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Evaluation of the Biological Effects of a Natural Extract of Chayotte (Sechium edule): A Radiolabeling Analysis

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Abstract: The aim of this study was to assess the effect of an extract of Sechium edule on the labeling of blood elements with 99mTc. A freshly extract of Sechium edule was administered to Wistar rats during 7 days. After that, samples (0.5 mL) of blood were incubated with stannous chloride (SnCl₂) 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 radioactivity (ATI%) was rated in RBC, IF-P and IF-C in a well counter. Due to the analysis of the results it was observed a decrease in the uptake of %ATI by the blood cells in the diabetic group treated with chayotte (89.96±5.16) in comparison to the diabetic group (97.16±1.26). Related to the %ATI binding in the IF-P it was noticed a decrease in the efficiency of radiolabeling in the diabetic group (from 80.22±5.50 to 71.16±1.97) and in the diabetic group treated with the extract (from 80.22±5.50 to 70.87±4.10). Due to the %ATI binding to the IF-C it was observed a decrease in the group treated with chayotte extract (from 91.08±4.10 to 76.26±2.20), in the diabetic group (from 91.08±4.10 to 71.26±2.46) and in the diabetic group treated with chayotte (from 91.08±4.10 to 71.51±3.38). In the light of the results the referred extract has an oxidant action. We suggest that the referred extract may induce the generation of activity metabolites with direct action on the labeling process probably acting in the cell membrane and in the binding sites protein together with an oxidative stress present in diabetes.

Key words: Chayotte, red blood cells, plasma proteins, diabetes, 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 neurodegenerative 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 radionuclide in nuclear medicine procedures and it has also been used in basic research. Many drugs and vegetable extracts have been

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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 radiolabeled drug 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 chayote, a subtropical vegetable with potent diuretic action, is a cucurbitaceae 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, 1994; Early and Sodee, 1995). Nevertheless, there is not a well established in vitro model to study the interaction of therapeutic drugs with radiopharmaceuticals. According to Roush (1996) it is suggested that oxidative stress may be resulted by the exposition to some drugs, ionizing radiation and deficiency of folic acid. Insulin resistance, characterized by an inexorable decline in skeletal muscle glucose utilization and/or an excessive hepatic glucose production, constitutes a major pathogenic importance in a cluster of clinical disorders including diabetes mellitus, hypertension, dyslipidemia, central obesity and coronary artery disease. A novel concept suggests that heightened state of oxidative stress during diabetes contributes, at least in part, to the development of insulin resistance (Bitar et al. 2005).

According to Love and Oldford (2005) cardiovascular disease (CVD) and diabetes are growing public health burdens and remain one of the leading causes of morbidity and mortality in Canada. It has become increasingly evident that individuals who present with a cluster of metabolic disorders, known as the metabolic syndrome, are at an increased risk of developing both CVD and type 2 diabetes. Some studies suggested that maternal diabetes can affect the embryology environmental and this fact could help to elucidate that the oxidative stress may be related to the disturb of the gene expression which is essential in the control of the ontogenetic processes. Bruce (2003) has described that aging is accompanied by decreased specific activity in many enzymes, altered heat stability, and increased carbonyl content of proteins. The nonenzymatic reaction of carbohydrates with amino groups of proteins (glycation) can give rise to advanced glycation end-products (AGEs). These AGEs increase with aging and are implicated in diabetes, eye disorders, and amyloid accumulation. Many extracellular matrix proteins exhibit increased cross-linking with age. Driven by hyperglycemia and oxidant stress, AGEs form to a greatly accelerated degree in diabetes. Within the vessel wall, collagen-linked AGEs may trap plasma proteins, quench nitric oxide (NO) activity and interact with specific receptors to modulate a large number of cellular properties. Specifically, the interaction of AGEs with vessel wall components increases vascular permeability, the expression of procoagulant activity and the generation of reactive oxygen species (ROS), resulting in increased endothelial expression of endothelial leukocyte adhesion molecules (Basta et al. 2004). Then, we have evaluated the influence of a chayote extract on the labeling of RBC and plasma proteins with 99mTc using in vivo and in vitro studies.

MATERIALS AND METHODS

To prepare the macerated of the referred vegetable, it was also used 50 g of the skin of the chayote with 500 mL of water which was triturated with a domestic electric extractor. This macerated was filtered and the watery extract was obtained.

Diabetes induction: The injection of Streptozotocine was realized in the ventral region next to the alba line with a unique dose of 30 µg kg⁻¹ by body weight dissolved in saline solution or in a same volume of citrate (control group). In a period of 2 h after the injection the rats were maintained without water and after that it was added sugar high quantities in their drinking during 1.5 h. After 48 h of the induction it was performed the rate of sugar tests by tail puction. It was considered diabetic the rats with rate of sugar rates above 180 mg dL⁻¹.

