Vol.50, Special Number : pp.123-128, September 2007
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

Effect of an Extract of *Artemisia vulgaris* L. (Mugwort) on the i*n vitro* Labeling of Red Blood Cells and Plasma Proteins with Technetium-99m

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ABSTRACT

The aim of this work was to evaluate the effect of an extract of the Artemisia vulgaris L. (mugwort) on the labeling of blood constituents with technetium-99m (99mTc). Blood samples from Wistar rats were incubated with a mugwort extract and the radiolabeling of blood constituents was carried out. Plasma and blood cells were separated by centrifugation. Aliquots of plasma and blood cells were also precipitated with trichloroacetic acid and centrifuged to isolate soluble and insoluble fractions of plasma and blood cells. Radioactivity in each fraction was counted and the percentages of radioactivity (%ATI) was calculated. Mugwort extract decreased significantly ($p<0.05$) the %ATI on the blood compartments and on the blood cells proteins (insoluble fraction). The analysis of the results indicates that the extract could have substances that could interfere on the transport of stannous through the erythrocyte membrane alterating the labeling of blood cells with 99mTc.

Key words: *Artemisia vulgaris* L., Technetium-99m, Blood constituents, labeling

INTRODUCTION

Artemisia vulgaris L. (mugwort), belonging to the family of Asteraceae, is a perennial weed growing wild and abundantly in temperate and cold-temperature zones of the word (Cui, 1989). In Traditional Chinese Medicine, mugwort has been used as an analgesic agent and in conjunction with acupuncture therapy (Yoshikawa et al., 1996), to treat the neonatal jaundice (Fok, 2001), gastric ulcers (Repetto et al., 2002), hepatitis (Tan et al., 1999) and convulsive crisis (Hickey et al, 2004).

Mugwort leaves and stem are used medicinally as a bitter digestive tonic, uterine stimulant and antirheumatic (Hickey et al., 2004). Some reports have revealed that mugwort is a potent immunomodulatory (Schmid-Grendelmeier et al., 2003), antihypertensive (Tigno et al., 2000), antinflammatory (Tigno and Gumila, 2000),

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antioxidant (Luo et al., 2007) and hepatoprotective agent (Gilani et al., 2005).

Antitumoral activity has been reported to artemisic acid and artemisinin B extracted from mugwort (Sun et al., 1992). Insect repellent and fumigant activity has been found in essential oils from mugwort (Wang et al., 2006). The insecticidal activity of essential oils from mugwort has been evaluated on *Aedes aegipti* (Chantraine et al., 1998). In addition, anti-viral activity has also been described to extracts of this plant (Tan et al., 1998).

Phytochemical studies have identified more than 20 flavonoids in mugwort extracts (Lee, 1998). Some flavonoids as well as acetylenes, coumarins, sesquiterpene lactones, and volatile oil components have previously been reported from mugwort (Marco et al., 1990). The most abundant compounds were eriodicyol and luteolin (Lee, 1998).

Despite the beneficial effects described by users, the indiscriminate use of this and other plants and infusions without medical advice/criteria can be dangerous. Several cases of biological effects of medicinal plants have been reported and drug interactions and side effects of these products are not completely known (Rotblatt and Ziment, 2002).

Molecules or cells labeled with technetium-99m (99mTc) are radiopharmaceuticals used in nuclear medicine procedures as imaging agents (Saha, 2004; Bernardo-Filho et al., 2005).

The labeling of red blood cells with 99mTc is a technique based on the reducing agent (stannous chloride) action on pertechnetate ion (Bernardo-Filho et al., 1983; Sampson, 1996, Saha, 2004).

Several factors can influence the labeling of blood constituents with 99mTc (Bernardo-Filho et al., 2005). Some investigators have reported that phytotherapeutic agents could alter the labeling of blood constituents with 99mTc (Oliveira et al., 2002; Santos-Filho et al., 2004; Abreu et al., 2006). The aim of this study was to evaluate the influence of mugwort extract on the labeling of blood constituents with 99mTc.

MATERIALS AND METHODS

The protocol was approved by the Ethical Committee of animal care in experiments of the *Universidade Federal de Pernambuco*.

