

## Effect of a Peel Passion Fruit Flour (*Passiflora edulis f. flavicarpa*) Extract on the Labeling of Blood Constituents with Technetium-99m and on the Morphology of Red Blood Cells

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### ABSTRACT

*Passiflora edulis f. flavicarpa* (maracujá) is a fruit consumed in Brazil and worldwide. Blood constituents labeled with technetium-99m (<sup>99m</sup>Tc) are used in nuclear medicine. The effect of *P. flavicarpa* extract on the radiolabeling of blood constituents and on red blood cells morphology was evaluated. Blood samples from Wistar rats was incubated with *P. flavicarpa* extract. After that, the labeling of blood constituents with <sup>99m</sup>Tc was carried out. Samples of plasma and blood cells were precipitated with trichloroacetic acid to isolate the soluble and insoluble fractions of plasma and blood cells. The radioactivity in each fractions was counted and the percentage of radioactivity was determined. Blood smears were also prepared to morphological evaluation and perimeter/area ratio determination. *P. flavicarpa* extract altered ( $p < 0.05$ ) the fixation of <sup>99m</sup>Tc on plasma proteins and the perimeter/area ratio of red blood cells. Substances present in *P. flavicarpa* extract could affect the labeling of blood constituents with <sup>99m</sup>Tc acting in specific targets as membrane of red blood cells.

**Key words:** Labeling, Blood, *Passiflora edulis f. flavicarpa*, Radiobiocomplexes, technetium-99m

### INTRODUCTION

Passion fruit, *Passiflora edulis f. flavicarpa* (*P. flavicarpa*) is a fruit widely consumed worldwide and in Brazil is commonly known as “maracujá”. The pulp of this fruit contains ascorbic acid and carotenoids (Talcoot et al., 2003). The passion fruit peel corresponds to 60% of the fruit and it contains pectin (21.5%), tryptophan, grass acid and

sulphured aminoacids (Guertzenstein, 1998). It has been reported some benefic effects of the *P. flavicarpa* flour in Wistar diabetic rats, such as cholesterol level reduction, increased glucose tolerance, prolonging gastric-emptying half-time, (Guertzenstein, 1998) increased protein metabolism (Pirman et al., 2007).

There are also studies in human suggesting an effect of pectin in reduction of cholesterol

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(Guertzenstein, 1998; Schwiezer et al., 1991), glucose blood levels (Schwartz et al., 1988) and glucose absorption in human intestine (Fuse et al., 1989).

Technetium-99m ( $^{99m}\text{Tc}$ ) is the most utilized radionuclide in nuclear medicine to obtain single photon emission computed tomography (SPECT). Its wide use in nuclear medicine is due to its optimal characteristics (half-life of 6h, gamma ray energy of 140 keV, easily obtained from a  $^{99}\text{Mo}/^{99m}\text{Tc}$  generator, minimal dose to the patient and negligible environmental impact) (Hladik III et al., 1987). The chemical form of the  $^{99m}\text{Tc}$  obtained from the generator is sodium pertechnetate ( $\text{Na}^{99m}\text{TcO}_4$ ). This ion has an oxidation number +7, which is practically nonreactive and can not label any compound (Saha, 2004). The labeling of some biocomplexes (Moreno et al., 2004; Bernardo-Filho et al., 2005) needs a reducing agent and stannous chloride ( $\text{SnCl}_2$ ) is the most utilized with this purpose (Saha, 2004).

Red blood cells (RBC) labeled with  $^{99m}\text{Tc}$  are used in research (Moreno et al., 2004; Bernardo-Filho et al., 2005; Santos-Filho et al., 2005; Freitas et al., 2007) and in many procedures of nuclear medicine, including studies for spleen localization, gastrointestinal bleeding sites, blood cells mass localization, cardiovascular system studies, placental studies (Saha, 2004).

Plasma proteins (macroaggregated albumin) can be labeled with  $^{99m}\text{Tc}$  and it can be used for lung perfusion imaging (Saha, 2004).

