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A PROPOLIS EXTRACT AND THE LABELING OF BLOOD CONSTITUENTS WITH TECHNETIUM-99m

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Since ancient times propolis has been employed for many human purposes because to their favourable properties. Blood constituents labeled with technetium-99m (^{99m}Tc) have been used in nuclear medicine procedures. Some authors have reported that synthetic or natural drugs can interfere with the labeling of blood constituents with ^{99m}Tc. The aim of this work was to evaluate the action of a propolis extract on the labeling of blood elements with ^{99m}Tc. Samples of whole blood of male Wistar rats were incubated in sequence with an aqueous propolis extract at different concentrations, stannous chloride and ^{99m}Tc, as sodium pertechnetate. Blood samples were centrifuged to separate plasma and blood cells, soluble and insoluble fractions of plasma and blood cells were also separated after precipitation in trichloroacetic acid solution and centrifugation. The radioactivity was counted and the percentage of incorporated radioactivity (%ATI) for each fraction was calculated. The data obtained showed that the aqueous propolis extract used decreased significantly the %ATI in plasma proteins at higher concentration studied. Results suggest that at high concentration the constituents of this extract could alter the labeling of plasma proteins competing with same binding sites of the ^{99m}Tc on the plasma proteins or acting as antioxidant compounds.

Keywords: Propolis – blood constituents – plasma proteins – technetium-99m

INTRODUCTION

Propolis, or bee glue, is a yellow-green to dark brown resin, actively secreted or exuded material collected by worker bees from leaf buds of numerous tree species [14, 16]. This resin is masticated, salivary enzymes added and the partially digested material is mixed with beeswax and used in hive [5, 46].

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Since ancient times propolis has been used as natural substance to numerous applications such as anti-putrefactive to embalm cadavers, antiseptic and cicatrizant, antimycotic, astringent as well as antipiretic and antibacterial agent [16]. The knowledge about propolis advanced and its other medicinal properties such as anti-viral [64], hepatoprotective [19, 31], anti-inflammatory [16, 39, 70] and antitumoral [29, 51, 68], treatment of gastroduodenal ulcers [44] and antioxidant [1, 2, 47, 62] have been described. Interest in dental medicine is considerable also due its anaesthesic effect and propolis is used in toothpaste and mouthwash preparations to treat gingivitis, cheilitis and stomatitis [17, 37, 43].

The composition of propolis depends of its source but in general it is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% others substances [18].

Flavonoid pigments are the principal compounds in propolis and correlate reasonable well with those present in the plants from which honeybees collect propolis [14]. Although ethanol extract of propolis is the most common, extracts with others solvents have been carried out and more than 200 constituents were identified [46]. Analysis of different extract has showed hydroquinone (0.1%), caffeic acid and its esters (2–20%) and quercetin (0.1–0.7%) [4, 5, 32–34].

In nuclear medicine, gamma emitters, as technetium-99m (^{99m}Tc), are widely used to label molecular and cellular structures (radiopharmaceuticals or radiobiocomplexes). These labeled structures can be utilized in obtaining images by single photon emission computerized tomography (SPECT) that indicates the physiological status of a tissue [26, 38, 63]. Others medical images, as X-ray radiography, computerized tomography (TC) or magnetic resonance (MR) are, in general, associated only with changes in anatomical structures.

^{99m}Tc, as sodium pertechnetate, has also been also used to label structures with biological interest [11, 12, 48, 57, 58] in basic research. The use of this radionuclide in nuclear medicine is due to its optimal physical characteristics (small half-life physics and biological, low gamma energy emission of 140 keV, availability from ^{99}Mo/^{99m}Tc generator and negligible environmental impact) [26, 38, 63].

Labeled red blood cells with ^{99m}Tc came into wide use in clinical nuclear medicine for several important applications, including imaging of cardiovascular system [52], peripheral arterial blood flow [36], evaluation of gastrointestinal bleeding [53, 71, 72], measurement of red cell volume [38], hepatic hemangiomas [3, 69], renal carcinoma [20] and splenic reticuloendothelial system [41, 67]. This labeling depends on several intracellular labeling steps, such as: (i) reduction of ^{99m}Tc (pertechnetate ion) by Sn^{2+} (stannous ion), (ii) transport mechanisms in which Sn^{2+} and ^{99m}Tc reach the internal compartment of the RBC (iii) subsequent binding to hemoglobin. The band-3 anion transport system and calcium channels may be the ways that ^{99m}Tc and Sn^{2+} ions, respectively, reach the interior of the red blood cells [15, 24, 35].

Some synthetic drugs [9, 28, 30, 65) as well as natural drugs [25, 50, 55, 56] have been reported to affect the labeling of blood constituents with ^{99m}Tc.

The aim of this work is to evaluate the effect of an aqueous propolis extract on the labeling of blood constituents with ^{99m}Tc.

