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Guava extract (*Psidium guajava*) alters the labelling of blood constituents with technetium-99m*

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Abstract: *Psidium guajava* (guava) leaf is a phytotherapic used in folk medicine to treat gastrointestinal and respiratory disturbances and is used as anti-inflammatory medicine. In nuclear medicine, blood constituents (BC) are labelled with technetium-99m (^{99m}Tc) and used to image procedures. However, data have demonstrated that synthetic or natural drugs could modify the labelling of BC with ^{99m}Tc. The aim of this work was to evaluate the effects of aqueous extract of guava leaves on the labelling of BC with ^{99m}Tc. Blood samples of *Wistar* rats were incubated with different concentrations of guava extract and labelled with ^{99m}Tc after the percentage of incorporated radioactivity (%ATI) in BC was determined. The results suggest that aqueous guava extract could present antioxidant action and/or alters the membrane structures involved in ion transport into cells, thus decreasing the radiolabelling of BC with ^{99m}Tc. The data showed significant (P<0.05) alteration of ATI in BC from blood incubated with guava extract.

Key words: Antioxidant, Blood constituents, *Psidium guajava*, Technetium-99m
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INTRODUCTION

Psidium guajava (guava), belonging to the family of *Myrtaceae*, is a native of tropical America and has long been naturalized in Southeast Asia. The positive effects of guava extracts on human ailments have been described (Lozoya, 1999).

The pharmacological actions and the medicinal uses of aqueous extracts of guava leaves in folk medicine include the treatment of various types gastrointestinal disturbances such as vomiting, diarrhea, inhibition of the peristaltic reflex, gastroenteritis, spasmolytic activity, dysentery, abdominal distention, flatulence and gastric pain (Lutterodt, 1992; Aguilar *et al*., 1994; Lozoya *et al*., 1994). These extracts have also been indicated to cause disturbances of the central nervous system: insomnia, convulsions and epilepsy (Lutterodt and Maleque, 1988; Meckes *et al*., 1996). Bronchitis, asthma attacks, cough, pulmonary diseases could be also treated with guava teas (Batick, 1984; Khan and Ahmad, 1985) and could also be useful as anti-inflammatory and hemostatic agent (Liu, 1988).

Moreover, aqueous extracts of guava leaves were described to be effective against a number of microbial strains: *Aeromonas hydrophila*, *Shigella* spp. and *Vibrio* spp. (Chulasiri *et al*., 1986), *Staphylococus aureus* and *β-streptococcus* group A (Jaiarj *et al*., 1999), *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* (Abdelrahim *et al*., 2002). In addition,

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anti-rotavirus activity has also been reported to exist in these extracts (Gonçalves *et al*., 2005).

Phytochemical studies have identified more than 20 compounds in guava extracts (Osman *et al*., 1974; Begum *et al*., 2002). The major constituents of its leaves were identified to be tannins, β-sitosterol, maslinic acid, essential oils, triterpenoids and flavonoids (Osman *et al*., 1974; Arima and Danno, 2002; Begum *et al*., 2002; 2004).

Technetium-99m (^{99m}Tc) has been the most used radionuclide in nuclear medicine diagnosis procedures (single photon emission computed tomography, SPECT) and in labelling of molecular and cellular structures that can be used as radiopharmaceuticals (radiobiocomplexes) (Saha, 2003).

Red blood cells labelled with ^{99m}Tc (^{99m}Tc-RBC) are radiobiocomplexes widely used in clinical nuclear medicine for several important applications (Wong *et al*., 2004; Zaman *et al*., 2004; Olds *et al*., 2005; Harel *et al*., 2005; Artiko *et al*., 2004; Verdu *et al*., 2005; Cortes *et al*., 2003; Jin *et al*., 2004; Slart *et al*., 2004).

Sequential steps of the intracellular labelling process of blood constituents include: (1) transmembrane transport of reducing agent (Sn^{2+}) and $^{99m}TcO_4^-$ ions into the internal compartment of the RBC, (2) reduction of ^{99m}Tc ($^{99m}TcO_4^-$) by Sn^{2+}, and (3) subsequent binding of the reduced ^{99m}Tc to hemoglobin (Dewanjee *et al*., 1982). The band-3 anion transport system and calcium channels may be involved in transport of these ions (Callahan and Rabito, 1990; Gutfilen *et al*., 1992; Sampson, 1996).