Sequential steps of the intracellular labeling process of the RBC include: (i) transmembrane transport of stannous and pertechnetate ions into internal compartment of RBC; (ii) reduction of  $^{99m}\text{Tc}$  ( $^{99m}\text{TcO}_4$ ) by the  $\text{SnCl}_2$  and (iv) binding of the reduced  $^{99m}\text{Tc}$  to hemoglobin (Callahan and Rabito, 1990).

Due the importance of the labeled blood constituents in nuclear medicine, the aim of this study was to evaluate the effect of a *P. flavicarpa* extract on the labeling of blood constituents with  $^{99m}\text{Tc}$  and on the morphology of red blood cells using an *in vitro* experimental model.

## MATERIALS AND METHODS

*Wistar* male rats (250-300g, 3-4 months) were obtained from the Laboratório de Radiofarmácia Experimental (Departamento de Biofísica e Biometria, Universidade do Estado do Rio de Janeiro, RJ, Brazil). They were maintained under controlled room conditions ( $22\pm 5^\circ\text{C}$ , 12 h of light/dark cycle), water and normal diet (*ad libitum*). Experiments were conducted in accordance with the Department Committee of Animal Care of this University (Protocol number CEA/133/2006).

A commercial of Peel Passion Fruit Flour was obtained from A.S.S. Neto's Alimentos LTDA., Rio de Janeiro, Brazil, (Lot 0001415).

In the preparation of the extract, 0.5g of the flour was diluted to 10mL in saline solution (0.9% NaCl) and shaken (2 minutes). The preparation was centrifuged (clinical centrifuge, 5 minutes, 1500 rpm). The supernatant solution was retired and considered 100% (50mg/mL) solution. The concentrations 25mg/mL, 12.5mg/mL, 6.25mg/mL and 3.12mg/mL were also obtained with successive dilutions in saline solution.

A spectrophotometric analysis (Analyser, 800M, São Paulo, Brazil) of *P. flavicarpa* extract was carried out. The absorbance at 480 nm was considered the marker of the quality control of this extract. All the prepared extracts to be used in the experiments must have the optical density close to 0.18 (Fig. 1) to be used in the experiments.

Blood samples (0.5mL) were incubated with 100 $\mu\text{L}$  of different concentrations of *P. flavicarpa* extract for 60 minutes at room temperature. As control, samples of heparinized whole blood were incubated with 0.9% NaCl solution. After this period of time, 0.5mL of stannous chloride (Sigma Chemical Co., USA) (1.2 $\mu\text{g}/\text{mL}$ ) solution (freshly prepared) were added and incubated for more 60 minutes. Then, 0.1mL (3.7MBq) of  $^{99m}\text{Tc}$ , as sodium pertechnetate, (*Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, São Paulo, Brazil*) was added and the incubation continued for more 10 minutes. These samples were centrifuged and plasma (P) and cells (BC) were separated. Samples (20 $\mu\text{L}$ ) of P and BC were also precipitated with 1ml of 5% trichloroacetic acid (TCA) and insoluble (IF) and soluble fractions (SF) isolated. The radioactivity of the samples was determined in a well counter (Automatic Gamma Counter, C5002, Packard, USA). After that, the percentage of radioactivity

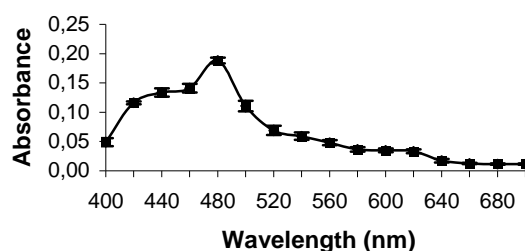
(%ATI) was calculated as described elsewhere (Bernardo-Filho et al. 1983).

