

In vitro assessment of the effects of a Chayotte (*Sechium edule*) extract (decoct) on the Morphology of Red Blood Cells and on the Labeling of Blood Elements with Technetium-99m

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Abstract

Natural products are increasingly used around the world in traditional medicine as drugs. Some of the constituents of natural herbal products may also cause adverse side effects. We evaluated the effect of one such product, *Sechium edule*, a Chayotte extract on the morphology of red blood cells and on the efficacy of labeling of RBC with Technetium-99m (Tc-99m). Blood samples from Wistar rats were first treated with Chayotte extract (decoct) in variable concentrations of 100, 50, 25, 12.5 and 6.25% (v/v) for durations of one hour each. Labeling of the treated RBCs was done with Tc-99m. The labeling procedure consisted of incubation of Chayotte extract treated RBCs with stannous chloride for over 1 hour, followed by addition of Tc-99m pertechnetate and continuing the incubation for a further period of 10 minutes. Subsequently Plasma and blood cells were isolated. Samples of plasma and blood cells were precipitated with trichloroacetic acid, followed by separation of soluble (SF) and insoluble fractions (IF). Evaluation of RBC morphology was done by a video optical microscope using image pro-plus program. Results of this study revealed significant alteration in the morphology of the RBCs as a result of Chayotte treatment at higher concentration. However there was no significant change in the efficacy of labeling of blood elements with Tc-99m. This phenomenon may be attributed to a stabilizing effect of the herb extract on the RBC membrane.

Key words: Chayotte extract, red blood cells, Technetium-99m

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Introduction

Medicines based on natural products or herbal extracts are being used increasingly around the world in the treatment of a number of diseases, as they are considered to be highly effective and believed to have few side-effects (1). However a few of the traditional Chinese herbal medicines have been reported to cause serious hematological side effects (2).

Tc-99m labeled RBCs are being used routinely in diagnostic nuclear medicine in the evaluation of cardiac function, diagnosis of gastrointestinal bleeding, detection of haemangiomas, calculation of blood volume or determination of RBC life-span. Many drugs and vegetable extracts have also been reported to affect the radio-labeling of blood elements with Tc-99m as well as the bioavailability of sodium pertechnetate (3, 4). *Sechium edule* (Chayotte) a sub-tropical vegetable, a cucurbitaceous species with potent diuretic and anti-hypertensive actions, has been used for several decades now as a food item or as drug in popular medicine (5).

Several side-effects of Chayotte have been reported. Severe hypokalemia has been reported in pregnancy with the intake of Chayotte preparations. But prompt reversal of this abnormality has also been reported following withdrawal of the herbal medicine (6,7). Alteration in the bio-distribution of Tc-99m pertechnetate as well as abnormal morphology of red blood cells leading to suboptimal radio-labeling of blood elements with Tc-99m have also been reported (8).

The effects of several other natural products on the radio-labeling of blood elements have been studied by several researchers. Several herbal extracts like *Bolax gummifera*, *Fucus vesiculosus*, *Paullinia cupana*, *Thuya occidentalis*, *Nicotiana tabacum* and *Maytenus ilicifolia*, which are being used in the treatment of a variety of clinical conditions, have been reported to alter the morphology of red blood cells, consequently resulting in reduced labeling efficacy of red cells with Tc-99m (9-17).

Because of its ideal physical properties and attractive imaging characteristics, Tc-99m has been used most widely in diagnostic nuclear medicine and clinical research (18,19). As has already been cited above, there are several

clinical applications of Tc-99m-labeled red blood cells (Tc-99m RBC). Technetium labeling of the RBCs can be achieved by either in vivo or in vitro techniques (20, 21). Although the techniques have been in use for several decades, there is no established model to study the influence of drugs (synthetic or natural) on the labeling of blood elements. In the present study we have tried to evaluate the influence of a Chayotte extract on the labeling of blood elements with Tc-99m, as well as on the morphology of the red blood cells, using an in vitro technique.