MATERIALS AND METHODS

Animals

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

Preparation of propolis aqueous extract

Propolis was purchased (commercial propolis) (Orient Mix Fitoterápicos do Brasil Ltda.). The extract was prepared adding 400 mg of propolis in 10 ml of saline solution (NaCl 0.9%). After agitation during 2 minutes this mixture was filtered, centrifuged (2000 rpm, 10 min) to obtain the final extract. This preparation of the extract was considered 40 mg/ml.

Radiolabeling in vitro *of blood constituents*

Heparinized blood (500 µl), was withdrawn from Wistar rats and incubated with 100 µl of propolis extract at different concentrations (2.5; 5.0; 10.0; 20.0 and 40.0 mg/ml) or with a saline solution alone, as control, for 1 hour (room temperature). Afterwards, 500 µl of stannous chloride (1.20 µg/ml) was added and the incubation continued for further 1 hour. After this period, 100 µl of ^{99m}Tc (3.7 MBq) as sodium pertechnetate ($Na^{99m}TcO_4$), recently milked from a ^{99}Mo/^{99m}Tc generator (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, São Paulo, Brasil) were added and the incubation was continued for 10 minutes. These samples were centrifuged in a clinical centrifuge (1500 rpm, 5 min) and aliquots of 20 µl of plasma (P) and blood cells (BC) were isolated. Another aliquots of 20 µl of P and BC were separated and precipitated in 1.0 ml of trichloroacetic acid (5%) and centrifuged (1500 rpm, 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 elsewhere [8, 10].

Statistical analysis

Data are reported as means ± SD of %ATI and compared between the treated (n = 4 for each extract concentration) and control group (n = 10) by one-way analysis of variance – ANOVA, followed by Bonferroni post test, with a $p < 0.05$ as significant

level. InStat Graphpad software was used to perform statistical analysis (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California, USA).

RESULTS

Figure 1 shows the ATI% in blood cells (BC) and plasma (P) from blood treated with different concentrations of propolis extract. The analysis of these data indicates that propolis extract did not alter the distribution of radioactivity in these two compartments (BC and P).

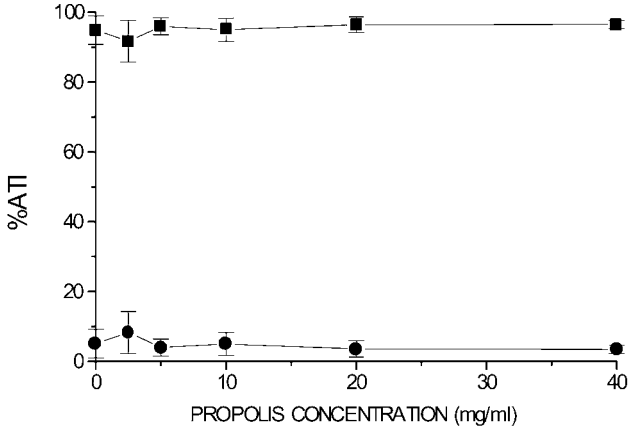

Fig. 1. Effect of propolis extract on the distribution of the ^{99m}Tc in the plasma (P) and blood cells (BC) compartments in the radiolabeling procedure of blood elements. Heparinized blood samples of Wistar rats were incubated with different concentrations of propolis extract (1 h) and after with $SnCl_2$ (1.20 μg/ml, 1 h) and in sequence with $Na^{99m}TcO_4$ (3.7 MBq, 10 min). After centrifugation, plasma (P) and blood cells (BC) were isolated, the radioactivity was counted and the ATI% calculated. (●) P and (■) BC

Figure 2 shows the ATI% in insoluble (IF-BC) and soluble (SF-BC) fractions isolated from blood cells separated from blood treated with different concentrations of propolis extract. The analysis of data indicates that propolis extract did not significantly alter the radioactivity uptake in insoluble blood cell fraction.

Figure 3 shows the ATI% in insoluble (IF-P) and soluble (SF-P) fractions isolated from plasma separated from whole blood treated with different concentrations of propolis extract. The analysis of this data indicates that propolis extract significantly ($p < 0.05$) reduced the radioactivity uptake in IF-P in the highest concentration studied (40.0 mg/ml).

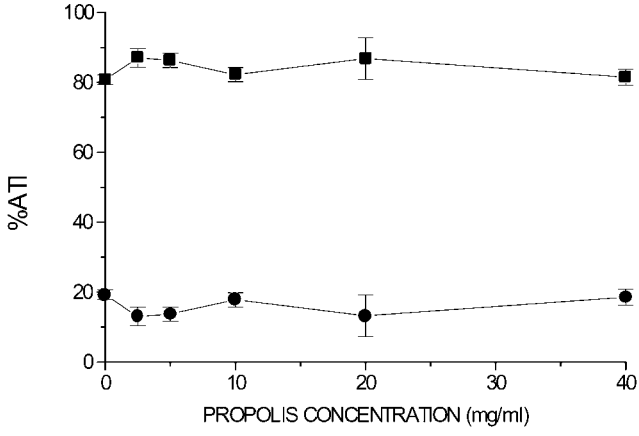

Fig. 2. Effect of propolis extract on uptake of ^{99m}Tc by insoluble (IF-BC) and soluble (SF-BC) fractions of blood cells (BC), in the radiolabeling procedure of blood elements. Heparinized blood samples of Wistar rats were incubated with different concentrations of propolis extract (1 h), after with $SnCl_2$ (1.20 µg/ml, 1 h) and in sequence with $Na^{99m}TcO_4$ (3.7 MBq, 10 min). Insoluble and soluble fractions of blood cells (IF-BC and SF-BC) were obtained by precipitation with trichloroacetic acid (5%) and centrifugation (1500 rpm, 5 min). The radioactivity in these fractions were counted and the ATI% were calculated. (●) SF-BC and (■) IF-BC