The morphometry study of the red blood cells was also performed with the samples of blood incubated with *P. flavicarpa* extract (all concentrations). One drop of each sample was smeared in glass slides (5 slides for each sample) and the May-Grünwald-Giemsa method was performed (Junqueira and Carneiro, 2004). The smears were evaluated under a light microscope of clear field (Eclipse E 400<sup>TM</sup>), in the immersion objective (100x), with photographer ocular. Quantification of the data was realized by Software image pro plus (media Cyberbetics) Statistical analysis (Kruskal-Wallis with post-test Dunns,  $p < 0.05$ ) was used to compare the experimental data with the controls.

## RESULTS

The Fig. 1 shows the absorbance spectrum of a *Passiflora edulis f. flavicarpa* extract. The data in this figure indicate that the main absorbance peak (0.18) of this *P. flavicarpa* extract occurs at 480 nm.

Table 1 shows the effect of the *P. flavicarpa* extract on the distribution of the radioactivity in plasma and cellular compartments. The results indicate no significant ( $p > 0.05$ ) alteration of the distribution of <sup>99m</sup>Tc between plasma and cellular compartments.



**Figure 1** - The absorption spectrum of a *P. flavicarpa* extract

**Table 1** - Effect of *P. flavicarpa* extract on the distribution of radioactivity between plasma and cellular compartments of the whole blood.

<i>P. flavicarpa</i> (mg/ml)	ATI% Plasma	ATI% Blood Cells
Control	4.48 ± 1.79	95.52 ± 1.79
3.0	3.19 ± 2.30	96.81 ± 2.30
6.0	2.02 ± 0.48	97.98 ± 0.48
12	2.84 ± 1.68	97.16 ± 1.68
25	2.61 ± 2.95	97.39 ± 2.95
50	4.17 ± 3.28	95.83 ± 3.28

Blood samples were incubated with *P. flavicarpa* extract for 60 minutes and the radiolabeling procedure was carried out. The radioactivity of plasma (P) and cells (BC) was determined and the percentage of radioactivity (%ATI) was calculated.

Table 2 shows the fixation of the radioactivity on the plasma proteins. The results indicate that there is a significant decrease ( $p < 0.05$ ) of the fixation of <sup>99m</sup>Tc on the insoluble fraction of plasma at the highest concentrations.

Table 3 shows the fixation of the radioactivity on blood cell proteins. The results indicates no significant ( $p > 0.05$ ) alteration on the fixation of <sup>99m</sup>Tc on soluble and insoluble fractions of the blood cells.

The qualitative analysis of the shape of red blood cells from blood samples treated with *P. flavicarpa* extract has shown important morphological alterations in the red blood cells. Some blood cells have presented spikes on the membrane (Fig. 3). These alterations were not found when blood samples were incubated with 0.9% NaCl solution, which presented cells with a normal and characteristic shape (Fig. 2).

A morphometric parameter (perimeter/area ratio) of the red blood cells was also significantly altered ( $p < 0.05$ ) when blood samples were treated with *P. flavicarpa* extract (Table 4).

**Table 2** - Effect of *P. flavicarpa* extract treatment on the fixation of radioactivity on the plasma proteins.

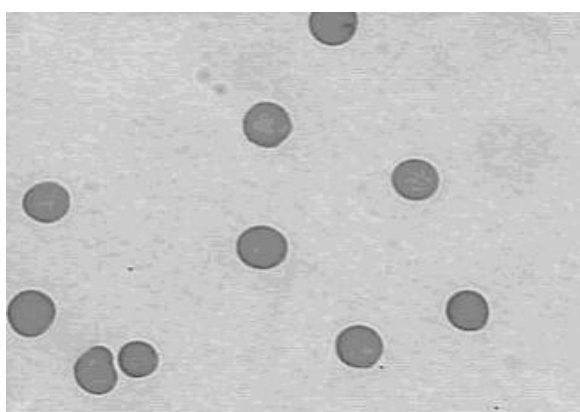
<i>P. flavicarpa</i> (mg/ml)	ATI%	ATI%
	Insoluble Fraction	Soluble Fraction
Control	72.77 ± 3.40	27.23 ± 3.40
3.0	72.37 ± 1.59	27.63 ± 1.59
6.0	67.36 ± 5.61	32.64 ± 5.61
12	67.93 ± 1.79	32.07 ± 1.79
25	*64.06 ± 3.14	35.94 ± 3.14
50	*53.98 ± 6.78	46.02 ± 6.78