Materials and Methods

Chayotte was procured from a local market in Rio de Janeiro. Chayotte extract was prepared by mixing 50g of the skin of Chayotte with 500 ml of water in an electric extractor. This preparation was filtered and the concentration of the filtered product was considered 100%. The extract was then assessed for presence of toxic substances like pesticides using the acetylcholinesterase inhibition technique (22). In this method, brain acetylcholinesterase was used as an in vitro detector of organophosphorus and carbamate insecticides. Acetylcholinesterase was extracted from a rat brain microsomal fraction using Triton X-100 and was incubated with the Chayotte extract. 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)

Heparinized whole blood was withdrawn from Wistar rats. Samples of 0.5 ml of blood were incubated with 100 µl of variable concentrations (100, 50, 25, 12.5 and 6.25% v/v) of Chayotte extract for 1 hour at room temperature. Samples of heparinized blood (0.5ml) incubated with normal saline (NaCl 0.9%) acted as control. After that 0.5 ml of stannous chloride (1.2 µg/ml), as SnCl₂.2H₂O (Reagen, Quimibrás Indústrias Químicas SA, Brazil) was added and the incubation continued for another 1 hour. This was followed by addition of freshly eluted Tc-99m (0.1 ml), as sodium pertechnetate, from a Mo-99/Tc-99m generator (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, Brazil), and continuation of incubation for another 10 minutes. These samples were then centrifuged, and plasma and blood cells were separated. Samples (20 µl) of plasma and blood cells were then precipitated with 1 ml of 5% trichloroacetic acid (TCA) followed by separation of soluble fractions (SF) and insoluble fractions (IF). The radioactivity in plasma, blood cells, soluble fraction in plasma (SF-P), insoluble fraction in plasma (IF-P), soluble fraction in blood cells (SF-BC) and insoluble fraction in blood cells (IF-BC) were determined in a well counter. Statistical analysis was done using the Kruskal Wallis test.

Preparation of blood smears

Homogenized blood was used to prepare smears on glass slides. Care was taken to conserve the morphology of red

blood cells and other elements. May-Grünwald-Giensa staining was used to stain the slides (23). The smears were examined under optical microscope of clear field (Eclipse E 400TM), in the immersion objective (100x), with photographer ocular.

Morphometric analysis

The aim of this study was to evaluate the effect of variable concentrations of Chayotte extract on the morphology of red blood cells. The following parameters were analyzed: surface area (µm²), maximum diameter (µm), minimum diameter (µm), perimeter (µm) and sphericity (as a percentage of the number of cells counted per unit area). The quantification of the data was done using the Software image pro plus (media Cybernetics). The process included capture of images in the gray schedule of 256 tons, transformation of the images from gray schedule to binary schedule according to the calibration threshold to detach red blood cells and counting of cells using the function count/size of the software program. The program allowed to title the objectives by size and shape; automatic measurement of area, perimeter and the sphericity (4); the gauges were impressed in the archives ASCII, exported to electronics tables and formatted to the programs STATISTICA e SPSS where the statistical analyses were performed.

For the morphometric analysis the following tools were used: (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 and (vii) printer HP 692C.

Results

There was no significant change in the labelling efficiency of Tc-99m with RBCs, plasma proteins or red cell proteins. Table 1 shows the effect of variable concentrations of Chayotte extract on the labeling efficiency of Tc-99m with the RBCs. Results revealed no significant change in labelling efficiency when compared with the control. Table 2 shows the effect of Chayotte extract on the labeling of Tc-99m with plasma proteins. The results clearly indicate that

Sechium edule	Blood Cells	Plasma
	Percentage radioactivity	
Control	94.81 ± 2.57	5.19 ± 2.57
6.25%	90.94 ± 6.18	9.06 ± 6.18
12.5%	3.93 ± 2.93	6.07 ± 2.93
25%	92.65 ± 3.45	7.35 ± 3.45
50%	90.68 ± 5.66	9.32 ± 5.66
100%	93.04 ± 4.97	6.96 ± 4.97

Table 1 Effect of a Chayotte extract on the labeling of blood cells and plasma with Tc-99m

Sechium edule	Insoluble fraction Percentage radioactivity	Soluble fraction
Control	77.67 ± 7.44	22.33 ± 7.44
6.25%	81.53 ± 4.45	18.47 ± 4.45
12.5%	76.96 ± 8.63	20.01 ± 8.63
25%	72.22 ± 10.40	27.78 ± 10.40
50%	74.03 ± 9.76	25.97 ± 9.76
100%	72.69 ± 9.55	27.31 ± 9.95

Table 2 Effect of a Chayotte extract on the labeling of plasma proteins with Tc-99m.

