Pakistan Journal of Nutrition 2 (4): 249-253, 2003

Evaluation of the Biological Effects of a Natural Extract of Chayotte (*Sechium edule*): A Molecular and Cellular Analysis

G.F. Diré[1], E.A.C. Lima[1], M.L. Gomes[1], S. Moreno[1], M.V.C. Faria[1], R.L. Jales[2], M.T.J.A. Catanho[3] and M. Bernardo Filho[1]

[1]Universidade do Estado do Rio de Janeiro, Instituto de Biologia Roberto Alcantara Gomes, Departamento de Biofísica e Biometria. Av. 28 de setembro, 87, Rio de Janeiro, RJ, Brasil 20551-030

[2]Universidade Federal do Rio Grande do nOrte, Faculdade de Farmácia, Natal, Rio Grande do Norte, Brazil

[3]Universidade Federal de Pernambuco, Departamento de Biofísica, Recife, PE, Brazil

E-mail: gdire@hotmail.com

Abstract: Natural products have been widely used by human beings. However, sometimes the biological effects of these products are not fully known. It is concerned that many natural medicines may contain potentially toxic ingredients and contaminants such as heavy metals. Red blood cells (RBC) and plasma proteins labeled with technetium-99m (99mTc) have several clinical applications and it has been reported that some natural products are capable of reducing the efficiency of this radio labeling. The aim of this work was to assess the oxidant or antioxidant action of the agents presents in the extract of *Sechium edule* verifying its effects on the labeling of blood elements with 99mTc and on the inhibition of the activity of acetyl cholinesterase (AchE). A freshly extract of *Sechium edule* was administered to *Wistar* rats during 15 and 60 days. After that, samples (0.5ml) of blood were incubated with stannous chloride ($SnCl_2$) and 99mTc. The blood was centrifuged and plasma (P) and RBC were isolated. P and RBC were also precipitated with trichloroacetic acid and soluble (S) and insoluble (I) fraction (F) were determined. The results have shown that the referred extract was able to reduce the radio labeling in BC to 15 days of treatment (from 98.77%± 0.45 to 90.35%± 5.04) to 60 days of treatment (from 98.77%± 0.45 to 53.53%± 0.91), in IF-BC (from 90.36%± 2.34 to 21.20%± 2.36) and in IF-P to 15 days of treatment (77.20%± 2.19 to 53.26%± 6.69) to 60 days of treatment (77.20%± 2.19 to 11.01%±3.22). The analysis of toxicology was performed by the determination of acetyl cholinesterase activity. In the light of the results the referred extract has not shown inhibitory action due to the activity of the enzyme. In the light of the results obtained we suggest that the referred extract may induce the generation of activity metabolites with oxidant properties with direct action on the labeling process.

key words: Chayotte, red blood cells, plasma proteins, acetyl cholinesterase, technetium-99m

Introduction

Natural products are widely used as food or food additives, or as a substance in medicinal treatment for humans. Medicinal plants are widely used worldwide for the treatment of many diseases. Aqueous extracts of many plants are widely used in therapy as complementary medicines (Oliveira *et al.*, 2003). Traditional Chinese herbal medicines (TCHM) are increasingly used throughout the Earth, as they are considered to be effective and to have few side-effects. Contaminants of TCHM include heavy metals and undeclared drugs. Biological effects of metals have been reported as the effect of the transition metals which catalyze free radical production that can be related to aging processes and neurodegenarative diseases such as Alzheimer's disease, Parkinson's disease, and others (Silva *et al.*, 2002). The toxicity of these contaminants and additives, and the toxic effects of the herbal ingredients have important implications during the perioperative period. The anesthetist must consider the potential for drug interactions and systemic adverse effects of these natural products (Kam and Liew, 2002).

Technetium-99m (99mTc) has been the most utilized radio nuclide in nuclear medicine procedures and it has also been used in basic research. Many drugs and vegetable extracts have been reported to affect the biodistribution of different radiopharmaceuticals (Early and Sodee, 1995; Braga *et al.*, 2000). Natural and synthetic drugs can alter the labeling of red blood cells with technetium-99m (99mTc) (Braga *et al.*, 2000; Oliveira *et al.*, 2003). When a radio nuclide 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 (Hesslewood and Leung, 1994; Sampson, 1996).

