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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

Effect of Medicinal Plants on the Parasitemia of *Trypanosoma Cruzi* and on the Biodistribution of Sodium Pertechnetate ($Na^{99m}TcO_4$)

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ABSTRACT

Artemisia vulgaris (AV) is an antihelmintic and antimalarial drug; Aloe vera(babosa) acts as antidiabetic, laxative and anti-inflammatory;Benznidazole (BZ) is a trypanocidal of Trypanosoma cruzi (TC). Technetium-99m (^{99m}Tc) has been used in nuclear medicine to obtain diagnostic images. This study evaluated the plant effects in TC parasitemia and on the biodistribution of ^{99m}Tc in mice. Twenty mice were infected by TC. At the peak of parasitemia, 5 mice received babosa; 5 received AV and 5 received BZ. The parasitemia was determined at 0, 2, 4 and 6 h of drugs administration. Five infected mice without drugs, 5 mice without TC and the group treated with AV, received ^{99m}Tc. The radioactivity was calculated. Infected mice that received babosa reduced significantly ($p<0.05$) the TC parasitemia. The percentage of activity per gram (%ATI/g) decreased significantly on the AV group. These results indicate that babosa possibly is an anti-TC drug and AV reduces the %ATI/g probably due to its biological effects.

Keywords: *Aloe vera*, *Artemisia vulgaris*, technetium-99m, *Trypanosoma cruzi*, parasitemia, biodistribution

INTRODUCTION

The Chagas' disease is a protozoan infection caused by the parasite *Trypanosoma cruzi* and transmitted by the depositing of metacyclic tripomastigotes, eliminated in the feces and urine of the several species of triatomine bugs, during the hematophagism phase (Cançado, 2005; Coura and Castro, 2002). It is an endemic Latin America parasitosis which affects 18 million individuals, with 300 thousand new cases every year (WHO, 2003) and persists for the lifetime of the human/mammalian host. This disease is

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characterized by an acute phase with detectable parasitemia and a long-lasting asymptomatic phase, generating megacolon, megaesophagus and chagasic heart disease (Santos et al., 2005; Teixeira et al., 2006). Treatment includes eradicating the parasite with Benznidazole, commercially known as Rochagan®, a drug with specific anti-*T. cruzi* activity *in vivo* and *in vitro*, available in Brazil since the 1970's (Coura and Castro, 2002). The use of certain plants as phytotherapy has been a millennial practice in folk medicine. Its use has gained enormous popularity around the world, as modern medicine is beyond the reach of many people. The *Artemisia vulgaris* and *Artemisia annua* are examples of ancient plants in Chinese medicine that has shown to be very effective against *Plasmodium falciparum* and *P. vivax*, malaria parasites in humans (Meschinick. and Dobson, 2001). *A. vulgaris* is metabolized and eliminated rapidly from the human organism and induce a rapid reduction of these species of *Plasmodium* (Meschnick, 1998; Meschinick and Dobson, 2001). However, there are no reports in the scientific literature about its tripanosomicidal action (anti-*T. cruzi*). Another example of phytotherapy, widely used in Brazilian folk medicine, is the *Aloe vera* plant, known as "babosa". It is a tropical or sub-tropical plant from North Africa and has been used over the years to treat various ailments and have been referred to as the "miracle" plant. It has been suggested that the extract of the plant promotes healing of diseases through the complex synergistic interaction of many substances, and some specially prepared *A. vera* extracts possess some biological activities such as antiinflammation, anti-cancer, anti-diabetes, macrophage activation, combat gastrointestinal infections and urinary infections, as an analgesic and more (Reynolds and Dweck, 1999). However, its effect anti-*T. cruzi* is not known until the moment. Studies are being developed to discover drugs that provoke the complete eradication of the *Trypanosoma cruzi*, not only through the elimination of tecidual forms (amastigotes) such as blood (tripomastigotes) and the 100% cure of cases in Brazil.

