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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

Uncaria Tomentosa Extract: Evaluation of Effects on the *in Vitro* and i*n Vivo* Labeling of Blood Constituents with Technetium-99m

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ABSTRACT

The influence (in vivo and in vitro) of an Uncaria tomentosa extract (Cats claw) on the labeling of red blood cells (RBCs) and plasma and cellular proteins with technetium-99m (Tc-99m) was evaluated. For the in vivo treatment, animals were treated with Cats claw. For the in vitro treatment, heparinized blood was incubated with Cats claw before the addition of stannous chloride ($SnCl_2$) and Tc-99m. Samples of plasma (P) and RBCs were separated and also precipitated with trichloroacetic acid. The soluble and insoluble fractions of P and RBCs were isolated. The analysis of the results of the in vivo study, indicates that there is no significant alteration on the uptake of Tc-99m by the blood constituents, but it significantly decrease ($p<0.05$) the labeling of blood constituents by in vitro methods. These effects could be due to chelation of stannous and /or pertechnetate ions and blockage of the Tc-99m bindings sites.

Keywords: *Uncaria tomentosa*, technetium-99m, radiolabeling, blood constituents

INTRODUCTION

Medicinal plants have been used in many cultures. Biological effects due to the use of medicinal plants have been reported (Rotblatt et al., 2002). *Uncaria tomentosa*, also known as unha de gato ('Cats claw' in English), has become widely known as an useful plant in ethnomedicine. This plant is traditionally used to treat arthritis and rheumatism, ulcers and other disorders of the gastrointestinal tract, asthma, gonorrea, dengue, dysentery and tumors (Rotblatt et al., 2002; Pilarski et al., 2006; Reis et al., 2008). No side effects were reported in the few human studies, and no adverse effects were found in rodents (Rotblatt et al., 2002). *Uncaria*-drugs interactions have not been well reported, but one *in vitro* study

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revealed Cats claw inhibition activity on the 3A4 isozime of cytochrome P450 (Reis et al., 2008).

There are some studies about the effect of natural products on the labeling of blood constituents using technetium-99m (Tc-99m) (Lima et al., 2002; Oliveira et al., 1997; Braga et al., 2000; Oliveira et al., 2002; Oliveira et al., 2003; Dantas et al., 2005; Oliveira-Fernandes et al., 2005; Abreu et al., 2006; Neves et al., 2007; Rebello et al., 2007; Sinzinger et al., 2007). It has been reported that *Fucus vesiculosus* (Oliveira et al., 2003), *Ginkgo biloba* (Moreno et al., 2004), *Psidium guajava* (Abreu et al., 2006), *Vellozia pusilla* (Dantas et al., 2005) and *Arctium lappa* (Neves et al., 2007) extracts are capable of altering the radiolabeling of blood constituents.

The alteration of fixation of Tc-99m on the blood constituents promoted by some drugs has been associated with a possible oxidation of the stannous ion (Bernardo-Filho et al., 2005).

The aim of this work was to evaluate the effect of an *Uncaria tomentosa* (*U. tomentosa*) extract on the labeling of blood constituents with Tc-99m using *in vivo* and *in vitro* models.