Several authors have reported the effect of artificial and natural drug on the radiolabelling process (de Oliveira *et al*., 2003a; 2003b; Frydman *et al*., 2004; Moreno *et al*., 2004; Fonseca *et al*., 2005). The drugs could alter the labelling of blood constituents acting as antioxidant agent, modify the membrane structure or decrease the efficiency of transmembrane transport system of stannous and pertecnetate ions into cells. In this context, compounds in aqueous extracts of guava leaves have been reported to present antioxidant action and some of their pharmacological effects could be related to interaction in calcium channels (Morales *et al*., 1994; Qian and Nihorimbere, 2004). However, to the authors' knowledge, there is no report on the possible effects of guava extracts on radiolabelling of blood constituents as RBC or plasma proteins.

This work was aimed at evaluating the effects of aqueous extract of guava leaves on the labelling of blood constituents with technetium-99m.

MATERIALS AND METHODS

Animals

Adult male *Wistar* rats (3~4 months of age, body weight 250~350 g) were maintained in a controlled environment and allowed free access to water and food and ambient temperature was kept at (25±2) °C. Experiments were conducted in accordance with the rules of the Institutional Committee of Animal Care.

Preparation of guava extract

The guava extracts were prepared with leaves of *Psidium guajava* L. (Conde Garcia *et al*., 2003) obtained from the Campus of the Federal University of Sergipe (Aracaju, Brazil), and collected between June and July of 2000 (plants were free of toxic compounds). A botanic specimen was compared with the exsicata at Federal University of Sergipe Herbarium, Brazil (code ASE 03304, collectors T.S. dos Santos and V.S. Paes, collectors No. 4122, registering book No. 1, page 138, exsicata No. 04531).

To prepare the extracts, 20 mg of dry leaves were ground in 2 ml of 0.9% NaCl (100 °C, 5 min). The crude extract was filtered, centrifuged (1500 r/min, 5 min) to obtain the final extract. The supernatant was considered as the concentration at 10 mg/ml of guava leaves.

Radiolabelling in vitro of blood constituents

Heparinized blood (500 μl), was withdrawn from the *Wistar* rats and incubated with 100 μl of guava extract at different concentrations (6.25, 12.5, 25.0, 50.0 and 100.0 mg/ml) or with a saline solution alone, as control, for 1 h (room temperature). After this the radiolabelling of blood constituents with ^{99m}Tc ($Na^{99m}TcO_4$, Institute of Energetic and Nuclear Research, National Commission of Nuclear Energy, São Paulo, Brazil) was performed as described in (Bernardo-Filho *et al*., 1983). The samples were centrifuged (1500 r/min, 5 min) and aliquot of plasma (P) and blood cells (BC) were isolated. Another aliquots of P and BC were separated and pre-

cipitated in trichloroacetic acid (5%) and centrifuged (1500 r/min, 5 min) to isolate soluble (SF) and insoluble fractions (IF). The radioactivity in P, BC, SF-P, IF-P, SF-BC and IF-BC were determined in a well counter (Packard, model C5002, Illinois, USA) and the percentage of incorporated radioactivity (%ATI) was calculated as described in (Bernardo-Filho *et al*., 1983; 1986).

Statistical analysis

Data obtained are reported as means±standard deviation of %ATI. To compare the treated (5 blood samples for each extract concentration) and control groups (10 blood samples), the one way analysis of variance (ANOVA) test was performed to verify possible statistical difference between the experimental groups. After this, a rigorous statistical post test (Tukey) was chosen to identify the *P* value ($P<0.05$ as lesser significant level) and compare each treated group with control. InStat GraphPad software was used to perform statistical analysis (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California, USA).

RESULTS

Fig.1a shows the %ATI in insoluble (IF-P) and soluble (SF-P) fractions isolated from plasma separated from whole blood treated with different concentrations of guava extract. Analysis of these data indicates that guava extract significantly ($P<0.05$) reduced the radioactivity uptake in IF-P at the highest concentration studied (100.0 mg/ml) but no alteration was obtained with lesser concentration used.

