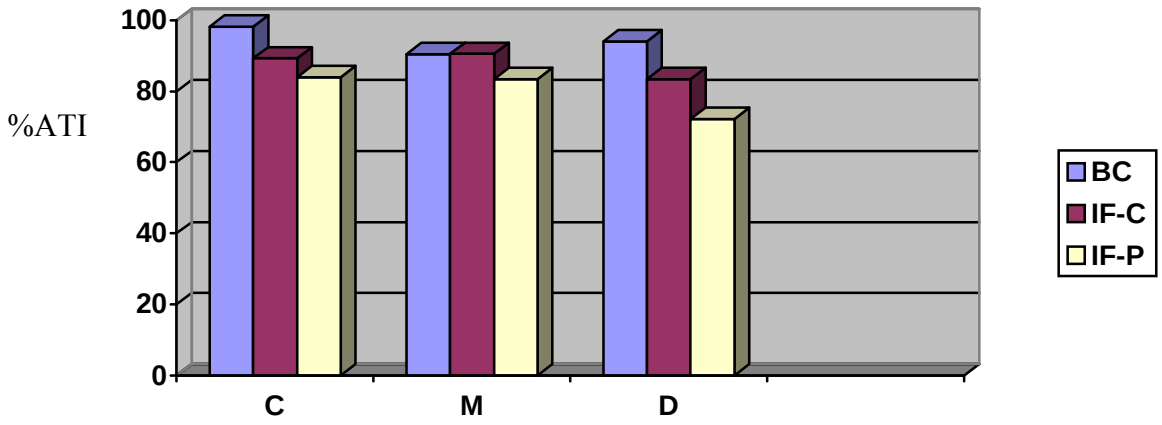
Table 1 - Effect of cayotte extract on the biochemistry of blood

Compostos	Control	Treated (macerated)	Treated (decoct)
Uric acid (mg/dL)	1.80 ± 0.45	1.50 ± 0.29	2.12 ± 0.50
Albumin (g/dL)	3.30 ± 0.07	3.30 ± 0.20	3.46 ± 0.11
Cholesterol (mg/dL)	70.20 ± 7.79	71.80 ± 4.14	86.60 ± 8.80
Creatinine (mg/dL)	0.44 ± 0.05	0.34 ± 0.05	0.29 ± 0.01
Glucose (mg/dL)	118.40 ± 10.69	97.20 ± 4.32	150.00 ± 10.58
Globulin (g/dL)	3.52 ± 0.13	3.08 ± 0.19	3.32 ± 0.27
HDL (UI)	25.40 ± 2.30	27.20 ± 3.56	32.60 ± 4.09
Triglicerídics (mg/dL)	50.40 ± 6.06	42.60 ± 8.90	44. 80 ± 8.04

In these samples of blood (n=10) were determined the concentrations of the blood compounds. The animals were treated during 15 days with chayotte extracts. The animals of control group received water. The blood was withdrawn in the morning period after a break of 8 hour on an empty stomach.

Table 2- Effect of the chayotte extract on the labeling of blood elements with 99mTc.

Animals have received water (control- C) or chayotte extracts (macerated- M or decoct- D) for 15 days.



Samples of whole blood were withdrawn from the animals and incubated stannous chloride. After that, 99mTc was added. The ATI% was calculated to blood cells (BC), insoluble fraction (IF) of plasma (P) and (C).

Table 2 - Effect of chayotte extract (100%) in the gauging of tail pressure in rats treated during 15 days.

Groups	Pressure Systolic (mmHg)	Pressure Diastolic (mmHg)
Control	204.75 ± 7.88	123.80 ± 9.12
Treated (macerated)	217.57 ± 9.11	84.40 ± 3.85
Treated (decoct)	121.75 ± 7.61	79.52 ± 2.33

The results were obtained by the gauging of tail pressure of rats which were treated and no treated with chayotte extracts.