Mugwort was collected and a specimen was identified by Professor *Iracema Loiola* of the *Universidade Federal do Rio Grande do Norte, Rio Grande do Norte*, Brazil. A voucher specimen was deposited in the *Universidade Federal do Rio Grande do Norte, Rio Grande do Norte*, Brazil, and has received the number UFRN-3872.

Wistar male rats (3-4 months, 300 ± 50 g) were maintained in a controlled environment and allowed free access to water and food and ambient temperature was kept at 25 ± 2 °C.

In the preparation of the mugwort extract, 100 ml of boiled saline (0.9% NaCl) solution was added to 1 g of leaves. After that, the preparation was keeped in infusion for 5 min. The preparation was filtered and the supernatant was considered to be 10 mg/ml. Stannous chloride ($SnCl_2$) was purchased from Sigma Chemical Co., St Louis, USA.

Heparinized whole blood was withdrawn from *Wistar* rats. Blood samples (500µl) were incubated with 100µl of mugwort extract at different concentrations (0.62, 1.25, 2.50, 5.00 and 10.00 mg/ml) or with saline solution alone, as control, for 1 h (room temperature). Then, 0.5 ml of a stannous chloride solution (1.2 µg/ml) freshly prepared was added and the incubation continued for another 1 hour. After that, 99mTc (0.1 ml; 3.7MBq), in the form of sodium pertechnetate ($99mTcO_4Na$) recently milked from a 99Mo/99mTc generator (*Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, São Paulo,* Brazil) was added and the incubation continued for 10 min, as previously described (Bernardo-Filho et al., 1983). These blood samples were centrifuged (1500 rpm, 5 min) to plasma and blood cells separation. Aliquots of plasma and blood cells were also precipitated in 5% trichloroacetic acid and centrifuged (1500 rpm, 5 min, clinical centrifuge) to isolate soluble and insoluble fractions. The radioactivity in each fractions was counted in a well counter (Automatic Gamma Counter, C5002, Packard, USA). The percentage of radioactivity (%ATI) in each fraction was calculated

The data are reported as media ± standard deviation of %ATI. Statistical analysis was performed by Kruskal-Wallis and Mann Whitney test. A $p<0.05$ was considered as statistical significance.

RESULTS

Table 1 shows the distribution of radioactivity in cellular and plasma compartments from blood incubated with mugwort extract. The results indicate that there was a significant (p<0.05) decrease in the radioactivity uptake by cellular compartment in the presence of mugwort extract when compared with the control (blood samples incubated with saline solution).

Table 2 shows the fixation of radioactivity on soluble and insoluble fractions of blood cells from blood samples incubated with mugwort extract. The data indicate that there was a significant (p<0.05) reduction in the radioactivity fixation on insoluble fraction of blood cells.

Table 3 shows the fixation of radioactivity on soluble and insoluble fractions of plasma proteins from blood samples incubated with mugwort extract. The results indicate that there was no significant (p>0.05) alteration of radioactivity fixation on the fractions of plasma when compared with control.

Table 1 - Effect of the mugwort extract on the distribution of the radioactivity between cellular and plasma compartments

Mugwort	**%ATI**	
(mg/mL)	**BC**	**P**
0.0 (control)	98.32±0.52	1.68±0.52
0.62	92.58±1,23	7.42±1.23
1.25*	81.50±1.67	18.50±1.57
2.50*	73.29±3.95	26.71±3.95
5.00*	70.42±3.47	29.58±3.47
10.00*	68.01±3.73	31.99±3.73

Blood samples from *Wistar* rats were incubated with mugwort extract for 1 hour. Then, radiolabeling procedure of blood constituents was carried out. The samples were centrifuged to plasma (P) and blood cells (BC) separation. Radioactivity in each fraction was counted and the %ATI was calculated. (*) p<0.05 when compared with control.

Table 2 - Effect of mugwort extract on the fixation of radioactivity on fractions of blood cells

Mugwort	**%ATI**	
(mg/mL)	**IF-BC**	**SF-BC**
0.0 (control)	95.46±1.78	4.54±1.78
0.62*	83.66±2.95	16.34±2.95
1.25*	76.44±2.46	23.56±2.46
2.50*	69.76±4.46	30.24±4.46
5.00*	72.92±0.81	27.08±0.81
10.00*	67.83±2.38	32.17±2.38

Blood samples from *Wistar* rats were incubated with mugwort extract and labeling of blood constituents with 99mTc was performed. Insoluble (IF) and soluble (SF) fractions from blood cells (BC) were isolated, radioactivity was counted and the percentage of the radioactivity (%ATI) was calculated. (*) p<0.05 when compared with control.

