Journal of Food Technology 2 (2): 76-82, 2004
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In vivo Evaluation of the Effect of a Chayotte (*Sechium edule*) Extracts (decoct and macerated) on the Radiolabeing of Blood Constituents and on the Histological Modifications on the Morphology of Red Blood Cells

G. Diré, E. Lima, M. Gomes and M. Bernardo-Filho
UERJ, IBRAG, Departamento de Biofísica e Biometria, Av. 28 de setembro, 87, Rio de Janeiro, RJ, Brasil, 20551-030

Abstract: Traditional Chinese Medicine is largely based on experience and is guided by a holistic concept. Theories such as the "yin-yang" theory and "five-elements" theory embrace the view that treatment is targeted at correcting an underlying imbalance. Ying-yang literally means "opposites" and refers to opposing influences such as positive and negative forces. The five-element theory is named "Wu Shing" in Chinese and expounds that everything is maintained in kinetic balance under the movement of five elements. Prescription of herbs based on these theories may comprise a single herb or more commonly a mixture of herbs in differing amounts. The constituents of herbal products can cause adverse effects. Although natural products are widely used as food or as medicines for humans they can alter the labeling of blood constituents with technetium-99m (99mTc) as wells as the shape of red blood cells. 99mTc is one of most used radionuclide in nuclear medicine and in basic research. It was related by some authors that natural and synthetic drugs may alter the process of radiolabeling. *Sechium edule* (chayotte) is a vegetable very spent in the human nutrition as the popular medicine due its properties such as the diuretic and the hypotensor effects. We evaluated the influence of a chayotte (*Sechium edule*) extracts (decoct and macerated) on the morphology of red blood cells and on the radiolabeling of blood elements. In our study, blood was withdraw from Wistar rats which were treated with chayotte extracts (0.1g mL^{-1}) during 15 days. After that, blood was incubated with stannous chloride (SnCl2) for 1 hour. Elapsed this time 99mTc as Sodium pertechnetate (NaTcO4) was added and the incubation continued for more 10 minutes. Plasma (P) and blood cells (BC) were isolated, also precipitated with trichloroacetic acid (TCA 5%) and soluble (SF) and insoluble fractions (IF) were separated. 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 using image pro-plus program. In our results it was observed that the referred extracts were capable to alter the radiolabeing of blood elements together with the morphology of red blood cells. Similar results were noticed in a *in vivo* study with a Mentha crispa L. estract (mint). The effect of the chayotte extracts probably, could be explained by the generation of activity metabolites with oxidant properties which might be altering the stabilizing of the red blood cell membrane as the radiolabeling of blood elements.

Key words: Chayotte, red blood cells, morphology, technetium-99m, *In vivo*, histology

