BASIC RESEARCH

An experimental model to study the effects of a senna extract on the blood constituent labeling and biodistribution of a radiopharmaceutical in rats

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ABSTRACT *Cassia angustifolia Vahl* (senna) is a natural product that contains sennosides, which are active components that affect the intestinal tract and induce diarrhea. Authors have shown that senna produces DNA (deoxyribonucleic acid) lesions in *Escherichia coli* cultures and can act as an antifungal agent. Natural drugs can alter the labeling of blood constituents with technetium-99m (^{99m}Tc) and can affect the biodistribution of radiopharmaceuticals. In this work, we have evaluated the influence of a senna extract on the radiolabeling of blood constituents and on the biodistribution of the radiopharmaceutical sodium pertechnetate ($Na^{99m}TcO_4$) in *Wistar* rats. Twelve animals were treated with senna extract for 7 days. Blood samples were withdrawn from the animals and the radiolabeling procedure was carried out. The senna extract did not modify the radiolabeling of the blood constituents. A biodistributional assay was performed by administering $Na^{99m}TcO_4$ and determining its activity in different organs and in blood. The senna extract altered the biodistribution of $Na^{99m}TcO_4$ in the thyroid, liver, pancreas, lungs and blood. These results are associated with properties of the chemical substances present in the aqueous senna extract. Although these assays were performed in animals, our findings suggest that caution should be exercised when nuclear medicine examinations using $Na^{99m}TcO_4$ are conducted in patients who are using senna extract.

KEYWORDS: *Cassia angustifolia Vahl*; Biodistribution; Radiolabeling; Rats; Technetium-99m.

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INTRODUCTION

Cassia angustifolia Vahl (senna) is a plant that belongs to the Fabaceae family. This branching shrub, which is found in abundance throughout South India, can grow up to 1.8 m in height. Extracts of this plant are used in folk medicine to treat certain gastrointestinal disorders.[1,2] Hydroxyanthracene glycosides, also known as senna sennosides, have been reported to stimulate the peristalsis of the colon and alter colonic absorption and secretion, which results in fluid accumulation and expulsion.[3] Researchers[2] have suggested that a normal daily dose of senna in adults consists of two tablets with a sennoside content of 18 mg per 90 mg tablet. Other investigations[4] have shown that sennosides induce diarrhea through changes in the intestinal tract. The laxative effect of this natural product has been linked to its content of anthraquinone glycosides.[5] The antifungal activity[6] of senna has been demonstrated previously and linked to a triterpenoid glycoside present in the butanolic seed extracts of senna. In addition, a study has demonstrated that an aqueous extract of senna can produce DNA (deoxyribonucleic acid) lesions but cannot induce cytotoxic or mutagenic effects in *Escherichia coli* cultures; the senna extract has also exhibited an antioxidant/antimutagenic effect in *Escherichia coli* cultures.[7]

Nuclear medicine images have allowed health professionals to measure physiological processes and identify changes related to various diseases. Disease can alter the biodistribution of a radiopharmaceutical, and the analysis of scintigraphic images can help physicians identify altered biological activity and diagnose clinical disorders.[8,9] However, other factors such as drug interactions (with natural or synthetic compounds)[12,13] can alter the biodistribution of a radiopharmaceutical.[10,11] If these drug interactions are not anticipated, poor image quality in the nuclear medicine examinations could lead to a misdiagnosis with a possible need to repeat the examination, thereby increasing the radiation exposure to the patient and the staff.[11,14]

Molecular and cellular structures labeled with technetium-99m (^{99m}Tc) have been used in scintigraphy and single photon emission computed tomography (SPECT) procedures to label radiopharmaceuticals.[8] Blood constituents labeled with ^{99m}Tc have been used for imaging applications in the cardiovascular system, to detect gastrointestinal hemorrhage and to locate intramuscular hemangioma.[8,15]

Natural products are widely used around the world for a variety of medical and domestic applications. However, some of the biological effects and biochemical properties of these products are not yet completely understood. Thus, experimental models can be used to improve our understanding of the cellular and systemic mechanisms of action and biological effects of these natural products. The aim of this work is to evaluate the effects of a senna extract on the labeling of blood constituents with ^{99m}Tc and on the biodistribution of the radiopharmaceutical sodium pertechnetate in *Wistar* rats.