The chayotte, a subtropical vegetable with potent diuretic action, is a cucurbitaceus species which is used as food or as medication in popular medicine (Flores, 1989). Guppy *et al.*, 2000, related the hypotensor effect of this fruit. Jensen and Lai, 1986, have described the diuretic effect of chayote.

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 been labeled with 99mTc for *in vitro, in vivo* or *in vivo*/*in vitro* techniques (Srivastava and Straub, 1990; Bernardo-Filho *et al*., 1994; Early and Sodee, 1995). Nevertheless, there is not a well established *in vitro* model to study the interaction of therapeutic drugs with radiopharmaceuticals. Then, we have evaluated the influence of a chayotte extract on the labeling of RBC and plasma proteins with 99mTc using *in vivo* and *in vitro* studies and the effect of this extract on the activity of acetyl cholinesterase (AchE).

Materials and Methods

To prepare the macerated of the referred vegetable, it was also used 50 g of the skin of the chayotte with 500 mL of saline solution 0.9% which was triturated with a domestic electric extractor. This macerated was filtered and the watery extract was obtained. The extract was administrated to the animals during 60 days.

Samples of 0.5 ml of blood from *Wistar* rats treated or not with the referred extract were incubated with 0.5 ml of stannous chloride (1.2 µg/ml), as $SnCl_2.2H_2O$ for 1 hour at room temperature. 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 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, as previously reported (Bernardo-Filho *et al.*, 1994). A statistical analysis (Mann Whitneyt test, n=5) was utilized to compare the experimental data.

The enzymatic activity (AchE activity) was determined by the modified method of Ellman, 1961. 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 ^{o}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 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 (Moura, 1998).

Results

The presence of toxic compounds was tested and we did not find them in the preparations of chayotte used in our experiments. Table 1 shows the fixation of the radioactivity on blood elements isolated from samples of whole blood (from animals that have received chayotte extract The analysis of the results indicates that there is a decrease ($p<0.05$) on the labeling of blood elements.

Discussion

Extracts of medicinal can also alter the labeling of blood elements with 99mTc. We agree with Hesslewood and 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. There are concerns that some natural medicines may contain potentially toxic ingredients and contaminants such heavy metals (Kam and Liew, 2002). Some substances may alter the labeling of blood constituents with 99mTc (Oliveira *et al.*, 2003). In this study it was verified that in the samples of chayotte extract analyzed was not find the presence of toxic compounds. Diré *et al.*, 2001, have related that chayotte extract is capable of altering the biodistribution of sodium pertechnetate. Lima *et al.*, 2001, described that an extract of cauliflower (*Brassica oleracea*) was not capable of altering the biodistribution of the referred radiopharmaceutical. Some authors have related that natural extracts may alter the labeling of blood elements with 99mTc (Braga *et al.*, 2000). In the labeling process of blood constituents with 99mTc is needed a reducing agent, and probably the stannous ion would be oxidized. In *in vitro* studies was verified that the 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 (Mart.) Coville* (Costa *et al.*, 2002) and *Ginkgo biloba* (Moreno *et al.*, 2002*),* possibly, would have oxidants compounds, and the labeling of blood elements decrease in the presence of these extracts. In a research was verified that *Paullinia cupana* extract was capable of altering the radio labeling of blood (Oliveira *et al.*, 2002). In other *in vitro* study with *Fucus vesiculosus* extract was noticed that the referred extract has induced alterations on the labeling of blood elements with 99mTc (Oliveira *et al.*, 2003). In a *in vivo* studies In this study it has demonstrated that the chayotte extracts were capable of altering the radio labeling of blood elements. Similar results were observed with an extract of *Solanun melongena*

Table 1: Effect of chayotte (*in vivo*, 15 and 60 days of treatment) on the labeling of red blood cells (BC), insoluble fraction of the red blood cells (IF-C) and in the insoluble fraction of the plasma (IF-P) with 99mTc