Fig.1b shows the %ATI in insoluble (IF-BC) and soluble (SF-BC) fractions isolated from blood cells separated from blood treated with different concentrations of guava extract. Analysis of these data indicates that incubation with guava extract at low concentrations (6.0 and 12.5 mg/ml) did not reduce the %ATI of IF-BC. However, when the highest concentrations (25, 50 and 100 mg/ml) were used significant alterations of radioactivity uptake in insoluble fractions of blood cells resulted.

Fig.1c shows the %ATI in blood cells (BC) and plasma (P) from blood treated with different concentrations of guava extract. Analysis of these data indicated that guava extract alters the distribution of

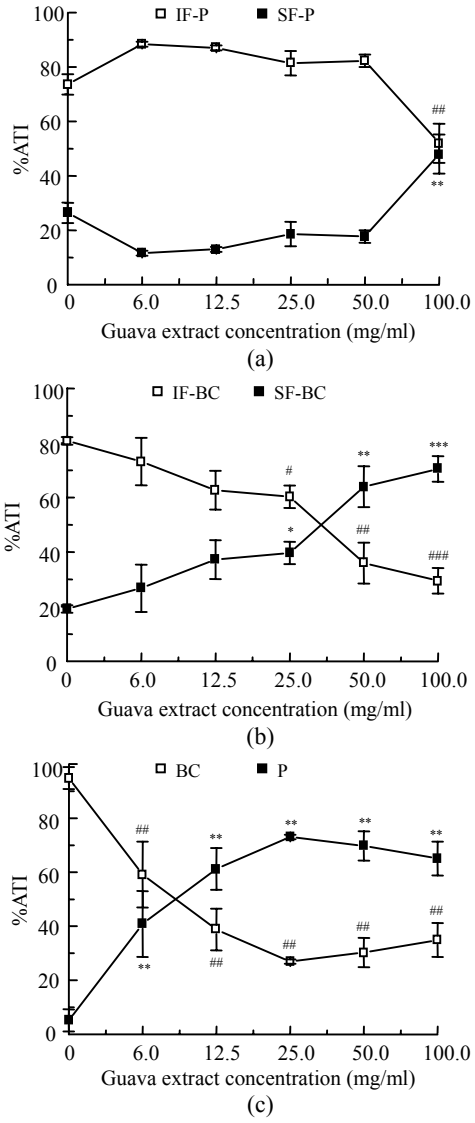

Fig.1 Effect of grava extract on (a) uptake of ^{99m}Tc by insoluble (IF-P) and soluble (SF-P) fractions of plasma (P), (b) uptake of ^{99m}Tc by insoluble (IF-BC) and soluble (SF-BC) fractions of blood cells (BC), and (c) the distribution of the ^{99m}Tc in the plasma and blood cells (BC) compartments, in the radiolabeling procedure of blood elements. Heparinized blood samples of *Wistar* rats were incubated with different concentrations of guava extract (1 h) and after with $SnCl_2$ (1.20 μg/ml, 1 h) and in sequence with $Na^{99m}TcO_4$ (3.7 MBq, 10 min). (a) Insoluble and soluble fractions of plasma (IF-P and SF-P) and (b) Insoluble and soluble fractions of blood cells (IF-BC and SF-BC) were obtained by precipitation with trichloroacetic acid (5%) and centrifugation (1500 r/min, 5 min). The radioactivity in these fractions was counted and the %ATI was calculated; (c) After centrifugation, plasma (P) and blood cells (BC) were isolated, the radioactivity was counted and the %ATI calculated

$^{\#}P\leq0.05$; $^{\#\#}P\leq0.01$; $^{\#\#\#}P\leq0.001$, when compared to control group of plasma; $^{*}P\leq0.05$; $^{**}P\leq0.01$; $^{***}P\leq0.001$, when compared to control group of blood cells

radioactivity in these two compartments (BC and P) at all guava extract concentrations studied. The data in this figure present an inverse dose-dependent relation with greater decreasing of cells %ATI at highest concentrations of guava extract.

DISCUSSION

Analysis of data presented in this study showed that aqueous guava extract could modify the fixation of ^{99m}Tc in plasma proteins only at high concentrations (Fig.1a). In cellular proteins, guava extract could affect the uptake of ^{99m}Tc at high but also at lesser concentrations (Fig.1b). However, the effects of this extract were more evident on the distribution of ^{99m}Tc between the blood compartments. The data analysis results suggest that aqueous extract of guava leaves used in this work could decrease the fixation of radioactivity in blood cell in a typical dose-dependent relation (Fig.1c).