Sechium edule	Insoluble fraction Percentage radioactivity	Soluble fraction
Control	91.26 ± 3.57	8.74 ± 3.57
6.25%	90.03 ± 2.63	9.97 ± 2.63
12.5%	89.15 ± 3.34	10.85 ± 3.34
25%	91.37 ± 3.44	8.63 ± 3.44
50%	91.94 ± 1.77	8.06 ± 1.77
100%	91.36 ± 2.29	8.64 ± 2.29

Table 3 Effect of a Chayotte extract on the labeling of cell proteins with Tc-99m

there was no significant change ($p > 0.05$) in the labeling efficiency of Tc-99m with plasma proteins. Table 3 shows the effect of Chayotte extract on the labeling efficiency of Tc-99m with blood cell proteins. Here also the results revealed that there was no significant change ($p > 0.05$) in the fixation of Tc-99m to the red cell proteins.

Concentration %	Perimeter/ Area (mm/ mm ²)
Control	0.72 ± 0.07
6.25	0.71 ± 0.03
12.5	0.74 ± 0.01
25	0.76 ± 0.04
50	0.81 ± 0.09
100	0.91 ± 0.08

Table 4 Effect of a Chayotte extract on the morphometry of red blood cells.

Table 4 shows the effect of a Chayotte extract on the morphology of red blood cells. The analysis of the results indicated that there were significant alterations ($p < 0.05$) in the morphology of the red blood cells.

No changes could be detected in the morphology of red cells in the control group incubated with saline (0.9% NaCl) (Figure 1A). On the other hand significant changes in the red cell morphology could be detected at all concentrations of incubation with Chayotte extract, e.g., 6.25, 12.5, 25, 50 and 100% (Figure 1B-F). The study of the morphology of the RBC revealed alterations in the shape of these cells. The morphometric results were compared employing the ANOVA and Dunnet tests.

Discussion

Drugs and extracts of medicinal plants used as drugs can alter the efficiency of radionuclide labelling as well as the morphology of red blood cells (3, 4, 9-16). *Sechium edule* (Chayotte) a sub-tropical vegetable with potent diuretic