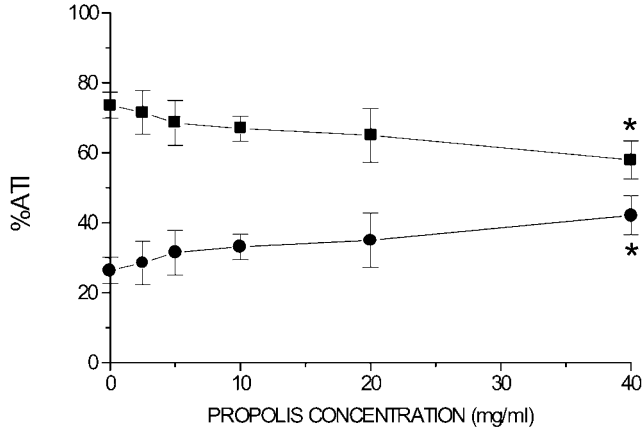

Fig. 3. Effect of propolis extract on uptake of ^{99m}Tc by insoluble (IF-P) and soluble (SF-P) fractions of plasma (P), in the radiolabeling procedure of blood elements. Heparinized blood samples of Wistar rats were incubated with different concentrations of propolis extract (1 h) and after with $SnCl_2$ (1.20 µg/ml, 1 h) and in sequence with $Na^{99m}TcO_4$ (3.7 MBq, 10 min). Insoluble and soluble fractions of plasma (IF-P and SF-P) were obtained by precipitation with trichloroacetic acid (5%) and centrifugation (1500 rpm, 5 min). The radioactivity in these fractions were counted and the ATI% were calculated. (●) SF-P and (■) IF-P. (***) $p \leq 0.001$, when compared to control group

DISCUSSION

There are numerous clinical and experimental studies about the effects and pharmacological interactions between natural and synthetic drugs. However, the data concerning interaction of diagnostic agents, including radiopharmaceuticals, with therapeutic drugs are relatively scarce. The development and use of *in vitro* tests can bring important information about the possible drug/radiopharmaceutical interactions.

In this study, the analysis of the results indicated that the aqueous propolis extract did not affect the distribution of technetium between cellular and plasma compartments or the binding of this radionuclide in cellular proteins (Figs 1, 2).

The absence of effect of the aqueous propolis extract on the radiolabeling of cells and cellular proteins is in contrast with propolis antibacterial effect by modification of structure and energy transducing system of the cytoplasmic membrane [21, 22, 49, 54]. In addition, propolis extracts have also been associated with the inhibition of a variety of cellular events. Thus, it has been described that propolis extract inhibits enzyme activity [22, 40] and DNA synthesis [66] and decreases DNA lesions induced by a chemical agent [60]. Other data suggested that propolis constituents can diminish the tumoral cells proliferation decreasing the cellular oxidative metabolism [29]. However, the absence of alteration of distribution of ^{99m}Tc between cellular and plasma compartments and labeling of cellular proteins may be related to type and source of propolis extracts. An aqueous propolis extract was used in this study while the data above cited were obtained with other extract type. In fact, data have demonstrated that the extract composition and biological effects can vary depending on the solvent used and of its source [14, 18, 23, 24].

On other hand, the analysis of Figure 3 shows that the aqueous propolis extract at the highest propolis concentration used (40 mg/ml) could decrease the labeling of plasma proteins with ^{99m}Tc. These data could be explained by interactions between substances present in the propolis extract and the plasma protein at same sites of ^{99m}Tc binding in these proteins, reducing the radiolabeling efficiency. In fact, by far the largest group of compounds isolated of propolis are flavonoid pigments and after their intestinal absorption and arrival to the blood stream these substances are transported and attached to serum albumin [37, 59]. Another hypothesis is the antioxidant property of some propolis constituents that can block the action of stannous ions in labeling procedure reducing the binding of ^{99m}Tc in plasma proteins. Part of flavonoids absorbed in the intestine are found in the blood in conjugates that retain their antioxidative properties [45]. This antioxidant property has been related to several effects of propolis extracts as anti-inflammatory [16, 39, 70], hepatoprotection [6, 7, 31, 60] and antitumoral activity [17, 29, 51, 62, 68].

In conclusion, our data suggest that propolis may alter the labeling of blood constituents when used at high concentration due its binding to plasma proteins and antioxidant property. Although the described experiments have been carried out with animals, precaution is suggested in interpretations in terms of nuclear medicine involving the labeling of blood constituents in patients treated with propolis.

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