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A N I N T E R N A T I O N A L J O U R N A L

Effects of *Cinnamomum Zeylanicum* Treatment on Radiolabeling of Blood Constituents and Morphology of Red Blood Cells in Wistar Rats

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ABSTRACT

The aim of this study was to evaluate the effect of in vivo treatment with an aqueous cinnamon extract on the labeling of blood constituents with ^{99m}Tc and on the morphology of red blood cells from Wistar rats. Animals were treated with cinnamon extract at different doses and for different periods of time. As controls, animals treated with 0.9% NaCl. Labeling of blood constituents with ^{99m}Tc was performed. Plasma, blood cells and insoluble fractions were isolated. Radioactivity in each fraction was counted and the percentage of radioactivity (%ATI) was calculated. Also, blood smears were prepared to morphological analysis of red blood cells from. Data showed that in vivo cinnamon extract did not significantly ($p>0.05$) modify the %ATI of blood constituents and morphology of red blood cells. The results suggest that in vivo aqueous cinnamon could not affect the membrane structures involved in transport of ions or the oxidation state of stannous and pertechnetate ions.

Keywords*:* blood constituents, *Cinnamomum zeylanicum*, technetium-99m

INTRODUCTION

Cinnamomum zeylanicum (cinnamon) belongs to the family Lauraceae, originates from the Ceylon is an important spice having wide applications in perfumery and beverages (Jayaprakasha et al., 2003). It has been recognized to have medicinal properties and posses beneficial effects on health (Khan et al., 2003). Chemical analysis indicated polyphenolic and benzene compounds and phenolic volatile oils (Shan et al., 2005).

Blood constituents labeling with technetium-99m (^{99m}Tc) have been utilized in nuclear medicine (Saha, 2004) and basic scientific research (Fonseca et al., 2007). The labeling process with ^{99m}Tc depends on a reducing agent; stannous chloride ($SnCl_2$) is the most used for this purpose (Saha, 2004). The band-3 anion and calcium channels

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may be the transport systems by which pertechnetate ion and stannous ions pass through of the red blood cell (RBC) membrane, respectively (Callahan and Rabito, 1990; Gutfilen et al., 1996).

Some authors have demonstrated some biological effects of drugs and herbal extracts using the labeling of blood constituents with ^{99m}Tc as an *in vitro* assay (Fonseca et al., 2007; Santos-Filho et al., 2008; Paoli et al., 2008). Alterations on the membrane of RBC could interfere on this radiolabeling procedure (Benarroz al., 2008).

Despite of some biological effects of cinnamon are described, there is few information about the interaction of cinnamon with ^{99m}Tc-radiopharmaceuticals (Benarroz et al., 2008). In this study we assessed the effect of the *in vivo* treatment with an aqueous cinnamon extract on the labeling of blood constituents with ^{99m}Tc and on morphology of RBC cells from *Wistar* rats.

MATERIALS AND METHODS

Plant material and preparation of the extracts

A commercial dried powder cinnamon was purchased from *Yoki Alimentos SA*, São Paulo, Brazil, lot number 04E05C. To prepare the extract, 2.4 mg of powder were dissolved with 20 mL of saline (0.9% NaCl), centrifuged (1500 rpm, 5 min) and the extract was considered to be 120 mg/mL.

Animals

Wistar rats (3-4 months, 250-300 g) were kept in constant environmental conditions (25±2 ºC, 12 h of light/dark cycle). The experimental procedures were in accordance with the Institutional Committee of Animal Care (*Comissão de Ética para o Cuidado e Uso de Animais Experimentais, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro*) with the protocol number CEA/134/2006.

In vivo treatments

The animals (n=12, 4 rats each group) were treated by an oral via for 60 minutes with cinnamon extract (1.5, 15.0 and 150.0 mg/kg). Other animals (n=12, 4 rats each group) were treated with the higher dose (150.0 mg/kg) for different periods of time (15, 60 and 120 minutes). Control group (n=8 rats) was treated with 0.9% NaCl. The animals were anesthetized and heparinized whole blood was withdrawn to experimental procedures.