Nuclear medicine (NM) is the medical specialty that uses radioactive isotopes to diagnose through images or therapy. The role of radionuclide technetium-99m (^{99m}Tc) in the diagnostic field of MN is already well established. This is due to its chemical versatility and nuclear properties such as the emission of a single photon gamma (140 keV) and with 89% abundance, perfect for obtaining the images in gamma cameras used in NM (Saha, 2004; Bernardo-Filho et al. 2005). Its short half-life (6 hours) is enough to acquire excellent studies of images, to prepare radiopharmaceuticals, to minimize the dose of radiation for the patient, to have an almost inexistent environmental impact, besides being an ideal radiotracer. The rapid growth of this field in the last decades is attributed to its ideal physicochemical characteristics and to it being easy to obtain from a portable generator of ^{99}Mo/^{99m}Tc in the form of sodium pertechnetate ($Na^{99m}TcO_4$) and can be lyophilized in *kits* to form labeled compounds with ^{99m}Tc in hospital or radiopharmacy clinic (Banerjee et al., 2001; Saha, 2004).

The aim of this study was to evaluate the effect of medicinal plant extracts on the parasitemia of *T. cruzi* and on the biodistribution of the $Na^{99m}TcO_4$ in mice infected with the *Y* strain of *T. cruzi*.

MATERIALS AND METHODS

Twenty-five male *Swiss* mice weighing 18-20g from Centro de Ciências da Saúde, Universidade Federal do Rio Grande do Norte (UFRN), Natal–RN, Brasil, were used. The protocol was conducted in accordance with Brazilian College of Animal Experimentation guidelines and was approved by the Research Ethics Committee of Onofre Lopes Hospital-UFRN (182/2008). The animals had free access to water and standard food for rodents (Labina Purina®) and were randomly allocated to 2 groups: control and treated. The animals were divided, randomly, in groups of 5 mice each. Twenty mice were infected intraperitoneally, with a suspension containing $1x10^5$ tripomastigotes blood parasites /mL of the *Y* strain of *T. cruzi*. Group 1 was used as the control group, being infected experimentally with *T. cruzi* and not treated. Group 2 was also a control group, but neither infected nor treated with drugs. Groups 3, 4 and 5 were infected with *T. cruzi* and received orally (gavage), respectively, 0.25mL of an aqueous *A. vera* (5mg/mL/day), 0.25mL of Benznidazole (5mg/Kg/day), diluted in sorbitol, and 0.25mL of hydroalcoholic extract of *A. vulgaris* (5mg/mL/day). To evaluate the parasitemia of each animal, whole blood of the mice was used and the parasites were counted according to Brener (1962). The mice were infected with *Y* strain of *T. cruzi* provided by the

René Rachou Research Center (CPqRR), FIOCRUZ, Belo Horizonte-MG. The parasitemic curve of the animals was tracked daily, from the 4º to the 12° day of the parasite infection, with the purpose to observe its growth and, thus, determine the parasitemic peak, which occurred between the 7^{th} and the 9^{th} days. The parasitemia of each animal was determined after 3 measuring in three observations. The number of circulating tripomastigotes was counted according to Brener (1962), which consisted in examining 5 μL of peripheri blood, taken by incision in the tail of each animal, in 50 field microscopes and using lamina and laminula, with increase of 400 times. After the counting of the parasites, the number found was multiplied by a correction factor corresponding to 80 (Brener, 1962). In this study, the parasitemia was achieved during the measure of time: 0 (before administering the drugs) and 2, 4 and 6 hours after its use. The group treated with Benznidazole was considered as the control group owing to its trypanosomicide action, which has been recognized since the 1970's. The results obtained in the parasitemia study were analyzed statistically by the parametric ANOVA test and the level of significance to $p<0.05$. Before the administration of the radiopharmaceutical sodium pertechnetate ($Na^{99m}TcO_4$), heparinized blood was withdrawn from infected and treated animals with each drug, and from infected and untreated animals (control group 1), by cardiac puncture, under anesthesia. The biochemical dosages were performed in automated equipment TermoKonelab 60i, Abbott and analyzed by Student's t-test, considering the level of statistical significance at $p<0.05$ in both tests. Statistica 6.0 software was used. Data were presented as mean ± standard deviation.