Introduction

An increasing number of people in the world are using traditional herbs medicines. Natural medicines may contain potentially toxic ingredients and contaminants such as heavy metal. In traditional Chinese herbal medicines (TCHM) may contain non-steroidal anti-inflammatory and anti-histamine drugs, steroids and oral hypoglycaemic agents (Kam & Liew, 2002). TCHM have been reported to cause serious hematological adverse effects (Azuno *et al.*, 1999). Some authors have reported that natural extracts have been affected the radiolabeling of blood elements with 99mTc as well as the bioavailability of sodium pertechnetate (NaTcO4) (Capriles *et al.*, 2002 and Diré *et al.*, 2002). *Sechium edule* (chayotte) a subtropical vegetable with potent diuretic action, is a cucurbitaceus species 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. The medicinal use of chayotte enclose the relief of diseases related to the kidneys, circulatory system, intestinal and coetaneous inflammation and to the cauterize the sores. The infusion of the leaves and of the skin bear a substance with cardiovascular properties indicated to the pulmonary ailment and intestinal inflammation (Jensen and Lai, 1986 and Flores, 1989). Gordon (2000) described the hypotensor effect of chayotte. Diré *et al.*, 2001 have noticed that chayotte extract (macerated) was capable of altering the biodistribution of sodium pertechnetate as the morphology of red blood cells in a qualitative analysis. In a in vitro study, Diré *et al.*, 2002 observed that the extracts (decoct and macerated) of chayotte were not capable of altering the radiolabeling of blood elements. The effects of natural products on the radiolabeling of blood elements have been studied by many researchers (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). 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. In a in vitro study developed by Oliveira *et al.* (2003), was remarked through a qualitative analysis that the *Fucus vesiculosus* extract altered the morphology of red blood cells. *F. vesiculosus* extract was capable of altering the labeling of blood elements with technetium-99m (99mTc). In other study, Oliveira *et al.* (2002), have shown that the Paullinia cupana extract have promoted alterations in the shape of red blood cells and on the radiolabeling of blood elements. Similar results were obtained by Braga *et al.* (2000), in a comparative study with Thuya occidentalis and Nicotiana tabacum. In a qualitative in vitro study, was noticed a lightly morphological alterations on the red blood cells due to treatment of blood with Maytenus ilicifolia. In this same study was verified that this extract has altered the labeling of blood constituents with 99mTc (Oliveira *et al.,* 2000). Moreno *et al.* (2002), demonstrated that an extract of Ginkgo biloba has been altered the radiolabeling of blood elements in an in vitro analysis. Like G. biloba extract the extract of Stryphnodendron adstringens (Mart.) Coville altered the efficiency of labeling of blood constituents with 99mTc in an in vitro assessment (Costa *et al.,* 2002). In other evaluation Santos *et al.* (2002), verified that an extract of Syzygium jambolanum has influenced the radiolabeling of blood constituents. 99mTc has been the most utilized radionuclide in nuclear medicine procedures (Saha, 1998) and it has also been used in basic research (Gutfilen et al, 1996). The wide utilized in nuclear medicine is due to its optimal physical characteristics as 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. There are 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 and Straub, 1992; Early and Sodee, 1995). In this assessment we have evaluated the influence of a chayotte extracts on the labeling of blood constituents with 99mTc and on morphology of the red blood cells.

Materials and Methods

Characterization of the Chayotte Sample: 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 mixtured 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 (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 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)

Preparing of the Extract: To prepare the decoct of cahyotte, this vegetable (50 g) was put in an Erlenmeyer with 500 mL of water and it was boiled on slow heat for ten minutes. After that, the solution was filtered and the watery extract was obtained. The same procedure was taken with the preparing of the macerated extract. It was used the skin (50g) of the chayote skin which were triturated in a liquidizer with 500 mL of water. The animals were treated during 15 days. In the control the animals just have received water backwards cahyotte extracts.

Radiolabeling Process: Samples of heparinized blood (0.5mL) from the animals treated and non-treated with the extracts were incubated with 0.5 mL of stannous chloride (1.2∝g mL^{-1}), as SnCl2.2H2O (Reagen, Quimibrás Indústrias Químicas SA, Brazil) during 1 hour. After this period of time, 99mTc (0.1 mL), as sodium pertechnetate, recently milked from a 99Mo/99mTc generator (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, Brazil), was added and the incubation continued for another 10 min. These samples were centrifuged (clinical centrifuge) and plasma (P) and blood These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Samples (20∝L) 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 percentage of radioactivity (%ATI) was calculated. Statistical analysis (Mann Whiteny test) was utilized to compare the experimental data.

Preparing of the Blood Smears: The blood homogenized was distended in the surface of a smear (26x76mm), after adequate cleaning, forming an angle of 45°. The distention was dried, agitating the smear in the air. The rapid desiccating is indispensable for a good conservation of the morphology of the cells and other elements.

Staining: May-Grünwald-Giensa method: The May-Grünwald-Giensa is a technique of staining which was utilized in this study. It has permitted us to visualize the cells through their distinct characteristics of staining, namely,

according to the affinity of various cellular compounds towards the used dyes (Junqueira and Carneiro, 1999).

Technique of Staining: The smears were putted upon a appropriated support and the staining technique followed:

i. distention with the dye of May-Grünwald-Giensa (Merck, Germany), for a period of 5 minutes, homogenized with a pipe;
ii. after that, the smear was covered with the dye of GIENSA (Merck, Germany), and again the surface of the smear was homogenized and left the dye acting for 10 minutes;
iii. the dye was despised and the smear was washed in the fluent water, dried in the room temperature in the vertical position.

The smears were evaluated under optical microscope of clear field (Eclipse E 400™), in the immersion objective (100x), with photographer ocular.