Radiolabeling procedure

Blood samples (n=10, for each dose) were incubated with $SnCl_2$ (1.20 μg/mL, 60 minutes). Afterwards, ^{99m}Tc (3.7MBq, $Na^{99m}TcO_4$), recently milked from a ^{99}Mo/^{99m}Tc generator (*Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear*, São Paulo, Brazil) was added (10 minutes). These samples were centrifuged (1500 rpm, 5 minutes) and aliquots of plasma (P) and blood cells (BC) were isolated. Another aliquots of P and BC were separated and also precipitated with 5% trichloroacetic acid and centrifuged (1500 rpm, 5 minutes) to isolate insoluble fractions (IF). The radioactivity in BC, IF-P and IF-BC was determined in a well counter (Packard, model C5002, Illinois, USA) and the percentage of radioactivity (%ATI) was calculated.

Morphological evaluation of red blood cells

Blood smears were prepared, dried, fixed and staining by May-Grünwald-Giensa method (Junqueira and Carneiro, 2002). Images of RBC were acquired (Optronics, Japan) from blood smears to qualitative morphology analysis under optical microscopy (x1000). To morphometric analysis of RBC the perimeter/area ratio was obtained from images by specific program (Image ProPlus Software).

Statistical analysis

The data are expressed as means ± SD of %ATI and perimeter/area ratio. The values were analyzed by one-way analysis of variance (ANOVA) with a $p<0.05$ as significant level. Statistical analysis was performed using InStat Graphpad software (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego, California, USA).

RESULTS

The figure 1 shows the ATI% of BC, IF-P and IF-BC from *Wistar* rats treated with cinnamon extract. Data indicate that there is no significant ($p>0.05$) modification on the uptake of radioactivity by BC and on the fixation of ^{99m}Tc on the IF-P and IF-BC from animals treated with cinnamon extract.

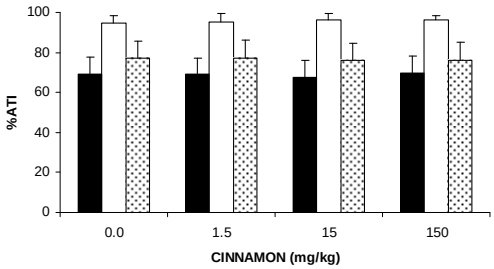

Figure 1 - Effect of *in vivo* treatment with cinnamon extract at different doses on the labeling of blood constituents with ^{99m}Tc. *Wistar* rats were treated with an aqueous cinnamon for 60 minutes, blood samples were withdrawn and the radiolabeling of blood constituents was carried out. Radioactivity was counted and the percentage of incorporated radioactivity (%ATI) was calculated. ( ) Blood cells, (■) insoluble fraction of plasma, (▒) insoluble fraction of blood cells.

Figure 2 shows the %ATI of BC, IF-P and IF-BC from *Wistar* rats treated with an cinnamon extract (150.0 mg/kg) for different periods of time. The data presented in this figure suggest that the extract has not significantly (p>0.05) altered the ^{99m}Tc-labeling of these blood constituents at the different times of treatment studied.

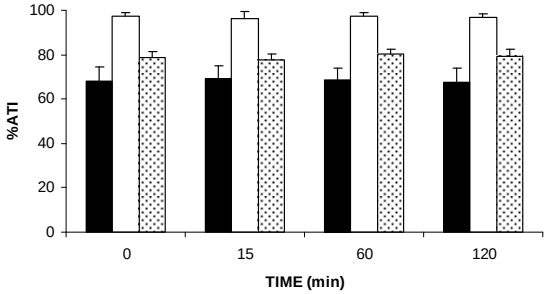

Figure 2 - Effect of *in vivo* treatment with cinnamon extract for different periods of times on the labeling of blood constituents with ^{99m}Tc. *Wistar* rats were treated with an aqueous cinnamon extract (150.0 mg/kg) for different periods of time. Blood samples were withdrawn and the radiolabeling of blood constituents was carried out. Radioactivity was counted and the percentage of incorporated radioactivity (%ATI) was calculated. ( ) Blood cells, (■) insoluble fraction of plasma, (▒) insoluble fraction of blood cells.

The figures 3 and 4 show the photomicrographs of the blood smears from *Wistar* rats treated with saline (control) and with an cinnamon extract at the higher concentration used (150.0 mg/kg), respectively. Qualitative morphological analysis suggests that the cinnamon extract has not induced important changes on the shape of the RBC.