MATERIALS AND METHODS

The experimental models used 12 male *Wistar* rats (weight 309±26 g) obtained from *Laboratório de Cirurgia Experimental, Universidade do Estado do Rio de Janeiro (UERJ)*, Brazil. The Committee on Animal Research of the UERJ approved the protocols used in this work (CEA/121/2006). The animals were maintained under normal environmental conditions (22±5℃, 12 h of light/dark cycle) with water and a normal diet. The animals were divided into treated (n = 6) and control (n = 6) groups. An extract prepared from a commercial sample of *Cassia angustifolia* Vahl (Laboratory of Lua de Maio, Brazil) was administered to the rats in the treated group. All of the experiments were carried out before the expiration date of this product.

The aqueous extract was freshly prepared using 400 mg of the herb and 10 mL of 0.9% NaCl. The solution was mixed in a vortex for 2 minutes and centrifuged (1500 rpm, 5 min, clinical centrifuge). The supernatant was approximately 40 mg/mL.

The supernatant solution was intragastrically administered (48 mg/kg/day) to the treated rats for 7 days. The control group received a saline solution (0.9% NaCl) in an identical manner.[16] The rats were observed daily to verify possible toxic effects.

To assess the effect of the senna extract on the labeling of blood constituents, samples of heparinized blood (0.5 mL) were collected from all animals on the seventh day and incubated with 0.5 mL of stannous chloride (1.2 μg/mL as $SnCl_2.2H_2O$; Sigma Chemical Co., USA) for 1 hour, as reported previously.[17] After this period of time, ^{99m}Tc (0.1 mL as sodium pertechnetate; 3.7 MBq) that was recently milked from a ^{99m}Mo/^{99m}Tc generator (Instituto de Pesquisas Energéticas e Nucleares, Brazil) was added, and the incubation was continued for an additional 10-min period. The samples were centrifuged (1500 rpm, 5 min, clinical centrifuge) and 20 μL of plasma (P) and blood cells (BC) were separated. Samples (20 μL) of P and BC were also precipitated with 1 mL of 5% trichloroacetic acid (TCA), and the soluble (SF) and insoluble (IF) fractions were separated. The radioactivity of the P, BC, IF-P, SF-P, IF-BC and SF-BC was counted using a well gamma-counter (Packard Instrument Company, mod C5002, USA). The percentage of radioactivity (%ATI) was calculated using a previously reported procedure.[13]

To evaluate the effect of the senna extract on the biodistribution of the radiopharmaceutical, 0.3 mL of the $Na^{99m}TcO_4$ radiopharmaceutical (3.7 MBq) was administered by the ocular plexus on the seventh day, as previously reported.[16] After 10 min, the animals were sacrificed, samples of blood were collected and the various organs (pancreas, thyroid, brain, testis, spleen, kidney, heart, stomach, lungs, liver, duodenum, large intestine, muscle and bone) were isolated. The mass of the organs was measured, and the ^{99m}Tc radioactivity of each organ was determined using a well gamma-counter (Packard Instrument Company, model C5002, USA). Samples of blood with a volume of 1 mL were considered to weigh 1 g. The percentage of radioactivity per gram of each organ (%ATI/gram) was calculated as described previously.[16]

All data were presented as mean ± standard deviation, and the statistical analysis of the results was performed using an unpaired t-test. The level of statistical significance was set at $p \leq 0.05$. InStat GraphPad software was used to perform the statistical analysis (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, USA).

RESULTS

The senna extract dose used in this study (i.e., 48 mg/kg/day) showed no laxative or toxic effects in the rats.

Table I shows the effect of the *Cassia angustifolia* Vahl aqueous extract on the distribution of radioactivity in blood cells and plasma constituents and in the insoluble and soluble fractions isolated from blood cells and plasma samples. The results indicate that the extract did not alter the distribution of ^{99m}Tc in the blood constituents. Moreover, the fixation of the radionuclide in the insoluble fraction of plasma and bold cells samples is not also altered.

Table II shows the effect of the aqueous extract of *Cassia angustifolia* Vahl on the biodistribution of $Na^{99m}TcO_4$ (%ATI/g) in *Wistar* rats that either received (treated group) or did not receive (control group) the extract. The data in this table indicate that the senna extract significantly altered ($p<0.05$) the % ATI/gram of the $Na^{99m}TcO_4$ radiopharmaceutical in the thyroid (from 5.64±2.27 to 3.16±1.50), liver (from 0.79±0.08 to 0.60±0.12), pancreas (from 0.62±0.23 to 0.39±0.09), lungs (from 0.89±0.14 to 0.69±0.10) and blood (from 1.37±0.23 to 0.90±0.24).