MATERIAL AND METHODS

Commercial *Uncaria tomentosa* (Cats claw, *Herbarium Foundation for Health and Research*, Brazil Lot n^{o} 923661) was purchased and aqueous preparations (32 mg/mL) were prepared using a NaCl 0.9 % solution. Female *Wistar* rats (2 month-old and 180-210g) were obtained from *Universidade do Estado do Rio de Janeiro*, Brazil). Experiments were conducted in accordance with the Committee of Animal Care (Giles, 1987). The preparation was administered to female *Wistar* rats (7 days, intragastric via). Tc-99m, as sodium pertechnetate (0.3 mL, 3.7 MBq, *Instituto de Pesquisas Energéticas e Nucleares, CNEN*, São Paulo, Brazil) freshly milked from a ^{99}Molybdenium/^{99m}Technetium generator was injected into the ocular plexus and the animals were sacrificed (after 10 minutes). For the *in vivo* study, blood samples (0.5 mL) were obtained from these rats treated with Cats claw (32 mg/mL, n=6). For the *in vitro* study, blood samples (0.5 mL, n=6) obtained from the rats were incubated (60 minutes) with 100 μL of the Cats claw (32 mg/mL). The blood samples (*in vitro* and *in vivo* studies) received 0.5 mL of freshly prepared stannous chloride solution (1.2 μg/mL, *Sigma Chemical Co. St Louis, USA*, Lot 65H26736), under vacuum conditions and the incubation was continued for 60 minutes. Then, 100 μL of Tc-99m (3.7 MBq/mL, *Instituto de Pesquisas Energéticas e Nucleares, CNEN*, São Paulo, Brazil, from a ^{99}Molybdenium/^{99m}Technetium generator as sodium pertechnetate) was added (10 minutes). The samples were centrifuged for 5 minutes, and plasma (P) and blood cells (BC) were separated. Samples (20 μL) of P and BC (20 μL) were also precipitated with 1 mL of trichloroacetic acid (TCA 5%) and soluble (SF) and insoluble fractions (IF) were separated by centrifugation. The radioactivity in BC, IF-P and IF-BC were determined in a sodium iodide well counter (Automatic Gamma Counter, C5002, Packard, USA) and the percent of administered radioactivity (% ATI) was calculated. The results are presented as mean and standard diversion (S.D.), with a statistical analysis performed (ANOVA test, Tukey-Kramer test and Dunnet test).

RESULTS

Table 1 shows the distribution of the radioactivity in RBCs, the insoluble fraction of plasma (IF-P) and of blood cells (IF-BC) from whole blood of animals treated with *Uncaria tomentosa* extract (Cats claw). The results indicate no significant alteration in the uptake of Tc-99m by the blood cells. The same result was found with samples of plasma proteins (insoluble fraction of plasma, IF-P) and of cell proteins (insoluble fraction of blood cells, IF-BC) obtained from animals treated (P>0.05, table 1).

Table 1 - *In vivo* Study: Effect of an *Uncaria tomentosa* extract on the labeling of blood cells (BC) with Tc-99m and on the distribution of this radionuclide in the insoluble fractions of the plasma (IF-P) and of the blood cells (IF-BC) obtained after the labeling.

U. tomentosa	**BC**	**IF-P**	**IF-BC**
Control	91.6±3.9	69.5±1.8	74.2±1.9
32 mg/mL	97±0.7	73.3±0.9	76.7±2.4

Table 2 shows a significant decrease in the Tc-99m uptake by RBCs from 98.6±1.9 to 55.4±0.7 ($p<0.05$) in samples treated *in vitro* with Cats claw. This table also shows that the % ATI in the IF-P and in the IF-BC obtained from this same blood significantly decreased ($p<0.05$) from 68.1±4.8 to 11.6±3.4 and from 76.7±2.4 to 19.8±4.4, respectively.

Table 2- *In vitro* Study: Effect of an Uncaria tomentosa extract on the labeling of blood cells with Tc-99m and on the distribution of this radionuclide in the insoluble fractions of the plasma (IF-P) and of the blood cells (IF-BC) obtained after the labeling.

U. tomentosa	**BC**	**IF-P**	**IF-BC**
Control	98.6±1.9	68.1±4.8	76.7±2.4
32 mg/mL	55.4±0.7	*11.6±3.4	19.8±4.4

**P<0.05.*

DISCUSSION

Medicine-*Uncaria* interactions have not been objectively evaluated in published peer-reviewed studies (Rotblatt et al., 2002; Pilarski et al., 2006; Reis et al., 2008). Thus, it is important to develop models that can describe the possible *in vitro* and *in vivo* drug interactions.