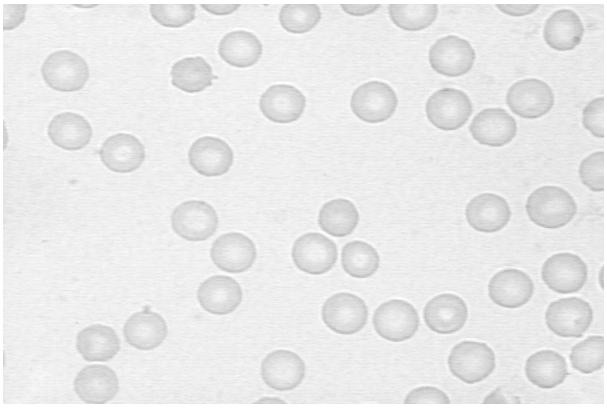

Figure 3 - Photomicrography of blood smear from *Wistar* rats treated with saline. *Wistar* rats were treated with saline (0.9% NaCl) for 1 hour, blood samples were withdrawn, and smears were prepared, dried and stained by May-Grünwald-Giemsa method. The slides were analyzed by optical microscopy (x1000).

Figure 4 - Photomicrography of blood smear from *Wistar* rats treated with cinnamon extract (150.0 mg/kg) for 60 minutes. *Wistar* rats were treated with saline (0.9% NaCl) for 1 hour, blood samples were withdrawn, smears were prepared, dried and stained by May-Grünwald-Giemsa method. The slides were analyzed by optical microscopy (x1000).

Figure 7 shows the perimeter/area ratio of RBC from *Wistar* rats treated with a cinnamon extract at different doses. Data indicate that the aqueous cinnamon extract has not significantly ($p>0.05$) modified the perimeter/area ratio of RBC.

The figure 5 shows the perimeter/area ratio of RBC from *Wistar* rats treated with a cinnamon extract (150.0 mg/kg) for different periods of time. These data confirm the absence of effects of the cinnamon extract used on the RBC morphology indicating no significant ($p>0.05$) modifications on the perimeter/area ratio.

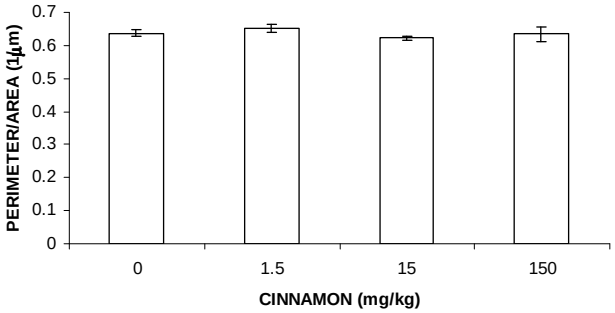

Figure 5 - Effect of *in vivo* treatment with cinnamon extract on the perimeter/area ratio of red blood cells. Morphometric measurements were performed to red blood cells from blood smears from *Wistar* rats treated with cinnamon extract for 60 minutes. A total of five fields per each slide and five slides to each extract dose were evaluated.

DISCUSSION

The data obtained in this work showed that cinnamon extract at different doses has not effects on the uptake of ^{99m}Tc by blood cells and fixation of ^{99m}Tc on plasma and blood cells proteins (Fig. 1). Also, no modifications on radiolabeling of blood constituents were obtained when animals were treated for up to 120 minutes with cinnamon extract at the higher dose used (Fig. 2).

It has been reported that *in vitro* natural or synthetic drugs could alter the labeling of blood constituents with ^{99m}Tc (Fonseca et al., 2005; Frydman et al., 2008a). However, other products are not able to interfere on this labeling process, as *Pfaffia sp*. (Fernandes et al., 2005). Recent data have suggested that *in vivo* acetylsalicylic acid treatment could alter the labeling of blood constituents with ^{99m}Tc (Fonseca et al., 2007).

Pharmacological effects of interest to human health have been reported with 1 up to 6 g per day of cinnamon as crude drug or extracts (Khan et al., 2003). These values are close to commonly-suggested doses (2 up to 4 g) to cinnamon as crude drug (WHO, 1999). In our experiments, animals were treated with cinnamon extract at doses similar to used by human beings as spices or herbal medicine. The absence of effects of the *in vivo* treatment with cinnamon extract are in disagree with previous data obtained in an *in vitro* study, whose data have suggested alterations on labeling of BC with ^{99m}Tc (Benarroz et al., 2008). This discrepancy could be explained by metabolic process of compounds in cinnamon extract that occurs when an *in vivo* treatment is performed. Other hypothesis could be the difference in the pharmacokinetic profile to each compound present in cinnamon extract (gastrointestinal absorption and plasma peak concentration) that did not occur in an *in vitro* treatment.