Table I - The effect of the senna extract on the ^{99m}Tc distribution for each blood compartment.

Compartments	Control group (%ATI)	Treated group (%ATI)
BC	93.18±1.94	92.17±0.90
P	6.82±1.94	7.83±0.90
IF-P	72.98±2.73	68.84±4.14
SF-P	27.02±2.73	31.16±4.14
IF-BC	78.44±2.09	76.08±5.94
SF-BC	21.56±2.09	23.92±5.94

Samples of blood from *Wistar* rats (treated and control) were incubated with stannous chloride and ^{99m}Tc was added. The samples were centrifuged, and plasma (P) and blood cells (BC) were separated. Other aliquots of P and BC were precipitated with trichloroacetic acid, and soluble (SF) and insoluble (IF) fractions were also separated and counted. The radioactivity was counted and the percentage of radioactivity (ATI%) was calculated.

Table II - The effect of the senna extract on the biodistribution of $Na^{99m}TcO_4$ in *Wistar* rats.

Organs	Control (%ATI/g)	Treated (%ATI/g)
Blood	1.37±0.23	0.90±0.24**
Bone	0.39±0.13	0.31±0.10
Brain	0.07±0.02	0.06±0.02
Duodenum	1.01±0.26	0.81±0.29
Heart	0.48±0.14	0.38±0.15
Kidney	0.66±0.15	0.65±0.12
Large intestine	0.40±0.16	0.39±0.06
Liver	0.79±0.13	0.60±0.12*
Lungs	0.89±0.14	0.69±0.10*
Muscle	0.17±0.02	0.16±0.02
Pancreas	0.62±0.23	0.39±0.09*
Spleen	0.51±0.11	0.47±0.09
Stomach	2.44±1.08	2.19±1.10
Testes	0.20±0.04	0.19±0.05
Thyroid	5.64±2.27	3.16±1.50*

Male *Wistar* rats were treated with an aqueous senna extract that was intragastrically administered daily. After seven days, $Na^{99m}TcO_4$ (3.7 MBq) was administered and the animals were sacrificed. The animals' organs were isolated, the mass of each organ was determined and the percentage of radioactivity per gram of each organ (%ATI/gram) was calculated (1 mL of blood was considered to weigh 1 g). Animals in the control group were treated with saline (0.9% NaCl). (*) $p<0.05$, (**) $p<0.01$ when compared with the control group.

DISCUSSION

Medicinal plants are used to treat a number of diseases around the world and help restore the quality of life of patients. Over the last few decades, considerable progress has been made towards exploring the biological activities of various plant-derived constituents (i.e., phytochemicals). These compounds have been isolated and their pharmacological properties have been evaluated.[3,5]

Leng-Peschlow[17] administered various fractions of senna to mice and identified components of *Cassia angustifolia* that exhibit laxative and acutely toxic effects. However, acute toxicity (24 hours) only occurs with high doses[18]. Sennosides administered orally were classified as not very toxic in rats and mice. The LD50 values were 5,000 mg/kg in both species. These findings are also reported by Morales et al.[19] The dose used in our work was 48 mg/kg/day and did not exhibit any laxative or toxic effects in the rats.

Several experimental models have been used to evaluate the properties of synthetic and natural drugs.[16,20-22] Assays using a radionuclide to study the *in vivo* and *in vitro* actions of medicinal substances have been published previously.[23,24]

The interaction between radiopharmaceuticals and the constituents of the extracts of medicinal plants can also interfere with the labeling[13,20] and biodistribution[11,16] of radiopharmaceuticals. Not recognizing this interference may lead to misdiagnosis along with a possible need to repeat the clinical examination, thereby increasing the radiation exposure to the patient and the staff. It is therefore important to evaluate the effects of natural products and to develop experimental assays to explain unexpected findings.[10,25]