After observation of the parasitemia and blood collection, a study of biodistribution of $Na^{99m}TcO_4$ was done. For this, 5 animals infected and treated with *A. vulgaris* were used, as well as, 5 infected and not treated (control group 1) and 5 not infected and not treated (control group 2). All of these animals received, by orbital plexus via, 0.1mL of $Na^{99m}TcO_4$ (3.7MBq), recently eluted from the generator of $^{99}Mo/^{99m}Tc$ (*Instituto de Pesquisas Energéticas e Nucleares*, São Paulo, Brasil). After 60 minutes, all animals were quickly killed under anesthesia. Samples were harvested from the brain, heart, intestines, spleen, liver, bladder, femur, lungs, kidneys and blood. The tissue samples were washed in 0.9% saline, weighed on a precision scale (Mark 160®, Bel equipment, Italy) and the percentage of radioactivity per gram of tissue (%ATI/g) was determined in an automatic gamma counter (Wizard 1470, Perkin-Elmer, Finland). The efficiency of the gamma counter was 86%, as specified by the manufacturer. The results of the study of the biodistribution were compared to their control groups and the statistical analysis was done using the non-parametric Mann-Whitney ($p<0.05$) test. Statistica 6.0 software was used.

RESULTS

Table 1 shows the parasitemia of the animals treated with *A. vera*, compared to those treated with benznidazole (control) and *A. vulgaris*. The analysis of the results shows a significant ($p<0.05$) decrease of counting of the parasites in all the times (0, 2, 4 e 6 hours). The values correspond to the mean±DP.

Table 1 - Parasitemia of mice infected with *Tripanosoma cruzi*, on times 0, 2, 4 and 6 hour, after administration of benznidazole, *A. vera* and *A. vulgaris*.

Hours	**Benznidazole (control)**	***Aloe vera (babosa)****	***Artemisia vulgaris* (*Artemisine*)***
0	165.80±20.30	150.00±50.80	164.30±13.70
2	198.50±12.00	151.80±38.80	300.00±39.60
4	138.80±17.10	122.00±16.16	392.80±55.20
6	33.50±11.90	25.25±1.14	400.80±82.50

Mean±DP. *, $p<0.05$.

Table 2 shows the effect of the *A. vulgaris* extract on the biodistribution of $Na^{99m}TcO_4$ in infected mice, 60 minutes after administration of the radiopharmaceutical. The values correspond to the mean±DP. A significant increase was observed ($p<0.01$) of the %ATI/g in spleen, brain, femur, liver, lungs and blood and a significant decrease of the %ATI/g ($p<0.01$) in heart, intestines, kidney and bladder, compared to the control groups 1 and 2.

In relation to biochemical dosages, there was a significant ($p<0.05$) decrease of blood levels of glucose and cholesterol in the group treated with

Table 2 – Effect of *A. vulgaris* on the biodistribution of $Na^{99m}TcO_4$ after 60 minutes of radiopharmaceutical administration.

	% ATI/g					
Organs	**Control 1**		**Control 2**		***A. vulgaris****	
Bladder	0.075	± 0.007	0.080	± 0.005	0.030	± 0.005
Blood	0.079	± 0.017	0.065	± 0.074	4.065	± 0.074
Brain	0.027	± 0.008	0.020	± 0.007	0.050	± 0.007
Femur	2.076	± 0.059	2.098	± 0.070	5.098	± 0.070
Heart	1.010	± 0.007	1.047	± 0.014	0.047	± 0.014
Intestine	2.013	± 0.034	2.036	± 0.008	0.036	± 0.008
Kidney	7.000	± 1.052	6.035	± 1.028	4.035	± 1.028
Liver	1.069	± 0.028	2.060	± 0.068	4.059	± 0.068
Lung	0.029	± 0.005	0.022	± 0.086	6.022	± 0.086
Spleen	0.035	± 0.010	0.000	± 0.015	0.086	± 0.015

Mean±DP. *, $p<0.05$.

Table 3 - Effect of *A. vulgaris* and *Aloe vera* extract on biochemical parameters of mice infected with *T. cruzi*.