The animals have been divided into 4 groups (control, diabetic, treated with chayotte and diabetic treated with the chayotte extract), each group with 4 animals. The extract has been administrated to the animals during 7 days. After this period of time Samples of 0.5 mL of blood from Wistar rats were incubated with 0.5 mL of stannous chloride (1.2 µg mL⁻¹), as SnCl₂, 2H₂O for 1 h at room temperature. After this period of time, 99mTc (0.1 mL), as sodium pertechnetate, was added and
RESULTS

Figure 1 shows the fixation of the radioactivity in the blood cells isolated from samples of whole blood from animals (normal and diabetic) that have received or not chayotte extract. Due to the analysis of the results it was observed a decrease in the uptake of %ATI by the blood cells in the diabetic group treated with chayotte (89.96±5.16) in comparison to the diabetic group (97.16±1.26). In the Fig. 2 it is shown the fixation of the radioactivity in the plasma proteins from samples of whole blood from animals (normal and diabetic) that have received or not chayotte extract. Related to the %ATI binding in the IF-P it was noticed a decrease in the efficiency of radiolabeling in the diabetic group (from 80.22±5.50 to 71.16±1.97) and in the diabetic group treated with the extract (from 80.22±5.50 to 70.87±4.10). In the Fig. 3 it is shown the fixation of the radioactivity in the cell proteins from samples of whole blood from animals (normal and diabetic) that have received or not chayotte extract. Due to the %ATI binding to the IF-C it was observed a decrease in the group treated with chayotte extract (from 91.08±4.10 to 67.26±2.20), in the diabetic group (from 91.08±4.10 to 71.26±2.46) and in the diabetic group treated with chayotte (from 91.08±4.10 to 71.51±3.38).

The animal have been treated with chayotte extract during 7 days. After this period of time, 99mTc (0.3 mL), as sodium pertechnetate, was injected by ocular plexus and the after 10 min samples of blood were withdraw. These samples were centrifuged and P and BC were separated. Samples (20 µL) of P and BC were diluted in 1 mL of distilled water. The radioactivity in P and BC were determined in a well counter. After that, the %ATI was calculated. Statistical analysis (Tukey test) was performed to compare the experimental data.

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These samples were centrifuged and P and BC were separated. Samples (20 μL) of P were also precipitated with 1 mL of TCA 5% and SF and IF were separated. The radioactivity in IF-P and SF-P were determined in a well counter. After that, the %ATI was calculated. Statistical analysis (Tukey test) was performed to compare the experimental data.

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**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/radiopharmaceutical 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 chayote extract analyzed was not find the presence of toxic compounds. Diré et al. (2001), have related that chayote 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 occidentalis (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 Paulinia cupana extract was capable of altering the radiolabeling 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 it has demonstrated that the chayote extracts were capable of altering the radiolabeling of blood elements. Similar results were observed with an extract of Solanum melongena (eggplant) which was capable of altering radiolabeling (Capriles et al., 2002). Moreno et al. (2002), eyed that in a in vitro study the extract of Ginkgo biloba altered the radiolabeling 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 radiolabeling process. Braga et al. (2000), in a in vitro study demonstrated that Periandus 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 chayote extracts were not capable of altering the radiolabeling of blood constituents. In the procedure of labeling RBC with 99mTc, the stannous and pertechnetate ions pass through the plasma membrane (Gutfielen et al., 1992). Then, as reported to the tobacco (Vidal et al., 1998) Maytenus ilicifolius (Oliveira et al., 2000), Sechium edule (Diré et al., 2001), Mentha crispa L. (Santos-Filho et al., 2002), Paulinia cupana (Oliveira et al., 2002), Ginkgo 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 chayote extract (macerated) has induced alteration on the shape of red blood cells. In this study the chayote extract did alter the radiolabeling of blood elements in a synergic action.
with diabetes status, in question to this fact, we can suggest that the chayote extract when it is administrated to the animals due to their possible metabolization it is not able to stabilize the active red blood cell membrane as well as it may induce the generation of active metabolites with oxidant properties as already reported to other natural product Maytenus icilifolia (Oliveira et al., 2000) and Fucus vesiculosus (Oliveira et al., 2003). These metabolites might be acting in the protein sites altering the efficiency of radiolabeling. Sohal and Weindruch (1996) have described that oxygen-derived species can react with macromolecules in a self-perpetuating manner, they create free radicals out of subsequently attacked molecules, which in turn create free radicals out of other molecules, thereby amplifying the effect of the initial free radical attack. According to Basta et al. (2004) the interaction of AGEs with vessel wall components increases the generation of ROS, this fact could support the decrease of radiolabeling in the insoluble fractions of blood elements. Meng et al. (1998) described that soluble plasma proteins, such as LDL and immunoglobulins IgG, are also entrapped and covalently cross-linked by AGES on collagen, this action may support the decrease of %ATI linked to IF-P.

CONCLUSIONS

We can speculate that chayote extract is capable of altering the efficiency of radiolabeling of the insoluble fraction of blood elements as well as the diabetes status. It may be suggested that the studied natural product, when metabolized, could be able to generate active metabolites with oxidant properties which probably would be responsible by the alterations of radiolabeling together with the oxidant stress linked to diabetes.

REFERENCES