Morphometric Analysis: The aim of this study was to evaluate the effect of the a chayotte extracts on the morphology of red blood cells. It was analyzed the following parameters: the area (∝m 2); maximum diameter (∝m); minimum diameter (∝m); perimeter (∝m); spherecity (no dimensional-[/]) e perceptual of area (no dimensional - [/]), which correspond to the percentage of the number of cells counted per area. The quantification of the data was realized by the following equipment: Software image pro plus (media Cybernetics), according to the procedures: (1) capture of images in the gray schedule of 256 tons; (2) transformation of the image from gray schedule to binary schedule according to the calibrating threshold to detach red blood cells; (3) counting of cells with the use of. the function count/size of the program which has permitted us: (i) title the objectives by the size; (ii) seep the objectives by the relation major and minor axis (iii) measure automatically the area, the perimeter and the spherecity; (4) the gauges were impressed in the archives ASCII, being exported to electronics tables and formatted to the programs STATISTICA® e SPSS® where the statistical tests were performed.
In the morphometric analysis were employed: (i) optical microscope of. clear field (Nikon); (ii) photographic ocular; (iii) video camera CCD Sony DXC-151 A; (iv) microcomputer Pentium MMX 166 MHz, with 64 Mb of RAM memory, 256 de memory Cache and equipped with frame grabber Matrix Vision to the capture and to process the image; (vi) auxiliary monitor SONY KX-14CP1, (vii) printer HP 692C.

Results

Table 1 has shown the effect of a chayotte extracts 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). The evaluation to the effect of the decoct reveals that there is also a slight decrease ($p<0.05$) in the uptake of 99mTc by the RBC (from 98.16 % ± 1.57 to 93.98% ± 0.93).

Table 1: Effect of a chayotte extracts on the labeling of blood cells and plasma with 99mTc

Sechium edule	Blood Cells Percentage radioactivity (%ATI)	Plasma
Control	98.16 ± 1.57	1.84 ± 1.57
Macerated	90.35 ± 5.04	9.65 ± 5.04
Decoct	93.98 ± 0.93	6.02 ± 0.93

Samples of blood from Wistar rats (treated and Control) were incubated with stannous chloride and 99mTc were added. The ATI% was calculated. A statistical analysis (Mann Whitney, n = 5) was used to compare the results.

Table 2 has shown the effect of a chayotte extracts 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). The analysis of. the results with the decoct reveals that there is a decrease in the IF-P (from 83.96 % ± 4.28 to 72.21% ± 2.69).

Table 2: Effect of a chayotte extract son the labeling of plasma proteins with 99mTc

Sechium edule	Insoluble fraction Percentage radioactivity (%ATI)	Soluble fraction
Control	83.96 ± 4.28	16.04 ± 4.28
Macerated	53.26 ± 6.69	46.74 ± 6.69
Decoct	72.21 ± 2.69	27.79 ± 2.69

Samples of blood from Wistar rats (treated and Control) were incubated with stannous chloride and 99mTc were added. The ATI% was calculated. A statistical analysis (Mann Whitney, n = 5) was used to compare the results.

Table 3: Effect of a chayotte extract on the labeling of cell proteins with 99mTc

Sechium edule	Insoluble fraction Percentage radioactivity (%ATI)	Soluble fraction
Control	89.23 ± 2.68	10.77 ± 2.68
Macerated	90.48 ± 3.25	9.52 ± 3.25
Decoct	83.34 ± 1.75	16.66 ± 1.75

Samples of blood from Wistar rats (treated and Control) were incubated with stannous chloride and 99mTc were added. The ATI% was calculated. A statistical analysis (Mann Whitney, n = 5) was used to compare the results.

Table 4: Effect of a chayotte extract on the morphometry of red blood cells

Concentration %	Perimeter/ Area (∝m/∝m 2)
Control	0.83 ± 0.03
Macerated	0.68 ± 0.07
Decoct	0.70 ± 0.04