Biochemical parameters	**Control**	***A. vulgaris***	***Aloe vera***
Cholesterol (mg/dL)	134.70±09.50	100.20±17.50*	68.75 ±4.35*
Glucose (mg/dL)	117.50±23.00	62.00 ±41.90*	113.00±10.58*
AST (U/L)	110.50±40.40	621.00±46.00*	955.00±90.00*
ALT (U/L)	74.70 ±10.40	423.80±10.63*	476.00±74.40*

Mean±DP. *, $p<0.05$.

A. vulgaris. A significant increase ($p<0.05$) was observed in the enzymes Aspartate amino transferase (AST) and Alanine amino transferase (ALT) in this group. The group treated with *A. vera* (babosa) also showed a significant decrease ($p<0.05$) in the dosages of glucose and cholesterol and a significant increase in the enzymes (AST and ALT). These results are observed in Table 3. The values correspond to the mean±DP.

DISCUSSION

For at least 30 years there has not been any new drug for the treatment of Chagas' disease, a fact that has instigated the search for new drugs for the treatment of this disease, envisioning alternatives with fewer side effects and greater effectiveness (Camandaroba *et al*, 2003). *A. vulgaris* or "Mugwort", as it is known in traditional Chinese medicine, is a plant widely used to treat diabetes and menstruation disorders. The infusion of their leaves presents potent action against intestinal parasites (Teixeira da Silva, 2004). In natural medicine (herbal) its extract has been widely used as anti-helmintic, anti-malaric, antiseptic, antispasmodic, antireumatic and antibacterial agent (Duke et al., 2002). The active components of *A. vulgaris* include: flavonoids, cumarinics, terpenes, lactones, volatile oils, inulin and traces of alkaloids (Haider et al., 2003; Teixeira da Silva, 2004; Judzentiene and Buzelyte, 2006). However, this study showed that the extract of *A. vulgaris* was not able to reduce the trypomastigote forms of *T. cruzi* in Swiss mice infected with this parasite,

despite the proven action, both of *A. vulgaris* as well as the *A. annua*, in reducing the parasitemia of another protozoan, the *P. falciparum*, in malaric patients (Meshnick and Dobson, 2001).

It is important to assess the interaction of the extract of *A. vulgaris* with the normal metabolism. Our data showed a high decline in blood glucose in animals treated with *A. vulgaris* extract, and also revealed an increase in liver enzymes (AST and ALT), indicating a possible liver injury induced by this extract. Meanwhile, scientific findings on the liver toxicity of *A. vulgaris* are scarce.

The extract of the plant promotes healing of diseases through the complex synergistic interaction of many substances, and specially prepared *Aloe vera* extracts, possess some biological activities such as antiinflammation, anti-cancer, anti-diabetes and macrophage activation, combat gastrointestinal infections and urinary infections, as an analgesic and more (Reynolds and Dweck, 1999). The data obtained from the *A. vera* (babosa) treatment in this study showed that its extract possibly has higher activity in reducing the parasitemia of animals infected with *T. cruzi* than that of benznidazole, a synthetic anti-*T. cruzi* drug used since the 1970's in Brazil.

The biochemical changes found in serum cholesterol and liver transaminases (AST and ALT) may be related to the biological, metabolic or toxic effects of "babosa".

According to Patel and Mengi (2008), the extract of *Aloe vera* possesses hipolipidemic, hipoglicemic and antitrombotic activities. This finding probably explains the low levels of cholesterol and glucose in mice infected and treated with *A. vera* in our study.

Several authors have demonstrated that the biodistribution of radiopharmaceuticals may be altered by natural and synthetic drugs, diets and surgery (Xavier Holanda et al., 2002; Bernardo et al., 2004; Santos-Filho et al., 2005; Holanda et al., 2006; Araújo-Filho et al., 2007). In this study, there was a significant increase in the %ATI/g of the $Na^{99m}TcO_4$ in the femur of mice treated with *A. vulgaris*, probably induced by the extract of this plant on the hydroxyapatite crystals, or the deposition of calcium phosphate in bone. We also observed a significant increase in the %ATI/g of the $Na^{99m}TcO_4$ in the liver, probably due to the metabolization of *A. vulgaris* in that organ.

