Influence of *Annona muricata* (soursop) on biodistribution of radiopharmaceuticals in rats[1]

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ABSTRACT

PURPOSE: To evaluate the effect of hydroalcoholic extract of *A. muricata* on biodistribution of two radiopharmaceuticals: sodium phytate and dimercaptosuccinic acid (DMSA), both labeled with ^{99m}technetium.

METHODS: Twenty four Wistar rats were divided into two treated groups and two controls groups. The controls received water and the treated received 25mg/kg/day of *A. muricata* by gavage for ten days. One hour after the last dose, the first treated group received ^{99m}Tc-DMSA and the second sodium ^{99m}Tc-phytate (0.66MBq each group), both via orbital plexus. Controls followed the same protocol. Forty min later, all groups were sacrificed and the blood, kidney and bladder were isolated from the first treated group and the blood, spleen and liver isolated from the second treated group. The percentage of radioactivity per gram of tissue (%ATI/g) was calculated using a gamma counter.

RESULTS: The statistical analysis showed that there was a statistically significant decrease ($p<0.05$) in the uptake of %ATI/g in bladder (0.11±0.01and1.60±0.08), kidney (3.52±0.51and11.84±1.57) and blood (0.15±0.01and 0.54±0.05) between the treated group and control group, respectively.

CONCLUSION: The *A. muricata* hydroalcoholic extract negatively influenced the uptake of ^{99m}Tc-DMSA in bladder, kidney and blood of rats.

Key words: Annona. Technetium Tc 99m Dimercaptosuccinic Acid. Radiopharmaceuticals. Biological Availability. Rats.

Introduction

Annona muricata L. (Annonaceae), commonly known as soursop, is found in Central America and South America, including the North, Northeast and Southeast regions of Brazil. Traditionally, the leaves are used for headaches, insomnia, cystitis, liver problems, diabetes, hypertension and as an anti-inflammatory, antispasmodic and antidysenteric drug. The decoction of the leaves have parasiticide, antirheumatic and antineuralgic effects when used internally, while the cooked leaves, applied topically, treat rheumatism and abscesses[1]. Currently, this plant has been also used as a natural treatment of parasitic infections and cancer[2].

The *A. muricata* has essential oils (β-caryophyllene, δ-cadinene and cadinol), chemical components such as alkaloids (reticulin, coreximine, coclarine and anomurine)[3] and antineoplastic substances such as acetogenins, which according Colman-Saizarbitoria *et al*.[4], exert a selective cytotoxicity on tumor cells without affecting normal cells. Popularly, soursop leaves are used to treat hypertension, inflammation, liver disease, cystitis, insomnia, headache, hyperglycemia, diabetes and parasitosis. The studies by Luna *et al*.[5] showed that extract of *A. muricata* was lethal to the freshwater snail, *Biomphalaria glabrata*, one of the main transmitters of schistosomiasis (*S. mansoni*) in Brazil, a parasitic endemic disease in our country. muricata has also been shown to be effective against multi-drug resistant (MDR) cancer cell lines.

Nuclear medicine images have allowed health professionals to measure physiological processes and identify changes related to various diseases. Diseases can alter the biodistribution of a radiopharmaceutical and the analysis of scintigraphic images can help physicians to identify altered biological activity and diagnose clinical disorders. However, other factors such as drug interactions (natural or synthetic compounds) can alter the biodistribution of a radiopharmaceutical[6,7]. 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[8].

In the applications in nuclear medicine diagnostic, the isotope technetium-99m (^{99m}Tc) has preferred choice. Technetium-99m may bind to different substrates or ligands, resulting radiopharmaceuticals with affinity for different organs, systems or receptors in the body[6-8]. Technetium-99m in the form of sodium pertechnetate, or labeled with other structures, such as sodium phytate (^{99m}Tc-sodium phytate) or dimercaptosuccinic acid (^{99m}Tc-DMSA) are the most frequently used radiopharmaceuticals in nuclear medicine[9].