Some authors have described that synthetic or natural products, can alter the labeling of blood constituents with Tc-99m (Braga et al., 2000; Gomes et al., 2002; Bernardo-Filho et al., 2005; Neves et al., 2007).

Moreno et al., (2004), have evaluated the effect of a *Ginkgo biloba* extract on labeling of blood constituents with Tc-99m using *in vitro* and *in vivo* study: a reduction of radiolabeling of blood cells was observed with the *in vitro* method (Moreno et al., 2004). *Psidium guajava* extract reduces the radioactivity uptake in IF-P (plasma proteins) and BC (blood cells) when used with an *in vitro* assay (Abreu et al., 2006). Neves et al., (2007) have reported that an *Arctium lappa* (burdock) extract significantly decrease the *in vitro* radiolabeling efficiency of blood cells. Rebello et al. (2007) studied the *in vitro* effect of a peel passion fruit flour on the labeling of blood constituents with Tc-99m. Their findings demonstrated a significant reduction of plasma proteins labeled with Tc-99m (Rebello et al., 2007).

Active chemical constituents of *U. tomentosa* include alkaloids, quinovic acid glycosides, polyhydroxylated triterpenes and several steroidal components (Rotblatt et al., 2002). These substances may be associated with the imunomodulatory, anti-inflammatory and anti-cancer properties, and also could be associated with the effect on the blood constituents radiolabeling. Therefore, the capacity of the *U. tomentosa* extract to inhibit the activity of the 3A4 isozime of cytochrome P 450 (Rotblatt et al., 2002) suggests that this extract has constituents that could be blockers of calcium channels (Klassen, 2001), preventing the entrance of stannous chloride in the cell. This mechanism may explain the effect on the radiolabeling of blood constituents promoted by the Cats claw (Table 2). As tannins (fractions of *U. tomentosa* extract) can precipitate proteins (Rotblatt et al., 2002), this also could explain the interference of the extract on the labeling of cells and plasma proteins. The *in vitro* effect of the *Uncaria tomentosa* extract on radiolabeling of blood constituents may be due to four possible mechanisms: (i) chelation of stannous and/or pertechnetate ions; (ii) competition of Tc-99m/stannous ions by the bindings sites; (iii) interference in the redox state of the labeling system, and (iv) blockage of calcium channels (Hesslewood et al., 1994; Bernardo-Filho et al., 2005). Results obtained from blood of animals treated with the extract, have shown that metabolization of *Uncaria tomentosa* extract after *in vivo* treatment was unable to alter the labeling of the blood constituents. Similar findings were reported for the *Pfaffia sp*. extract, wich also does not alter the labeling of blood constituents with technetium-99m when an *in vivo* model was employed (Oliveira-Fernandes et al., 2005).

In conclusion, the interference of *Uncaria tomentosa* components in the radionuclide labeling method may be explained by: chelation of stannous and/or pertechnetate ions; blockage of the Tc-99m binding sites and effect in the redox state of the labeling system.

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RESUMO

O objetivo do presente estudo foi avaliar a influência (*in vivo* e *in vitro*) de um extrato de *Uncaria tomentosa* (unha de gato) na marcação de hemácias e proteínas plasmáticas e celulares com tecnécio-99m (Tc-99m). Para o estudo *in vivo*, animais foram tratados com um extrato de unha de gato. Para o estudo *in vitro*, sangue heparinizado foi incubado com o extrato de unha de gato antes da adição de cloreto estanoso ($SnCl_2$) e Tc-99m. Amostras de plasma e células foram separadas e também precipitadas com ácido tricloracético. As frações solúveis e insolúveis foram isoladas. A análise dos resultados do estudo *in vivo*, indica que não houve alteração significante na captação de Tc-99m pelos constituintes sanguíneos, entretanto, no tratamento *in vitro*, ocorreu redução significante da marcação de constituintes sanguíneos. Esses efeitos poderiam ser justificados por quelação dos íons estanoso e pertecnetato e bloqueio dos sítios de ligação do Tc-99m.

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