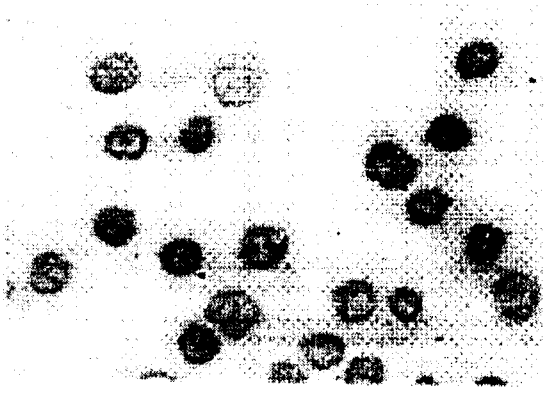

Fig. 1: Photomicrography of blood smears prepared with samples of whole blood used to label blood elements with 99Tc. Samples of whole blood from animals which just received water were incubated stannous chloride during 1 hour. After that, 99mTc, as sodium pertechnetate was added. Blood smears were carried out, dried, fixed and staining. Afterwards, the morphology of. red blood cells was evaluated under an optical microscope (x1000).

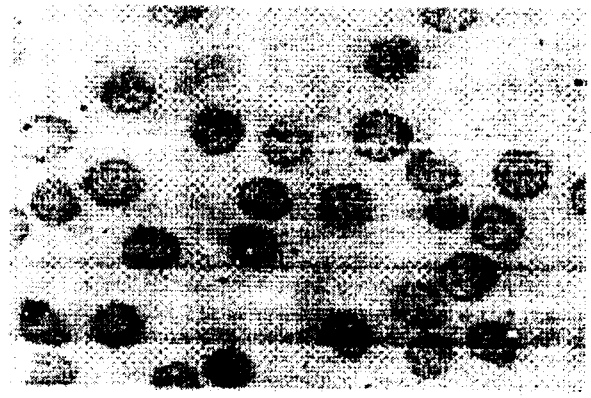

Fig. 2: Photomicrography of blood smears prepared with samples of whole blood used to label blood elements with 99Tc. Samples of whole blood from animals which just received chayotte extract (decoct) were incubated stannous chloride during 1 hour. After that, 99mTc, as sodium pertechnetate was added. Blood smears were carried out, dried, fixed and staining. Afterwards, the morphology of. red blood cells was evaluated under an optical microscope (x1000).

Table 3 has shown the effect of a chayotte extracts 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 although the analysis to the effect of the infusion reveals a decrease in the fixation of radioactivity in the insoluble fraction of the cell (from 89.23 % ± 2.68 to 83.34% ± 1.75).
Table 4 has shown the effect of a chayotte extracts on the morphology of red blood cells. The analysis of the results indicates that there is an alteration ($p < 0.05$) on the shape of the cells.
The blood smears were observed under optical microscope. In the treated group animals were treated with the chayotte extracts (decoct or macerated) during 15 days. Aliquots of blood were obtained by cardiac punch. The samples were incubated with stannous chloride during 1hour. Elapsed this period NaTcO4 was toted and the incubation continued for more 10 minutes. After this period of. time samples of blood were collect and the smears were prepared. The slides were dried, fixed and staining. After that, the morphology of. red blood cells was evaluated under optical microscope (x1000).The morphometric results were compared employing the ANOVA and Dunnet tests.

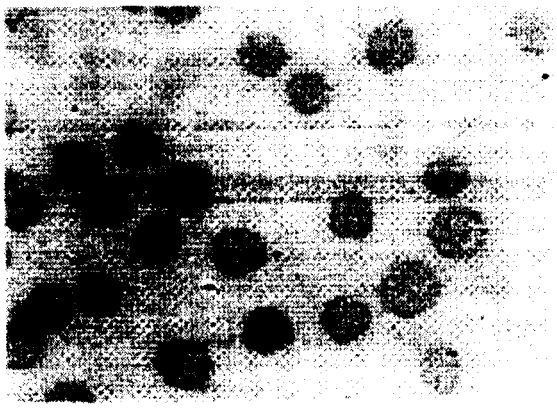

Fig. 3: Photomicrography of blood smears prepared with samples of whole blood used to label blood elements with 99Tc. Samples of whole blood from animals which just received chayotte extract (macerated) were incubated stannous chloride during 1 hour. After that, 99mTc, as sodium pertechnetate was added. Blood smears were carried out, dried, fixed and staining. Afterwards, the morphology of. red blood cells was evaluated under an optical microscope (x1000).