Dimercaptosuccinic acid labeled with technetium-99m (^{99m}Tc-DMSA) has been the radiopharmaceutical of choice for high-resolution images of the renal cortex and to estimate the function of the renal parenchyma with better sensitivity and specificity than other techniques[9]. The kidney uptake of ^{99m}Tc-DMSA correlates well with the effective renal plasma flow, glomerular filtration rate and creatinine clearance. Thus, the kidney uptake of ^{99m}Tc-DMSA provides a practical index for assessing renal function. Therefore, ^{99m}Tc-DMSA is currently the standard method for assessing renal lesions in children with infectious diseases of the urinary tract[9].

The radiocolloid sodium phytate labeled with technetium-99m (^{99m}Tc-sodium phytate) is a radiopharmaceutical that has been widely used to study the liver and spleen[10], especially for diagnosis and progression of disease liver. However, using this radiocolloid has also been used for biodistribution studies, which is useful for determining function parameters[10]. Biodistribution to the liver and bone marrow has shown good correlation with the severity of liver diseases such as cirrhosis and fibrosis of the organs and its prognosis. Thus, quantification of the uptake of ^{99m}Tc-phytate serves as an excellent index of liver function[10].

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 present study aimed to evaluate, in experimental animals, the influence of the hydroalcoholic extract of *Annona muricata* (soursop) on laboratory parameters and biodistribution of two radiopharmaceuticals: dimercaptosuccinic acid labeled with technetium-99m, which is widely used in renal scintigraphy, and sodium phytate labeled with technetium-99m very effective in liver and/or spleen scintigraphy.

Methods

The experimental protocol was submitted to Ethics Committee for Use of Animals in Research (CEUA/UFRN) and approved under the number 035/2012. This protocol followed guidelines for the care and use of laboratory animals.

Extract

The *Annona muricata* (soursop) extract (prepared from a 10% dye-mother solution and diluted in water, *Herbarium* Laboratory, Rio de Janeiro/Brazil) was administered orally to the rats in single

dose, during 10 days, in the treated groups. The control groups received water by the same way and period. All of the experiments were carried out before the expiration date of this product.

Surgery and sample collection

Twenty four male Wistar rats (100±20 g), were divided into two groups: control (n=12) and treated (n=12) groups. The treated group received daily 0.5 mL of soursop extract (25mg/kg/day) by gavage for ten days, and the control group received 0.5 mL of water by the same way and period. Treated animals were subdivided into two subgroups: first group (n=6) was submitted to radiopharmaceutical injection ^{99m}Tc-DMSA and the second (n=6) to ^{99m}Tc-sodium phytate. The controls also suffered the same division and treatment. These radiopharmaceuticals have been labeled with technetium-99m (^{99m}Tc-DMSA and ^{99m}Tc- sodium phytate with 0.66 MBq of radioactivity). The technetium-99m was eluted from a ^{99}Mo/^{99m}Tc generator produced by the Institute of Energy and Nuclear Research, São Paulo/Brazil and the *kits* of DMSA and sodium phytate were kindly donated by the *Liga Norteriograndense contra o Câncer, Natal/RN*. Each animal for group received 0.1 mL of specific radiopharmaceutical via orbital plexus, on the 10th day of treatment. After 40 minutes they received the specific dose of the radiopharmaceutical for each group, then, all the animals were sacrificed after anesthesia intramuscular of Ketamin 50mg/kg (Ketamina®- Pfizer do Brasil Ltda) associated with Xylazin 10 mg/ml (Ropum® - Bayer do Brasil Ltda). Blood and organ samples (spleen and liver, from the group treated with sodium phytate, and kidney and bladder, from the group treated with DMSA) were isolated, cleaned with 0.9% saline solution and the radioactivity of each organ was determined by means of an automatic gamma counter (1470 Wizard, Perkin Elmer, Finland) with automatic correction for decay and efficiency of 86%, in the Nucleus of Experimental Surgery- UFRN. The percentage of radioactivity per gram of each organ (%ATI/g) was calculated as described elsewhere Bernardo-Filho *et al.*[6].