Our data showed a significant decrease of %ATI/g of the radiopharmaceutical in the kidneys, bladder and intestines, possibly because these organs are the main route for the excretion of metabolites from *A. vulgaris* extract (Meshnick and Dobson, 2001). The changes found in other organs and tissues probably are due to the biological and metabolic effects of *A. vulgaris*. Further studies are necessary to explain the mechanisms of these effects.

The human American trypanosomiasis, a disease of high morbidity and mortality has been treated with unefficient drugs, and requires much research about new drugs and new measures for prevention and cure. In conclusion, the data of this work suggest that the drugs studied had anti-*T. cruzi* effect and changed the metabolism and biodistribution of pertechnetate in mice.

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RESUMO

A *Artemisia vulgaris* (AV) é uma planta com atividades antihelmíntica e antimalárica. *Aloe vera* (babosa) tem ação antidiabética, laxante e anti-inflamatória. Benzonidazol (BZ) é uma droga tripanosomicida contra o *Trypanosoma cruzi* (TC), agente da doença de Chagas. Tecnécio-99m (^{99m}Tc) tem sido usado na medicina nuclear para obtenção de imagens diagnósticas. Este estudo avaliou o efeito de plantas na parasitemia do TC e na biodistribuição do ^{99m}Tc em camundongos. Vinte camundongos foram infectados por TC. No pico da parasitemia, 5 camundongos receberam babosa; 5 receberam AV e 5 receberam BZ. A parasitemia foi determinada durante os tempos 0, 2, 4 e 6 horas após administração das drogas. Cinco camundongos infectados e não tratados, 5 camundongos não infectados e o grupo tratado com AV receberam ^{99m}Tc, na forma de pertecnetato de sódio. A radioatividade foi calculada. Os animais infectados que receberam babosa reduziram significativamente ($p<0.05$) a parasitemia. A porcentagem da radioatividade por grama (%ATI/g) diminuiu significativamente no

grupo tratado com AV. Estes resultados indicam que a babosa possivelmente é uma droga anti-TC e a AV reduz a %ATI/g provavelmente devido seus efeitos biológicos e/ou metabólicos.

Palavras-chave: *Aloe vera*, *Artemisia vulgaris*, tecnécio-99m, *Trypanosoma cruzi*, parasitemia, biodistribuição

REFERENCES

Araújo-Filho, I.; Rego A. C. M.; Brandão-Neto J.; Villarim-Neto A.; Egito E. S. T.; Azevedo I. M.; Medeiros A. C. (2007), Biodistribution of the Radiopharmaceutical Sodium Pertechnetate after Biliopancreatic Bypass with a Duodenal Switch. *Braz Arch Biol Technol*., **50**, 189-197.

Banerjee, S.; Raghavan M.; Pillai, A.; Ramamoorthy, N. (2001), Evolution of Tc-99m in Diagnostic Radiopharmaceuticals. *Sem Nucl Med*., **31**, 266-277.

Bernardo-Filho, M.; Santos-Filho, S. D.; Moura, E. G.; Maiworm, A. I.; Orlando, M. M. C.; Penas, M. E. (2005), Drug Interaction with Radiopharmaceuticals: a Review. *Braz Arch Biol Technol*., **48**, 13-27.

Bernardo, L. C.; Santos, A. E. O.; Mendes, D. C.; Ribeiro, C. K.; Gomes, M. L.; Diré, G.; Jesus, L. M.; Abreu, P. R. C.;Pereira, R.; Frydman, J. N. G.; Moura, R. S.; Bernardo-Filho, M. (2004), Biodistribution Study of the Radiopharmaceutical Sodium Pertechnetate in Wistar Rat Treated with Rutin. *Pak J Biol Sci*, **7**, 518-520.

Brener Z. (1962), Therapeutic activity and criterion of cure on mice experimentally infected with *Trypanosoma cruzi*. *Rev Inst Med Trop*., **4**, 389-396

Camandaroba, E. L. P.; Reis, E. A. G.; Gonçalves M. S.; Reis M. G.; Andrade S. G., (2003), *Trypanosoma cruzi*: susceptibility to chemotherapy with benznidazole of clones isolated from the highly resistant Colombian strain. *Rev Soc Bras Med Trop*, **36**, 201-209.