Some authors have described that natural and synthetic products can alter the labelling of blood constituents with ^{99m}Tc (Frydman *et al*., 2004; Valenca *et al*., 2005; Fonseca *et al*., 2005). The labelling of blood constituents could decrease due to the action of drugs in (1) binding at the same sites on the blood constituents, (2) direct inhibition (chelating action) of the stannous and pertechnetate ions, (3) direct oxidation or generation of free radicals that could oxidize the stannous ion, (4) antioxidant action impeding or decreasing the stannous ion oxidization, and (5) alteration of the plasma membrane structure or modifying the transport systems of stannous and pertechnetate ions into cells.

Several compounds (as flavonoids) present in guava extracts are transported in blood attached to plasma proteins (Podhajcer *et al*., 1980). The fixation of ^{99m}Tc in plasma proteins occurs at different proteins sites with albumin being the principal protein involved (Early and Soddee, 1995). The binding sites in these proteins could be the same binding sites of ^{99m}Tc and thus in the presence of guava extract compounds the plasma protein labelling with ^{99m}Tc could be reduced. However, the data shown in this work suggest that at only highest concentration of aqueous guava extract these compounds (flavonoids and others phenolic compounds) could compete with the binding of ^{99m}Tc with plasma proteins.

The antioxidant activity of some compounds could be used to prevent various chronic diseases such as heart-disease, diabetes, cancer, arterial thrombosis, cataract and may provide health-promoting effects (Kimura *et al*., 1985; Qian and Nihorimbere, 2004). Chemical analysis of guava extracts has revealed the presence of essential oils, tannins, saponins, carotenoids, flavonoids and triterpenes (Cuellar *et al*., 1984; Mercadante *et al*., 1999; Arima and Danno, 2002; Begum *et al*., 2002). The different phenolic phytochemicals in guava leaves have been described to possess antioxidant action and able to inhibit peroxidation reaction in living systems (Kimura *et al*., 1985; Vanacker *et al*., 1995; Qian and Nihorimbere, 2004). Thus, some compounds present in guava extracts can inhibit the oxidation of the stannous ions and to alter the labelling of plasma and cellular proteins with ^{99m}Tc as well as interfere with the distribution of this radionuclide between plasma and cellular compartments.

Moreover, data have demonstrated that aqueous and hidroalcoholic extracts of guava leaves present anti-microbial activity (Arima and Danno, 2002; Qa'dan *et al*., 2005). Flavonoid glycosides and flavonoids as guaijavarin and quercetin have been isolated and present activity against *Salmonella enteritidis* and *Bacillus cereus* (Arima and Danno, 2002). This anti-microbial activity may be related to action on plasma membrane modifying its structure and decreasing or impeding the transport of stannous and pertechnetate ions into blood cells that can alter the labelling efficiency of blood constituents with ^{99m}Tc. On the other hand, the above cited compound quercetin has been described to decrease the spasmolytic activity in experimental models (Morales *et al*., 1994). This effect was related to a possible narcotic (opioid) component in the guava leaves (Lutterodt and Maleque, 1988; Lutterodt, 1992) while other data have indicated inhibition of acetylcholine release in neuromuscular junctions by calcium-antagonistic action of quercetin decreasing the inward calcium membrane current leading to a decrease of smooth muscle contractile force (Lozoya *et al*., 1994; Morales *et al*., 1994; Re *et al*., 1999) and depression of myocardial inotropism (Conde Garcia *et al*., 2003). The radiolabelling process of red blood cells with ^{99m}Tc depends on the entry of stannous and pertech-

netate ions into these cells through ionic channels (Callahan and Rabito, 1990; Gutfilen *et al*., 1992). The action of quercetin and/or other compounds present in aqueous guava extract blocking and/or binding on calcium and anions (as band 3) channels could make difficult the transport of stannous ions through plasma membrane and to decrease the labelling of blood cells with ^{99m}Tc explaining in part the data obtained in this work.

In conclusion, our data suggest that aqueous extracts of guava leaves could present antioxidant action and/or effects on the membrane structures involved in ions transport altering the radiolabelling of blood constituents with ^{99m}Tc and that precaution should be taken in examinations of nuclear medicine based on this procedure in patients using guava extracts.

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