Laboratory analysis

Biochemical parameters were measured using the Konelab 60i spectrophotometer autoanalyzer, in the Onofre Lopes University Hospital. The serum levels of alanine aminotransferase (ALT), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), cholesterol and fractions (LDL and HDL), glucose, creatinine, triglycerides, total protein and fractions (albumin and globulin) were measured using the assay kit from *Weiner*, São Paulo, Brazil. All data were presented as mean±standard deviation.

Statistical analysis

Data were reported as means±SD. The %ATI/g was compared by Mann–Whitney test and biochemical parameters by the Student's t-test, considering $p<0.05$ statistically significant in both tests. Stat Graph Pad software was used to perform statistical analysis.

Results

The Table 1 shows the biodistribution of ^{99m}Tc-sodium phytate in organs of control group rats and treated group rats, 40 min after administration of the radiopharmaceutical. The statistical analysis showed that there was no statistically significant difference ($p>0.05$) in the uptake of radioactivity (% ATI/g) in the spleen, liver and blood, when compared the treated group and control group, despite of the decrease observed in the treated group.

TABLE 1 – Results of effect of *A. muricata* extract on the uptake of ^{99m}Tc-sodium phytate of Wistar rats**.**

Organs	%ATI/g		p-value*
	Control group	Treated group	
Spleen	5.61±0.87	3.40±0.53	0.3841
Liver	20.71±1.51	16.53±2.41	0.6066
Blood	0.90±0.05	0.12±0.08	0.8089

Data are reported as means±SD. *p>0.05, compared with control.

The Table 2 shows the biodistribution of ^{99m}Tc-DMSA in organs of control group rats and treated group rats, 40 min after administration of the radiopharmaceutical ^{99m}Tc-DMSA. The statistical analysis showed that there was a statistically significant decrease ($p<0.05$) in the uptake of radioactivity (% ATI/g) in the bladder, kidney and blood comparing the treated group and control group.

TABLE 2 - Results of effect of *A. muricata* extract on the uptake of ^{99m}Tc-DMSA of Wistar rats.

Organs	%ATI/g		p-value*
	Control group	Treated group	
Bladder	1.60±0.08	0.11±0.01	0.0341*
Kidney	11.84±1.57	3.52±0.51	0.0002*
Blood	0.54±0.05	0.15±0.01	0.0416*

Data are reported as means±SD. *p<0.05, compared with control.

The analysis of biochemical parameters (Table 3) shows that there was a statistically significant difference ($p<0.05$) only in the serum levels of LDL cholesterol ($p=0.0141$) between the treated group and control group. There was no alteration on hematological blood parameters of the treated group when compared with the control group (data no showed).

TABLE 3 - Results of effect of *A. muricata* extract on biochemical parameters of Wistar rats.

Serum Biochemical Parameters	Treated group	Control group	p-value*
Albumin (g/dL)	1.60±0.09	2.60±0.08	0.7043
Alanine aminotransferase (UI/L)	55.00±5.78	62.00±6.09	0.4590
Aspartate aminotransferase (UI/L)	181.00±12.65	136.00±9.22	0.6748
Cholesterol (mg/dL)	56.00±4.21	69.50±5.03	0.2198
Creatinine (mg/dL)	0.40±0.01	0.30±0.01	0.1011
Glucose (mg/dL)	86.00±6.90	66.50±5.69	0.1100
Globulin (g/dL)	2.10±0.99	2.15±0.89	0.7952
HDL cholesterol (mg/dL)	31.00±3.07	36.50±4.55	0.2048
LDL cholesterol (mg/dL)	16.00±2.44	22.00±3.01	0.0141*
Lactate dehydrogenase (UI/L)	1171.00±18.98	870.00±10.76	0.3889
Total protein (g/dL)	4.50±1.01	4.80±1.09	0.7646
Triglycerides (mg/dL)	76.00±6.11	62.00±5.07	0.1011

Data are reported as means±SD. *$p<0.05$, compared with control.

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[11]. *Annona muricata* is used in popular medicine to treat diabetes, cough and hypertension and has analgesic, anti-inflammatory and anticancer activities. The antitumor activity is related to the presence of acetogenins, characteristic of Annonaceae compounds that exert selective cytotoxicity on tumor cells without affecting normal cells[11].