Labeling blood constituents with ^{99m}Tc is a simple, convenient, and useful experimental model for the study of cellular transport phenomena and the biological effects of substances.[16,20-22] Natural product extracts could decrease the labeling of blood constituents due to the following four factors: (i) the presence of oxidant compounds that could oxidize the $SnCl_2$, (ii) the presence of chelating agents that could form a complex with $Na^{99m}TcO_4$ and $SnCl_2$, (iii) modifications induced in the plasma membrane and (iv) the competition among the cited ions for the same binding sites.[20,23] Through *in vitro* studies, researchers have already verified that extracts of *Ginkgo biloba*,[26] *Mentha crispa*,[27] *Fucus vesiculosus*[21], cinnamon[20] and *Cordia salicifolia*[13] decrease the radiolabeling of blood constituents. However, another study[28] of treatment with cauliflower extract showed no alterations in the uptake of ^{99m}Tc by the blood constituents. In *in vivo* studies of blood samples from animals treated with *Ginkgo biloba* extract, the effect of this natural product on the labeling of blood constituents with ^{99m}Tc was almost completely eliminated.[29] The extract of *Cassia angustifolia* used in the present study (Table I) did not alter the radiolabeling of the blood constituents, most likely because the generated metabolites did not exhibit oxidant properties. In this case, the reducing agent (stannous chloride) would not be oxidized by the metabolites of the *Cassia angustifolia* and the radiolabeling efficiency would be not altered. This phenomenon is also most likely associated with the findings obtained with the *Ginkgo biloba* extract.[29]

The biodistribution assay is another experimental model used to evaluate the interactions between radiopharmaceuticals and drugs. Biodistribution is related to the distribution, uptake, retention and elimination of radiopharmaceuticals and depends on several factors including regional blood flow tissue metabolism and binding to the blood constituents.[8] Altered biological behavior may also occur due to disease or interference caused by the pharmacodynamic effects of synthetic and natural drugs. An unknown interaction with radiopharmaceuticals can lead to a misdiagnosis along with a possible need to repeat the examination.[8] The radiopharmaceutical sodium pertechnetate is generally distributed throughout the vasculature and interstitial fluid and is concentrated in the stomach, intestinal tract, thyroid and salivary glands.[8,15] A biodistribution study has demonstrated an increase in the uptake of $Na^{99m}TcO_4$ in the liver[30] due to eggplant extract and an altered uptake in the duodenum, spleen, pancreas, stomach and blood[16] due to *Passiflora edulis flavicarpa* extract. However, cauliflower extract does not alter the biodistribution of $Na^{99m}TcO_4$ in mice.[28] In the present study (Table II), the aqueous senna extract decreased the uptake of the $Na^{99m}TcO_4$ radiopharmaceutical in the thyroid, liver, pancreas, lungs and blood. The action of the senna extract could generate metabolites capable of promoting morphological and physiological modifications in these organs and altering the biodistribution of $Na^{99m}TcO_4$ in the treated animals. The effects of the senna extract on the gastrointestinal tract have been previously characterized,[3,31,32] but its effects on other organs have not yet been completely established. In this work (Table II), no alteration of the gastrointestinal uptake of the $Na^{99m}TcO_4$ radiopharmaceutical was found after treatment with the senna extract. These results indicate that no physiological changes would be observed in the animal's gastrointestinal tissues due to treatment with the senna extract at the concentration used. No pathological changes were observed in these tissues under light microscopy examination at 3 hours and at 3, 4 and 6 weeks after the gastric administration of the senna extract.[32] However, the senna aqueous extract decreased the uptake of the $Na^{99m}TcO_4$ radiopharmaceutical in the thyroid, liver, pancreas, lungs and blood. The action of the

senna extract generates metabolites that alter the biodistribution of $Na^{99m}TcO_4$ in the treated animals and promotes morphological and physiological modifications in these organs; the same effects are most likely true during embryonic development.[33] The effect of the *Cassia angustifolia* extract on the thyroid, pancreas, lungs and blood is unclear. However, the decreased uptake of $Na^{99m}TcO_4$ in the liver could be due to the anti-hepatoma activity of the *Cassia angustifolia* extract; such activity has been described in studies with human liver cancer cell lines.[34]

The two experimental models used in this study suggest that substances in the aqueous senna extract would not alter the labeling of blood constituents with ^{99m}Tc but would alter the uptake of sodium pertechnetate in some organs. Although these assays were performed in animals, the findings suggest that caution should be exercised while interpreting the results of $Na^{99m}TcO_4$-based nuclear medicine examinations in patients using senna extract. Moreover, our findings reinforce the importance of experimental models that use a radionuclide to evaluate biochemical properties and to study the biological effects associated with synthetic and natural drugs.

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