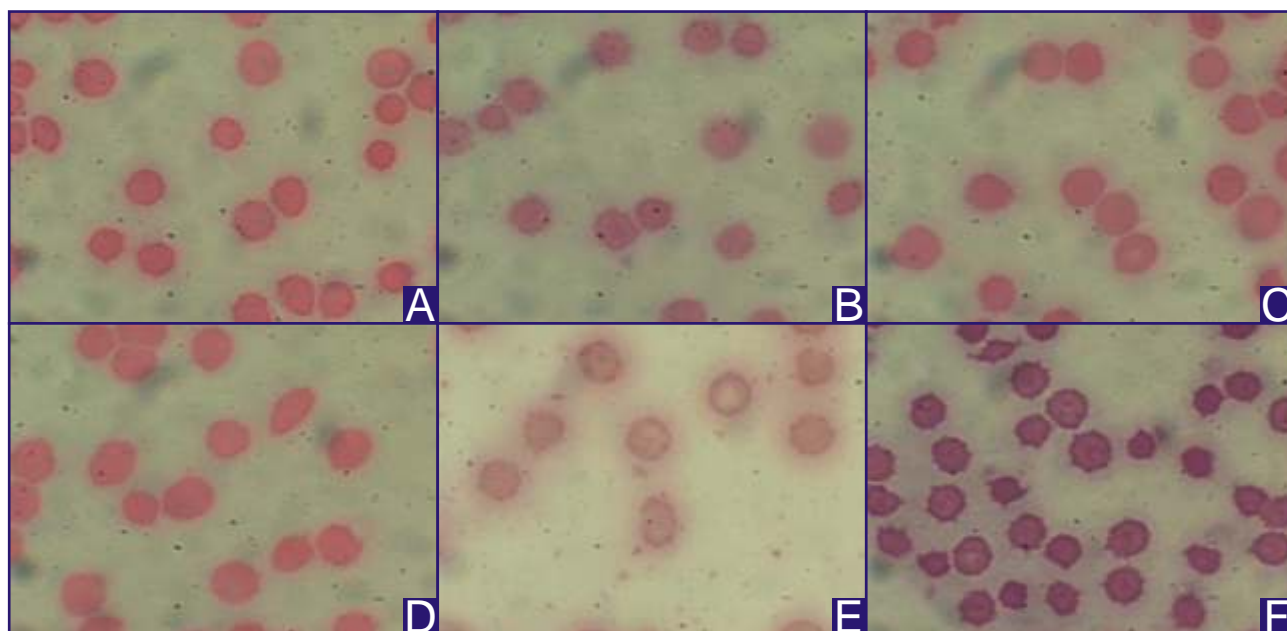


Figure 1 (A-F) shows the effect of a Chayotte extract on the morphology of red blood cells. The analysis of the results indicated that there were significant alterations ($p < 0.05$) in the morphology of the red blood cells treated with Chayotte extract. No changes could be detected in the morphology of red cells in the control group incubated with saline (0.9% NaCl) (A). On the other hand significant changes in the red cell morphology could be detected at all concentrations of incubation with Chayotte extract, e.g., 6.25, 12.5, 25, 50 and 100% (Figure 1B-F respectively). The study of the morphology of the RBC revealed alterations in the shape of these cells. The morphometric results were compared employing the ANOVA and Dunnet tests. The abnormalities in RBC morphology were more severe at higher concentrations of 12.5, 25 and 100% (D-F respectively).

action is a cucurbitaceous species which is used as food item or as drug in popular medicine. Chayotte has also been reported to have good antihypertensive effect. As its use has been widespread, we decided to evaluate its effect on RBC morphology and influence on the efficacy of Tc-99m labelling with RBCs (24). In this regard it is also important to develop models which would allow evaluation of biologic properties of natural herbal products. The evidence that herbal products may affect radiolabeling and alter the morphology of RBCs resulting in altered bio-distribution has come to light only recently. It is also important to know that such effects may create difficulty in interpretation of the scintigraphic data. A number of researchers have worked on the in vitro and in vivo evaluation of the effects of herbal products and other drugs on red cell morphology and radio-labeling (25-28).

In the labeling process of blood elements with Tc-99m, a reducing agent stannous chloride is needed, and probably the stannous ion would be oxidized. Several in vitro studies in the past have reported that extracts of *Thuya occidentalis* (9) *Nicotiana tabacum* (10) and *Maytenus ilicifolia* (13) contain oxidant compounds, and these compounds affect the labeling efficacy of blood elements with Tc-99m. *N. tabacum* and *T. occidentalis* have been reported to alter the shape of red blood cells and affect radiolabeling (12). In another qualitative study, Oliveira et al, have shown that the extract of *M. ilicifolia* could alter the morphology of red blood cells (13). In few other studies the extracts from *Paullinia cupana*, *Fucus vesiculosus*, *Mentha crispera* L. (mint) and *Piper methysticum* (Kava Kava) were also shown to affect radio-labeling of blood elements as well as altering the RBC morphology (15, 16, 31). On the other hand a few authors have reported no adverse effect either on RBC labeling or morphology by herb extracts like *Peumus boldus* (12) or extracts from cauliflower (14) or even Chayotte extracts (4), which is the main focus of our current study.