There are over 250 known species of Annonaceae, but *Annona muricata* is one of the most used in Brazil for its local availability, widespread use and alleged healing powers[1]. However, liver toxicity is a potential complication of these herbal compounds that may lead to liver failure, as demonstrated in studies described by Holanda *et al.*[7] with the extract of *Aloe vera*, a medicinal plant widely used by the general population, but despite having facilitated the uptake of sodium pertechnetate ($Na^{99m}TcO_4$) in rats organs, it was responsible to increasing the levels of hepatic enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), indicating a possible liver damage. However, in the present study, the *A. muricata* extract did not significantly alter the levels of AST and ALT, demonstrating no hepatotoxicity.

Adeyemi *et al.*[12] showed in their study that there was a significant reduction in the concentration of blood glucose in diabetic rats treated with *Anonna muricata* (soursop), a result not found in our study, possibly due to the low extract concentration used. High levels of unsaturated fatty acids seem to play important role in improving the lipid profile, both in humans and in animals, being appointed more substantial effect on reducing low density lipoprotein (LDL), considered a cardiovascular risk factor[13]. The study done by Adewole and Ojewole[14] showed that diabetic rats treated with *streptozotocin* showed a decrease in levels of total cholesterol, LDL and triglycerides. Our study showed that treatment of normal rats (non-diabetic) with *Anonna muricata* also causes a significant decrease in the levels of LDL cholesterol ($p<0.05$).

Several experimental models have been used to evaluate the properties of synthetic and natural drugs[6-8]. The biodistribution of radiopharmaceuticals in the animal organism can be changed with concomitant use of drugs natural and/or synthetic, certain diets, parasitic infections, the use of cigarettes and surgery, as demonstrated in studies described by Araújo-Filho *et al.*[15]and Rêgo *et al.*[16]. Changes in biodistribution of sodium pertechnetate ($Na^{99m}TcO_4$) in organs of rats were observed after treatment with natural products such as *Artemisia vulgaris* extract[17] and *Aloe vera* extract[7].

Nuclear medicine imaging using dimercaptosuccinic acid radiopharmaceutical labeled with technetium-99m (^{99m}Tc-DMSA) is currently the standard method for assessing renal lesions especially in children with infectious diseases of the urinary tract[9]. Study described by Açucena *et al.*[18] showed that splenectomy in rats altered renal function and uptake of ^{99m}Tc-DMSA. Stokland *et al.*[9] showed that decreased uptake of ^{99m}Tc-DMSA in acute

pyelonephritis is caused by dysfunction of renal tubular cells and ischemia. Cortical lesions are characterized by defects in single or multifocal uptake of ^{99m}Tc-DMSA, but can often be observed decrease in uptake diffuse[9]. In our study, it was demonstrated that soursop extract significantly decreased the uptake of ^{99m}Tc-DMSA in kidney, bladder and blood, probably due to biological and/or morphological changes caused by this extract.

Sodium phytate labeled with ^{99m}Tc (^{99m}Tc-sodium phytate) is a colloid that has been used as an agent for scintigraphic imaging of the liver and spleen. Parameters such as dimensions and capacity of the liver uptake of the radiopharmaceutical have been used for diagnosis of hepatic disease and its progression and for evaluation of liver function. However, this radiocolloid has also been used for biodistribution studies, which is useful for determining function parameters[19].

Study in splenectomized rats showed that the biodistribution of ^{99m}Tc-phytate to the liver was higher than in non-splenectomized rats, meaning that the operative process improved the hepatic uptake of radiotracer. According to these authors, this result coincided with the improvement of liver function validated by normal liver enzymes ALT and AST found in splenectomized rats compared with controls. In our study we demonstrated no statistically significant changes in hepatic and splenic uptake of the radiopharmaceutical sodium phytate labeled with technetium-99m, after the use of *A. muricata* extract. This fact leads us to believe that this extract, at the studied concentration, does not interfere with normal biodistribution of ^{99m}Tc-sodium phytate in organs of *Wistar* rats.

Conclusions

The results of this study suggest that the *A. muricata* hydroalcoholic extract negatively influenced the uptake of ^{99m}Tc-DMSA in bladder, kidney and blood of rats.

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