Cançado J. R. (2002), Long term evalution of etiological treatment of Chagas disease with benznidazole. *Rev Inst Med Trop*., **44**, 29-37

Coura J. R.; Castro S. L. (2002), A critical rewiew on Chagas disease chemotherapy. *Mem Inst Oswaldo Cruz*, 97, 3-24.

Duke, J. A.; Godwin, M. J. B.; Du Cellier, J.; Duke, P. N. K. (2002), *Handbook of medicinal herbs*, 2nd ed. CRC Press, Washington, D.C.

Haider, F.; Dwivedi, P. D.; Naqvi, A. A.; Bagchi, G. D. (2003), Essential oil composition of *Artemisia vulgaris* harvested at different growth periods under Indo-Gangetic plain conditions. *J Essen Oil Res*., **15**, 376–378.

Holanda, C. M. C. X.; Holanda-Leite, R. C.; Nunes, R. A. S. N.; Oliveira, H. A.; Catanho, M. T. J. A.; Souza, G. M. L.; Bernardo-Filho, M. (2006), Effect of antimalarial drugas on the bioavailability of the methylenediphosphonic acid labeled with technetium99m (99mTc-MDP) in *wistar* rats. *Braz Arch Biol Technol.*, **49**, 207-214.

Judzentiene, A.; Buzelyte, J. (2006), Chemical composition of essential oils of Artemisia vulgaris L. (mugwort) from plants grown in North Lithuania. *Chemija*, **17**, 12–114.

Meshnick, S. R. (1998), From quinine to qinghaosu: historical perspectives. In *Malária: Parasite Biology, Pathogenesis, Protection.* Sherman, I.W. (Ed.). ASM. Press. Washington, pp. 341-53.

Meshnick, S. R. and Dobson, M. J. (2001), The history of antimalarial drugs. In-*Antimalarial chemotherapy. Mechanism of Action, Resistance and New Directions in Drug Discovery*. Totowa, New Jersey, pp.15-25.

Patel, P. P.; Mengi, S. A. (2008), CU Shah College of Pharmacy, Mumbai, Maharashtra, India. Paper presented at 77th Congress of the European Atherosclerosis Society, 26–29 April, Istanbul, Turkey

Reynolds, T.; Dweck, A. C. (1999), *Aloe vera* leaf gel: a review update. *J Ethnopharmacol.*, **68**, 3 –37.

Saha, G. B. (2004), *Fundamentals of Nuclear Pharmacy*, Springer-Verlag, New York.

Santos, C. D.; Caldera, J. C.; Toldo, M. P. A.; Prado, J. C. (2005), *Trypanosoma cruzi*: effects of repetitive stress during the development of experimental infection. *Experim Parasitol*., **110**, 96 -101.

Santos-Filho, S. D.; Bernardo-Filho, M. (2005), Efeito de um extrato de Hipérico (*Hypericum perforatum*) na marcação in vitro de elementos sangüíneos com tecnécio-99m e na biodisponibilidade do radiofármaco pertecnetato de sódio em ratos *Wistar*. *Acta Cir Bras*., **20**, 76-80.

Teixeira, A. R. L.; Nascimento, R. P. J.; Sturn, N. R. (2006), Evolution and pathology in Chagas' disease: A review. *Mem Inst Oswaldo Cruz*, **101**,463-491.

Teixeira da Silva, J. A. (2004), Mining the essential oils of the Anthemideae. *Afr J Biotechnol*., **3**, 706–720

World Health Organization. Division of Control of Tropical Diseases [on line]. Disponível em: (http://www.who.int/tdr/diseases), acessado em: 11/10/2003.

Xavier-Holanda, C. M. C., Jales, R. L. C.; Catanho, M. T. J. A.; Holanda-Leite, R. C.; Brito, L. M. L.; Jales-Junior, L. H.; Brandão, K. C.; Amorim, L. F.; Brito, G. G. B.; Gomes, M. L.; Bernardo-Filho, M. (2002), Effects of the glucantime on the kinetic of biodistribution of radiopharmaceuticals in wistar rats. *Cell Mol Biol*., **48**, 761-765.

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