Blood samples were incubated for 60 minutes with *P. flavicarpa* extract and the radiolabeling procedure was carried out. Aliquots of plasma were precipitated with TCA to isolate the soluble and insoluble fractions of plasma. The radioactivity in each fraction was determined and the percentage of radioactivity (%ATI) was calculated. (\*)  $p < 0.05$  when compared with control group.

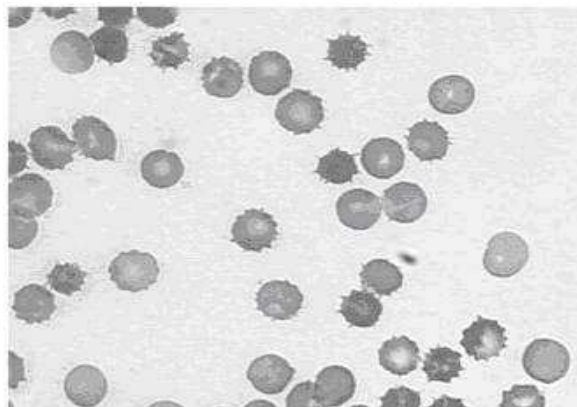
**Table 3** - Effect of *P. flavicarpa* extract treatment on the fixation of radioactivity on the blood cell proteins.

<i>P. flavicarpa</i> (mg/ml)	ATI%	ATI%
	Insoluble Fraction	Soluble Fraction
Control	92.82 ± 1.98	7.18 ± 1.98
3.0	93.29 ± 1.46	6.71 ± 1.46
6.0	93.46 ± 0.87	6.54 ± 0.87
12	93.43 ± 1.30	6.57 ± 1.30
25	92.99 ± 1.63	7.01 ± 1.63
50	90.61 ± 2.84	9.39 ± 2.84

Blood samples were incubated with *P. flavicarpa* extract for 60 minutes and the radiolabeling procedure was carried out. Aliquots of blood cells were precipitated with TCA to isolate the soluble and insoluble fractions of blood cells. The radioactivity in each fraction was determined and the percentage of radioactivity (%ATI) was calculated.



**Figure 2** - Photomicrography of blood smears from blood samples treated with NaCl 0.9%. Blood samples incubated with saline solution for 60 minutes. After that, smears were prepared, dried, fixed and staining. Morphology of red blood cells was evaluated under light microscopy (x1000)



**Figure 3** - Photomicrography of blood smears from blood treated with *P. flavicarpa* extract. Blood samples were incubated with *P. flavicarpa* extract (50mg/ml) for 60 minutes. After that, smears were prepared, dried, fixed and staining. Morphology of red blood cells was evaluated under light microscopy (x1000)

**Table 4** - The perimeter/area ratio of RBC incubated with PPF and labeled with  $^{99m}\text{Tc}$ .

<i>P. flavicarpa</i> (mg/ml)	Perimeter/area Ratio (1/ $\mu\text{m}$ )
0.0	0.42 $\pm$ 0.01
3.0	0.46 $\pm$ 0.01
6.0	0.42 $\pm$ 0.01
12	0.44 $\pm$ 0.01
25	*0.65 $\pm$ 0.04
50	*0.70 $\pm$ 0.09

Blood samples were incubated with *P. flavicarpa* extract for 60 minutes. After that, smears were prepared, dried, fixed and staining. Morphology of red blood cells was evaluated under light microscopy. After image caption, measurement of perimeter and area was performed and the perimeter/area ratio was determined.