Discussion

The morphology analysis of the cells of the various tissues have several studiousness. The red blood cells may have alterations in their morphology which can indicate states of abnormality of the organism. It is of the fundamental importance the acquaintance of these alterations so as to the clinical full measure can have more efficacy due to the diagnostic of the patient (Hoffbrand & Pettit, 1994). The labeling of blood elements with 99mTc has many applications. It is known that extracts obtained from medical plants can alter the labeling of blood elements with 99mTc as well as the morphology of red blood cells (Oliveira *et al.*, 1997; Vidal *et al.*, 1998; Reiniger *et al.*,1999 and Braga *et al.*, 2000; Oliveira *et al.*, 2000; Lima *et al.*, 2002; Oliveira *et al.*, 2002; Diré *et al.*, 2002, Capriles *et al.*, 2002 and Oliveira *et al.*, 2003). The evidence that drugs can affect either the radiolabeling as the biodistribution of red blood cells or the morphology of them in the context of nuclear medicine clinic has come to light only comparatively recently and it is an important factor in the interpretation of scintigraphic images. A great number of workers have turned their attention to in vitro and *in vivo* evaluation of drugs in the process to label blood cells and in the biodistribution of radiopharceutical (Hladik *et al.*, 1987; Hesslewood and Leug, 1994; Owunnwanne *et al.*, 1995 and Sampson, 1996). As an example an in vitro study with Wistar rats developed by Nigri *et al.* (2002), analyzing concentrations levels higher than the therapeutic levels in humans it was demonstrated that antiseizure drugs like phenobarbital, clonazepam and phenytoin have the capacity of altering the radiolabeling of blood elements. In other research Gomes *et al.* (2002), have demonstrated that a component of many chemotherapeutic regimens, mitomycin-C, has altered the bioavailability of technetium-99m-labelled sodium pyrophosphate in mice.

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 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. By of a qualitative analysis it was remarked that N. tabacum and T. occidentalis have altered the shape of red blood cells and the radiolabeling of blood elements (Braga *et al.*, 2000). In qualitative study, Oliveira et al (2000), have shown that the extract of M. illicifolia was only induced a lightly morphological alterations of red blood cells. In a research was verified that Paullinia cupana extract was capable to alter the radiolabeling of blood elements as well as to alter quantitatively the shape of red blood cells (Oliveira *et al.*, 2002). In other in vitro study with Fucus vesiculosus extract was noticed that the referred extract has induced a qualitative alterations on the morphology of red blood cells together with alterations on the labeling of blood elements with 99mTc (Oliveira *et al.*, 2003). In a in vivo studies Diré *et al.* (2002), have demonstrated that the chayotte extracts were capable to alter the radiolabeling of blood elements. Similar results were observed with an extract of Solanun melongena (eggplant) which was capable of altering radiolabeling of blood elements with 99mTc as well as the bioavailability of NaTcO4 (Capriles *et al.*, 2002).

Sastre *et al.* (1998), described that Ginkgo biloba, used for treating cognitive disturbances, is able to produce an anticoagulant effect by inhibition of platelet activating factor. In other study, Moreno *et al.* (2002), eyed that in a in vitro study the extract of Ginkgo biloba altered the morphology of red blood cells together with the radiolabeling of blood elements, the opposite, was observed in a *in vivo* study which this fact may be explained by the generate of metabolites *in vivo* without direct action on the morphology of red blood cells. It was reported by Santos-Filho & Ribeiro *et al.* (2002), that the extracts of Mentha crispa L. (mint) and Piper methysticum (Kava Kava) were capable to alter the morphology of red blood cells notwithstanding mint extract has also altered the radiolabeling process. Braga *et al.*, (2000), in a in vitro study demonstrated that Peumus boldus did not alter the labeling of blood elements with 99mTc. 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 radiolabeling of blood constituents. In the procedure of labeling RBC with 99mTc, the stannous and pertechnetate ions pass through the plasma membrane (Callahan and Rabito, 1990; Gutfilen *et al.*, 1993). 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 extracts did alter the radiolabeling of blood elements as well as the shape of red blood cells, in question to this fact, we can suggest like observed by Mongelli *et al.* (1997), in a study with Bolax gummifera extract, that the chayotte extracts when they are administrated to the animals due to their possible metabolization they are not able to stabilizer the active of red blood cell membrane entailing morphology alterations on the red blood cells.