In an in vivo study Diré et al have demonstrated that the Chayotte extracts adversely affected radio-labeling of blood elements (8). Another herbal extract, *Ginkgo biloba*, used as an anticoagulant (by virtue of its inhibiting effect on platelet activating factor) and also for the treatment of cognitive disturbances, has been reported to produce RBC morphology abnormalities in an in vitro study. However this could not be reproduced in vivo. (30).

In the process of labeling RBCs with TC-99m, the stannous and pertechnetate ions cross through the plasma membrane into the red blood cells (19, 32). It has been postulated that chemical substances present in some of the extracts combine with these ions and the resultant compounds affect labeling efficacy of Tc-99m with the RBCs. In the present study we observed definite morphological abnormalities in the RBCs caused by the Chayotte extracts, but this did not affect the overall labeling efficacy of Tc-99m with the RBCs. This is consistent with the results reported by Diré et al in their study (8). This dichotomy of effect abnormal

morphology but no effect on labeling efficiency, can be explained on the basis of previous reports by Mongelli et al (17) with *Bolax gummifera*, where the herb has been implicated to have a stabilizing effect on the red blood cell membrane, even though the actual morphology of the cell has been altered.

Conclusion

In general, we can conclude that *Sechium edule* extract is not capable of altering the labeling of blood elements with Tc-99m although it has altered the morphology of red blood cells. This fact could be due to the presence of compounds which were not strong enough to complex with pertechnetate and stannous ions but good enough to modify the architecture of plasma membrane without interfering in the transport of ions by the cell.

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References

1. Kam PCA, Liew S. Traditional Chinese herbal medicine and anaesthesia. *Anaesthesia* 2000; 57: 1083-1089.
2. Azuno Y, Yaga K, Sasayama T, Kimoto K. Thrombocytopenia induced by Jui, a traditional Chinese herbal medicine. *Lancet* 1999; 354: 304-305.
3. Capriles PV, Dias AP, Costa TE et al. Effect of egg-plant (*Solanum melongena*) extract on the in vitro labeling of blood elements with technetium-99m and on the bio-distribution of sodium pertechnetate in rats. *Cell Mol Biol* 2002; 48: 771-776.
4. Dire GD, Lima EA, Pereira MJS et al. Effect of a Chayotte (*Sechium edule*) extract on the labeling of red blood cells and plasma proteins with technetium-99m: in vitro and in vivo studies. *Cell Mol Biol* 2002; 48: 751-755.
5. Jensen LP, Lai AR. Chayote (*Sechium edule*) causing hypokalemia in pregnancy. *Am J Obst Gynecol* 1986; 5: 1048-1049.
6. Flores EM. The chayote, *Sechium edule* Swartz (Cucurbitaceae). *Rev Biol Trop* 1989; 37 Suppl 1:1-54. Review. Spanish.
7. Gordon EA. The antihypertensive effects of the Jamaican Cho-cho. *West. Indian Med J* 2000; 1: 27-31.
8. Dire G, Lima E, Mattos D et al. Effect of Chayotte (*Sechium edule*) extract on the biodistribution of technetium-99m and on the morphometry of red blood cells. *J Labelled Cpd Radiopharm* 2001; 44: 648-650.
9. Oliveira JF, Braga ACS, Avila ASR, Gutfilen B, Bernardo-Filho M. Effect of *Thuya occidentalis* on the labeling of red blood cells and plasma proteins with technetium-99m. *Yale J Biol Med* 1997; 69: 489-494.