Sechium edule	BC	IF-C	IF-P
Control	98.77 ± 0.45	90.36 ± 2.34	77.20 ± 2.19
100% (15 days)	90.35 ± 5.04	90.48 ± 3.25	53.26 ± 6.69
100 % (60 days)	53.33 ± 0.91	21.19 ± 2.36	11.01 ± 3.22

Samples of heparinized blood from Wistar Rats (treated or not with the extract) were incubated for 1 hour with stannous chloride (1.2 µg/ml) and 99mTc, as sodium pertechnetate were added. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Samples (20 µl) of BC were precipitated with trichloroacetic acid (TCA) 5% and soluble (SF) and insoluble fractions (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 (Mann Whitney test, n=5) was used to compare the results. The values are averages ± SDs.

Table 2: Detection of pesticide in the samples of chayotte

Samples	%Absorbance	AchE activity	[ ]equivalent of metil paration
Control	0.086	100	0
Watery	0.075	100	0
Dicloro			
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

To the watery phase the enzymatic preparation of the kit had been added. After that, the same enzymatic kit diluted twice was added. Elapsed 120 min, the reagent of color and the substratum have been added. The reaction of formation of the product was mediated in 412 nm. 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 the detection of the method.

(eggplant) which was capable of altering radio labeling (Capriles *et al.*, 2002). Moreno *et al.* (2002), eyed that in a *in vitro* study the extract of *Ginkgo biloba* altered the radio labeling of blood elements. It was reported by Santos-Filho *et al*. (2002), that the extracts of *Mentha crispa L*. (mint) were capable of altering the radio labeling process. Braga *et al.* (2000), in a *in vitro* study demonstrated that *Peumus boldus* did not alter the labeling of blood elements with 99mTc similar results were observed by Santos-Filho *et al.* (2002) with the Kava Kava (*Piper methysticum*) extract in a *in vitro* study. Lima *et al.* (2002) in a *in vivo* study have shown that an extract of cauliflower (leaf) was not capable of altering the labeling of blood elements with technetium-99m. Diré *et al.* (2002), in a *in vitro* study eyed that the chayotte extracts were not capable of altering the radio labeling of blood constituents. In the procedure of labeling RBC with 99mTc, the stannous and pertechnetate ions pass through the plasma membrane (Gutfilen *et al.*, 1992). Then, as reported to the tobacco (Vidal *et al.*, 1998) *Maytenus ilicifolia* (Oliveira *et al.*, 2000), *Sechium edule* (Diré *et al.*, 2001), *Mentha crispa L*. (Santos-Filho *et al.*, 2002), *Paullinia cupana* (Oliveira *et al.*, 2002), *Gingko biloba* (Moreno *et al.*, 2002) and *Fucus vesiculosus* (Oliveira *et al.*, 2003) extracts, histological alterations of red blood cells could be responsible for the modifications on the labeling of RBC with 99mTc. Furthermore, we can speculate that if the chemical compounds present in these extracts could complex with these ions as a chelating agent, this fact could explain the decrease in the fixation of radioactivity on the blood elements. Diré *et al.* (2001), in a qualitative analysis *in vivo*, have eyed that a chayotte extract (macerated) has induced alteration on the shape of red blood cells. In this study the chayotte extract did alter the radio labeling of blood elements, in question to this fact, we can suggest that the chayotte extract when it is administrated to the animals due to their possible metabolization it is not able to stabilizer the active of red blood cell membrane as well as it may induce the generation of active metabolites with activity like reactive oxygen species (ROS) as already reported to other natural product *Maytenus icilifolia* (Oliveira *et al.*, 2000) and *Fucus vesiculosus* (Oliveira *et al.*, 2003).

Conclusion: We can conclude that, depending on how the chayotte extract is administrated, due to the time of treatment, the labeling of RBC with 99mTc can be altered, as well as the fixation of radioactivity in the blood proteins. We suggest that the studied natural product,

when metabolized, could be capable of generating active metabolites with oxidant properties that could probably be responsible for the effects on the radio labeling blood elements.

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