Discussion:

A therapeutic drug is capable of modifying 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 (Reiniger et al, 1999; Sampson, 1996). We agree with Hesslewood & Leung (1994) 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 (Srivastava et al, 1992; Nigri et al, 2002; Gomes et al, 2002).

Sechium edule macerated and decoct extracts (100% v/v) were administrated orally to the animals during 15 days. It was observed an alteration on the labeling of blood constituents with 99mTc as well as in the levels of non electrolytic components of blood and in the diastolic blood pressure. Lima et al (2001) described that a leaf extract isolated from cauliflower (*Brassica oleracea*) which was administrated to the animals during the same time was not capable of altering the radiolabeling of blood elements. Braga et al (2000), described that *Peumus boldus* showed a rapid uptake of the radioactivity by blood cells in whereas there was a slight decrease in the amount of 99mTc radioactivity in the insoluble fraction of plasma. 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 was verified that extracts of *Thuya ocidentallis* (Oliveira et al, 1997), *Nicotiana tabacum* (Vidal et al, 1998), *Maytenus ilicifolia* (Oliveira et al, 2000), *Syzygium jambolanum*

(Santos et al, 2002), *Stryphnodendron adstringens* (Costa et al, 2002), *Mentha crispa L.*(Santos-Filho et al, 2002), *Ginkgo biloba* (Moreno et al, 2002), *Paullinia cupana* (Oliveira et al, 2002), *Solanum melongena* (Capriles et al, 2002), *Fucus vesiculosus* (Oliveira et al, 2003) possibly, would have oxidants compounds, and the labeling of blood elements decreased in the presence of these extracts.

The decrease of diastolic pressure observed by Gordon et al (2000), could be due to the action of metabolites which were produced by the possible metabolism of chayotte in liver. The diuretic effect described by Jensen & Lai (1986) have encouraged us to gauging the tail pressure of the animals treated during 15 days with chayotte extracts. The results obtained are according with the ones described by Gordon et al (2000). We observed a decrease in the diastolic pressure in the animals treated with both extracts (table 3).

The genotoxic effect of *Paullinia cupana* (Fonseca et al, 1995) and *Brassica oleracea* (cauliflower) (Lima et al, 2001), a 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 of altering the biodistribution of 99m-Tc-radiopharmaceutical as well as to alter the morphology of red blood cells (Diré et al, 2001). 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 blood biochemistry analysis. On the labeling of red blood cells is important to consider the homeostasis of the membrane for if the architecture of the membrane is changed the labeling pattern could be modified (Ammus & Yunis, 1989). Alterations on the shape of the red blood cells were found with blood treated with *Thuya occidentalis* (Oliveira et al, 1997), *Nicotiana tabacum* (Vidal et al, 1998), *Maytenus ilicifolia* (Oliveira et al, 2000), *Sechium edule* (Diré et al, 2001), *Mentha crispa L* (Santos-Filho et al, 2002), *Ginkgo biloba* (Moreno et al, 2002), *Paullinia cupana* (Oliveira et al, 2002) and *Fucus vesiculosus* (Oliveira et al, 2003). Mongelli et al (1997) have showed that *Bolax gummifera* extract was used as a treatment of wounds probably due its properties related to the stabilizing activity of the red blood cell membrane.

There is not a well established model to study the interaction of therapeutic drugs (natural or synthetic) with radiopharmaceuticals (Santos et al, 1995). Much discussion has centered on the fact that many reports are individual case studies and are rarely reported in the nuclear medicine literature. In order to make an accurate assessment of the impact of the studied natural product and other factors on cell labeling to nuclear medicine procedures, additional data would be required (Hladik et al, 1987; Hesslewood and Leug, 1994; Sampson, 1996).

Conclusion:

Concerning to the analysis of the results we suggest that *Sechium edule* extracts are capable of altering the labeling of blood elements with 99mTc as well as to induce the decrease of diastolic blood pressure and to alter the levels of non electrolytic components of blood. In this case, we suggest that these effects can be due to the generation of active metabolites *in vivo* with oxidant properties.

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5/13/2009