10. Vidal MV, Gutfilen B, Fonseca LMB, Bernardo-Filho M. Influence of tobacco on the labeling of red blood cells and plasma proteins with technetium-99m. *J Exp Clin Cancer Res* 1998; 17: 1-16.
11. Reiniger IW, Oliveira JF, Araujo AC, Bernardo-Filho M. Effect of *Peumus boldus* on the labeling of red blood cells and plasma proteins with technetium-99m. *Appl Rad Isotopes* 1999; 51:145-149.
12. Braga ACS, Oliveira MBN, Feliciano GD et al. The Effect of Drugs on the Labelling of Blood Elements with Technetium-99m. *Curr Pharm Design* 2000; 6: 1179-1191.
13. Oliveira JF, Braga ACS, Ávila ASR et al. Assessment of the effect of *Maytemus icilifolia* (espinheira santa) extracts on the labelling of red blood cells and plasma proteins with technetium-99m. *J Ethnopharmacol* 2000; 72: 179-184.
14. Lima EAC, Dire G, Mattos DMM et al. Effect of an extract of cauliflower (leaf) on the labeling of blood elements with technetium-99m and on the survival of *Escherichia coli* AB 1157 submitted to the treatment with stannous chloride. *Food chem Toxicol* 2002; 40: 919-923.
15. Oliveira JF, Avila AS, Braga ACS et al. Effect of extract of medicinal plants on the labeling of blood elements with Technetium-99m and on the morphology of red blood cells: a study with *Paullinia cupana*. *Fitoterapia* 2002; 73: 305-312.
16. Oliveira JF, Oliveira MB, Avila AS et al. Assessment of the effect of *Fucus vesiculosos* extract on the labeling of blood constituents with technetium-99m and the histological modifications on the shape of the red blood cells. *Food Chem Toxicol* 2003; 41: 15-20.
17. Mongelli E, Desmarchelier C, Coussio J, Ciccia G. Biological studies of *Bolax gummifera*, a plant of the Falkland Islands used as a treatment of wounds. *J Ethnopharmacol* 1997; 56: 117-121.
18. Saha GB. *Fundamentals of Nuclear Pharmacy*. Springer-Verlag, New York, 1998.
19. Gutfilen B, Boasquevisque EM, Bernardo-Filho M. Calcium channel blockers: interference on red blood cells and plasma proteins labelling with Tc-99m. *Rer Esp Med Nucl* 1992; 11:195-199.
20. Srivastava SC, Straub RF. Blood cell labelling with tc-99m: progress and perspectives. *Semin Nucl Med* 1990; 1: 41-51.
21. Early PJ & Sodee DB. *Principles and Practice of Nuclear Medicine*. 2nd Ed. New York, 1995.
22. Cunha Bastos VLF, Cunha Bastos JF, Lima JS. Brain acetylcholinesterase as an in vitro detector of organophosphorus and carbamate insecticides in the water. *Water Res* 1991; 7: 835-840.
23. Junqueira LC, Carneiro J. *Histologia Básica*. 9a ed. Rio de Janeiro: Guanabara Koogan, 1999.
24. Hoffbrand AV, Pettit JE. *Clinical Haematology*. 2nd. London, 1994.
25. Hladik III WB, Saha GB, Study KT. *Essentials of Nuclear Medicine*, Williams & Wilkins, Baltimore-London, 1987.
26. Hesslewood S, Leung E. Drug interactions with radiopharmaceuticals. *Eur J Nucl Med* 1994; 21:348-356.
27. Owunwanne A, Patel M, Sadek S. *The Handbook of Radiopharmaceuticals*. Chapman and Hall Medical, 1995.
28. Sampson CB. Complications and difficulties in radiolabelling blood cells: a review. *Nucl Med Commun* 1996; 17: 648-658.
29. Sastre J, Millan A. A Ginkgo biloba extract (Egb 761) prevents mitochondrial aging by protecting against oxidative stress. *Free Rad Biol Med* 1998; 24: 298-304.
30. Moreno SRF, Feliciano GD, Freitas RS et al. Effect of *Ginkgo biloba* on the labeling of blood elements with technetium-99m: in vitro study. *Rev Bras Farmacogn* 2002; 12: 62-63.
31. Santos-Filho SD, Ribeiro CK, Dire GF et al. Morphological Alterations on Red Blood Cells Labeled with Technetium-99m: the Effect of *Mentha crispa L.* (Hortela) and *Piper methysticum* (Kava Kava) Extracts. *Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine*, SG Editoriali, Padova 6: 503-505.
32. Callahan RJ, Rabito AC. Radiolabeling of erythrocytes with technetium-99m: role of band-3 protein in transport of pertechnetate across the cell membrane. *J Nucl Med* 1990; 31: 2004-2010.