## DISCUSSION

Although there is great variety of medicinal plants, there is not so much data relating natural products correct identification of their chemical composition, collateral effects, therapeutic properties and some biological properties (Messina, 2006). The radiolabeling of blood constituents has a great importance in nuclear medicine (Saha, 2004). Some authors have suggested that drugs could alter the radiolabeling of blood constituents (Moreno et al., 2002; Abreu et al., 2006; Silva et al., 2006), as well as, promote morphological alterations on the erythrocyte membrane (Braga et al., 2000; Oliveira et al., 2002; Oliveira et al., 2003; Santos-Filho et al., 2004; Moreno et al., 2004). Other studies report that some natural products could not interfere on the radiolabeling of blood constituents (Diré et al.,

2004; Fernandez et al., 2005) and could not modify the morphology of the red blood cells (Fernandez et al., 2005).

The analysis of the our results indicates that *P. flavicarpa* extract decreased the fixation of  $^{99m}\text{Tc}$  on plasma proteins and did not alter the labeling of blood cells with  $^{99m}\text{Tc}$ . It should be possible that the chemical compounds in *P. flavicarpa* extract could compete with the  $^{99m}\text{Tc}$  (pertechnetate) and/or stannous ions for the binding sites of the plasma proteins decreasing the fixation of  $^{99m}\text{Tc}$  on plasma proteins.

The morphological analysis showed an increase of the perimeter/area ratio of red blood cells from blood samples treated with *P. flavicarpa* extract (50mg/ml). Similar alterations were obtained when blood samples were treated with 25 mg/ml (data not shown). It could be suggested that some substances present in the *P. flavicarpa* extract

could promote morphological alterations on the membrane of red blood cells as shown in the Fig. 3. Alterations on the erythrocyte membrane have also been reported to other extracts as *Tobacco* (Braga et al., 2000), *Maytenus ilicifolia* (Oliveira et al., 2000), *Paulinia cupana* (Oliveira et al., 2002), *Coffea Arabica* (Oliveira et al., 2003), *Fucus vesiculosus* (Oliveira et al., 2003) and *Ginkgo biloba* (Moreno et al., 2004). In these studies when the morphological alterations occurred, the red blood cells labeling with  $^{99m}\text{Tc}$  was also altered by the extracts. *P. flavicarpa* extract is capable to modify the erythrocyte membrane morphology, but is not capable to modify the labeling of blood cells. It may possible that the morphological alterations produced by chemical compounds in *P. flavicarpa* extract on the membrane red blood cells could not interfere on the ions transport mechanism into the cell. In consequence the labeling of the blood cells would be not altered.

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## RESUMO

*Passiflora edulis* f. *flavicarpa* (maracujá) é um fruto consumido no Brasil e no mundo. O efeito de um extrato de farinha da casca de maracujá na marcação dos constituintes sanguíneos com tecnécio-99m e na morfologia de hemácias foi avaliado. Amostras de sangue de ratos Wistar, foram incubadas com extrato de *P. flavicarpa*. Em seguida, o procedimento de marcação de constituintes sanguíneos com  $\text{Tc-99m}$  foi realizado. Amostras de plasma e células sanguíneas foram separadas e alíquotas destas frações foram precipitadas com ácido tricloroacético para isolamento das frações solúvel e insolúvel do plasma e das células sanguíneas. A radiatividade em cada fração foi contada a porcentagem de radioatividade (%ATI) foi calculada. Distensões sanguíneas foram também preparadas para avaliação morfológica e da

relação perímetro/área de hemácias. O extrato de *P. flavicarpa* alterou a fixação do  $^{99m}\text{Tc}$  nas proteínas plasmáticas e a relação perímetro/área das hemácias. Substâncias presentes no extrato de *P. flavicarpa* poderiam afetar a marcação de constituintes sanguíneos com  $^{99m}\text{Tc}$  atuando em alvos específicos, como a membrana das hemácias.

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