DISCUSSION

Some authors have been reported that natural and synthetic drugs could alter the labeling of blood constituents with 99mTc (Frydman et al., 2004; Abreu et al., 2006; Fonseca et al., 2007). The labeling of blood constituents would decrease due to the action of drugs that could (1) bind at the same sites of 99mTc on the blood constituents, (2) directly inhibit (chelating action) of the stannous and pertechnetate ions, (3) oxidize or generate free radicals that could oxidize the stannous ion, and (4) alter the plasma membrane structure or modify the transport systems of stannous and pertechnetate ions into cells.

Table 3 - Effect of mugwort extract on the fixation of radioactivity on fractions of plasma

Mugwort mg/mL)	%ATI	
	IF-P	SF-P
0.0 (control)	75.44±1.91	24.56±1.91
0.62	73.03±2.64	26.97±2.64
1.25	76.84±0.87	23.16±0.87
2.50	69.39±3.37	30.61±3.37
5.00	75.53±3.01	24.47±3.01
10.00	72.26±5.19	27.74±5.19

Blood samples from *Wistar* rats were incubated with mugwort extract and labeling of blood constituents with 99mTc was performed. Insoluble (IF) and soluble (SF) fractions from plasma (P) were isolated, radioactivity was counted and the percentage of the radioactivity (%ATI) was calculated.

Luo et al. (2007) have demonstrated that aqueous and hidroalcoholic extracts of mugwort leaves present anti-microbial activity. If this activity was related with actions on the plasma membrane, this finding could be also related with effects on the membrane of red blood cells. A possible modication on the erythrocyte membrane could alter its structure, decreasing and/or blocking the transport of stannous and pertechnetate ions into blood cells. In consequence the labeling efficiency of blood constituents with 99mTc would be decreased.

Authors using mugwort have described that flavonoids have decreased the spasmolytic activity in experimental models (Lozoya et al., 1994) and have antioxidant and radical-scavenging properties (Miyake and Shibamoto, 1997). These findings can also aid to understand the effect of the extract on the labeling of blood constituents (Table 1 and 2).

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 (Losoya et al., 1994; Re et al., 1999; Morales et al., 1994) and depression of myocardial inotropism (Conde Garcia et al., 2003). As calcium and stannous ions are very similar (Gutfilen et al, 1992), these described actions of the mugwort extract on the calcium flow through the membrane could justify the decrease of the radiolabeling of blood cells with 99mTc.

The analysis of the results indicates that the extract could have substances that could interfere on the transport of stannous ions through the erythrocyte membrane. In consequence, it would be expected that the labeling of blood cells with 99mTc would be altered. Moreover, morphological experiments are ongoing to try to verify the action of this extract on the shape of the red blood cells.

RESUMO

O objetivo desse trabalho foi avaliar o efeito da *Artemisia vulgaris* L.(artemisa) na marcação dos constituintes sangüíneos com tecnécio-99m (99mTc). Amostras de sangue obtidas de ratos *Wistar* foram incubadas com um extrato de artemisa e o processo de radiomarcação dos constituintes sangüíneos foi realizado. Plasma e células sangüíneas foram isoladas por centrifugação. Alíquotas de plasma e células sangüíneas foram também precipitadas com ácido tricloroacético para isolamento de frações solúvel e insolúvel. A radiatividade em cada fração foi contada e as porcentagens de radioatividade (%ATI) foram calculadas. O extrato de artemisa diminuiu significantemente ($p<0,05$) a %ATI nas células sanguíneas e nas proteínas celulares. A análise dos resultados indicou que o extrato de artemisa apresentaria substâncias que interferir no transporte de íons estanoso e/ou pertecnetato através da membrana do eritrócito alterando a marcação das células sangúineas com 99mTc.

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Received: July 31, 2007;
Revised: August 08, 2007;
Accepted: Sepember 11, 2007.