Conclusion

Due to the results obtained in this study we can conclude that *Sechium edule* extracts are capable of altering the labeling of blood elements with 99mTc as well as the morphology of red blood cells. This fact could be related to the presence of compounds with oxidant properties which could be produced by the metabolization of the chayotte extracts. These compounds might be strong enough to complex with pertechnetate and stannous ions and involve modifications in the architecture of plasma membrane interfering in the transport of ions through the cell.

Acknowledgment

We thank to UERJ, CAPES, FAPERJ, CNPq for the financial support

References

Azuno, Y. *et al.*, 1999. Lancet 354: 304-305.
Braga, A. C. S. *et al.*, 2000. Curr. Pharmac. Design., 6: 1179-1191.
Callahan, R.J. and A.C.J. Rabito, 1990. Nucl. Med., 31: 2004-2010.
Capriles P.V.S.Z. *et al.*, 2002. Cell. Mol. Biol., 48:771-776.
Costa, T.E.M.M. *et al.*, 2002. Rev. Bras. Farmacogn., 12: 07-09.
Cunha Bastos, V.L.F. *et al.*,1991. Water Res., 7: 835-840.
Diré, G. *et al.*, 2001. J. Labelled Cpd. Radiopharm., 44: 648-650.
Diré, G. *et al.*, 2002. Cell. Mol. Biol., 48: 751-755.
Early, P.J. and D.B. Sodee, Principles and Practice of Nuclear Medicine. Mosby-Year
Flores, E.M., 1989. Rer. Biol. Trop., 1:1-54.
Gomes, M.L. *et al.*, 2002. Cell. Mol. Biol., 48: 757-759.
Gordon, E.A., 2000. West. Indian. Med. J., 1: 27-31.
Gutiflen, B. *et al.*, 1993. Biomed. Letter. 48: 305-309.
Gutfilen, B. *et al.*, 1996. Arq Biol Tech., 39: 69-74.
Hladik III, W.B., G.B. Saha and K.T. Study, 1987. Essentials of Nuclear Medicine Science. Willians and Wilkings, Baltimore-London.
Hesslewood, S. and E. Leung, 1994. Eur. J. Nucl. Med., 21: 348-356.
Hoffbrand, A.V. and J.E. Pettit, 1994. Clinical Hematology. 2 nd . London.
Jensen, L. P. and A. R. Lai, 1986. Am. J. Obstet. Gynecol., 5: 1048-1049.
Junqueira, L.C. and J. Carneiro, 1999. Histologia Básica. 9 a ed. Guanabara Koogan, Rio de Janeiro.
Lima, E.A.C. *et al.*, 2002. Food Chemical. Toxicol., 40: 919-923.
Mongelli, E. *et al.*, 1997. J. Ethnopharmacol. 56: 117-121.

Moreno, S.R.F. *et al.*, 2002. Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine 6. SGEditoriali, Padova.
Moreno, S.R.F. *et al.*, 2002. Rev. Bras. Farmacogn., 12: 62-63.
Nigri, F. *et al.*, 2002. Cell. Mol. Biol., 48: 793-801.
Oliveira, J.F. *et al.*, 1997. J. Biol. Med., 69: 489-494.
Oliveira, J.F. *et al.*, 2000. J. Ethnopharmacol., 72: 179-184.
Oliveira, J.F. *et al.*, 2002. Fitoterapia 73: 305-312.
Oliveira, J.F. *et al.*, 2003. Food Chem. Toxicol., 41: 15-20.
Owunwanne, A., M. Patel and S. Sadek, 1995. The Handbook of Radiopharmaceuticals. Chapman and Hall Medical.
Reiniger, I.W. *et al.*, 1999. Appl Radiat Isotopes., 51:145-149.
Saha, G.B., 1998. Fundamentals of Nuclear Pharmacy. Springer-Verlag, New York.
Sampson, C.B., 1996. Nucl Med Commun., 17: 648-658.
Santos, A.E.O., Dos *et al.*, 2002. Rev. Bras. Pl. Med., 5: 63-67.
Santos-Filho, S.D. and C.K. Ribeiro *et al.*, 2002. Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine 6. SGEditoriali, Padova.
Srivastava, S.C. and R.F. Straub, 1992. J. Nucl. Med., 33: 307-308.
Vidal, *et al.*,1998. J. Exp. Clin. Cancer. Res., 17: 1-16.
Book, New York, 1995.