RBC has been proposed as a prototypical cellular system regarding drug mediated plasma membrane effects (Li et al., 1999). It has been suggested that cinnamon extracts could affect the membrane of eukaryotic cells inhibiting the Na+ - K+- ATPase (Verspohl et al., 2005) and calcium currents (Su et al., 1999). The qualitative and quantitative (area and perimeter) analyses have been used to evaluate the alterations induced by drugs on membrane of RBC (Frydman et al., 2008b). However, aqueous cinnamon extract used could not cause morphological modifications on the membrane of RBC at different doses (Fig. 3, 4 and 5) or different period of treatment (Fig. 6 and 7).

These results are agreed with data obtained in an *in vitro* study when similar cinnamon extract was used (Benarroz et al., 2008).

In conclusion, our data suggest that *in vivo* aqueous cinnamon extracts could not alter the labeling of blood constituents with ^{99m}Tc and could not affect the membrane structures involved in transport of ions or the oxidation state of stannous and pertecnetate ions.

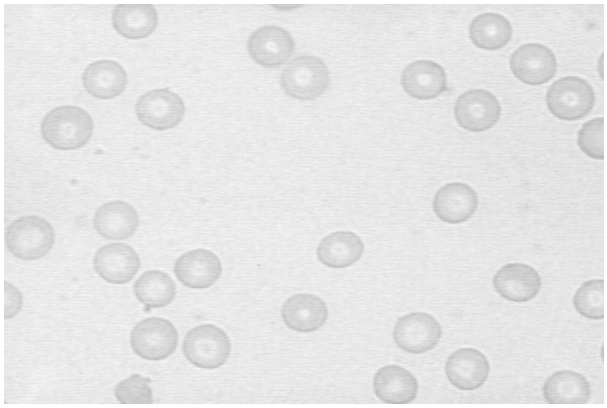

Figure 6 - Photomicrography of blood smear from blood from *Wistar* rats treated with a cinnamon extract (150.0 mg/kg) for 120 minutes. Blood samples were withdrawn; smears were prepared, dried and stained by May-Grünwald-Giemsa method. The slides were analyzed by optical microscopy (x1000).

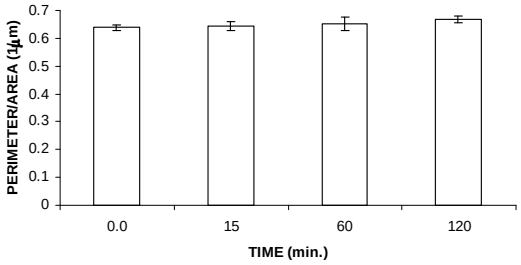

Figure 7 - Effect of *in vivo* treatment with a cinnamon extract for different periods of time on the perimeter/area ratio of red blood cells. Morphometric measurements of red blood cells from smears from *Wistar* rats treated with cinnamon extract (150.0 mg/kg) for different periods of time. A total of five fields per each slide and five slides to each extract dose were evaluated.

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RESUMO

O objetivo deste estudo foi avaliar efeitos do tratamento *in vivo* com um extrato aquoso de canela na marcação de constituintes sangüíneos com ^{99m}Tc e na morfologia de hemácias de ratos *Wistar*. Os animais foram tratados com diferentes doses ou por diferentes tempos com extrato de canela Como controles, animais tratados com NaCl 0,9%. A marcação de constituintes

sangüíneos com ^{99m}Tc foi realizada, plasma, células sangüíneas e frações insolúveis foram isoladas. A radioatividade em cada fração foi contada e a porcentagem de radioatividade (%ATI) foi calculada. Distensões sangüíneas foram preparadas para análise morfológica de hemácias. Os dados mostraram que o tratamento *in vivo* com extrato de canela não modificaria significativamente (p>0,05) a %ATI nos constituintes sangüíneos e a morfologia de hemácias. Os resultados sugerem que o extrato aquoso de canela não afetaria *in vivo* as estruturas da membrana envolvidas no transporte de íons ou o estado de oxidação dos íons estanoso e pertecnetato.

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