

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biological Effects of a Chayotte Extract in Wistar Rats with Induced Diabetes: A Radiopharmaceutically Analysis

^{1,2,4}G.F. Diré, ⁴J.S. Rodrigues, ⁴J.C.S. Oliveira, ⁴S.D.D. Vasconcelos, ⁴P.R.A. Siqueira,
⁴R.M. Duarte, ⁴M.C.L. Almeida, ²M.L. Fernandes and ^{1,3}M. Bernardo-Filho

¹Universidade do Estado do Rio de Janeiro, Instituto de Biologia Roberto Alcântara Gomes, Departamentos de Biofísica e Biometria. Av. 28 de setembro, 87, Rio de Janeiro, RJ 20551-030, Brazil

²Universidade do Estado do Rio de Janeiro, Faculdade de Ciências Médicas, Departamento de Patologia Geral. Av. 28 de setembro, 87, Rio de Janeiro, RJ 20551-030, Brazil

³Instituto Nacional do Câncer, Coordenadoria de Pesquisa, Praça Cruz Vermelha, 23, Rio de Janeiro, RJ, Brasil, 20230-130

⁴Universidade Estácio de Sá. Centro de Ciências da Saúde, Campus R9/Taquara. Laboratórios de Semiologia e Semiotécnica e Fisiologia do Exercício. Rua André Rocha, 838, Rio de Janeiro, RJ 22710560, Brazil

Abstract: The present research evaluated the influence of a chayotte (*Sechium edule*) extract (macerated) on the bioavailability of ^{99m}TcO₄Na as well as in the mass of the organs. In this study, in the biodistribution analysis, the ^{99m}TcO₄Na was administrated into female Wistar rats (diabetes and no diabetes induced) which had drunk or not the extract for 7 days. After 10 min, animals were sacrificed, the organs were isolated, the radioactivity determined in a well counter and the percentages of radioactivity per gram (%ATI/g) in the organs and mass of them (g) were calculated. The analysis of the results has indicated that in the diabetes group had been an increase in the uptake of ^{99m}TcO₄Na the in pancreas as well as in the diabetes groups treated with chayotte extract. The mass of the spleen, stomach, pancreas, heart and kidney has been altered due to the comparison of the groups. It is possible to suggest that some components of chayotte extracts present an oxidant power able to alter the biodistribution of ^{99m}TcO₄Na, as a tip, we speculate that the referred extract when metabolized in the liver may produce reactive metabolites with oxidant properties linked to the stress which is generated by diabetic status, this fact could justify by the increase of %ATI/g in the pancreas which probably may be due to the producing of AGEs in diabetes status as well as by the different molecular and cellular mechanisms related to the effects of the extract and diabetes would promote differences in the mass of the organs.

Key words: Chayotte, red blood cells, biodistribution, technetium-99m, *in vivo*, radiopharmaceutical

INTRODUCTION

The formation of Advanced Glycation End products (AGEs) is an important biochemical abnormality that accompanies diabetes mellitus and likely, inflammation in general. Recent studies have been indicating that the effects of AGEs on vessel wall homeostasis may account for the rapidly progressive atherosclerosis associated with diabetes mellitus. Driven by hyperglycemia and oxidant stress, AGEs form to a greatly accelerated degree in diabetes. Within the vessel wall, collagen-linked AGEs may trap plasma proteins, quench Nitric Oxide (NO)

activity and interact with specific receptors to modulate a large number of cellular properties. On plasma Low Density Lipoproteins (LDL), AGEs initiate oxidative reactions that promote the formation of oxidized LDL. Interaction of AGEs with endothelial cells as well as with other cells accumulating within the atherosclerotic plaque, such as mononuclear phagocytes and smooth muscle cells, provides a mechanism to augment vascular dysfunction. Specifically, the interaction of AGEs with vessel wall components increases vascular permeability, the expression of procoagulant activity and the generation of Reactive Oxygen Species (ROS), resulting

Corresponding Author: G.F. Diré, Universidade do Estado do Rio de Janeiro, Instituto de Biologia Roberto Alcântara Gomes, Departamentos de Biofísica e Biometria. Av. 28 de setembro, 87, Rio de Janeiro, RJ 20551-030, Brazil
Fax: +552122543532/ +552125876432

in increased endothelial expression of endothelial leukocyte adhesion molecules. AGEs potently modulate initiating steps in atherogenesis involving blood-vessel wall interactions, triggering an inflammatory-proliferative process and furthermore, critically contribute to propagation of inflammation and vascular perturbation in established disease (Basta *et al.*, 2004).

The development of diagnostic tools using radioisotopes is widely used in almost all hospitals nationwide. In nuclear medicine practice, physicians set norms for morphological or physiological function for each organ by diagnosing a large number of patients. For every procedure, there is diagnostic data for a range of normal variations familiar to physicians. Nuclear medicine practitioners interpret diseases on the basis of deviations from these limits. Radioisotopes provide vital information to help diagnosis and therapy of various medical diseases. Data on tissue shape, function and localization within the body are relayed by use of one of various radionuclides, which can either be a free chemical species or covalently bound to part of a larger organic or inorganic moiety. These images are generated by the distribution of radioactive decay of the nuclide (Saha, 1998). ^{99m}Tc -labeled human serum albumin scintigraphy is helpful to localize the protein-losing origins and surgery is an effective treatment for Cronkhite-Canada syndrome with protein-losing enteropathy (Tseng *et al.*, 2005). Various radiochemicals have been labelled with this nuclide. ^{99m}Tc has a half-life of 6 h and emits γ -rays of 140 keV with an abundance of 90%. ^{99m}Tc is primarily obtained from $^{99}\text{Mo}/^{99m}\text{Tc}$ generator and is eluted as sodium pertechnetate ($\text{Na}^{99m}\text{TcO}_4$). In this chemical form, it can be used to study brain and thyroid (Saha, 1998). Intravenously administered ^{99m}Tc -pertechnetate is loosely bound to plasma proteins and rapidly moves out of the intravenous compartment. The plasma half-time clearance is approximately 30 min. Approximately 30% of the administered dose is excreted within 24 h. The total urinary and fecal excretion of ^{99m}Tc activity is about 50% in 3 days and up to 70% after 8 days. ^{99m}Tc is also trapped by the thyroid gland and it passes into the small intestine. However in the brain, the blood-brain barrier prevents $\text{Na}^{99m}\text{TcO}_4$ from entering brain cells. The biodistribution and kinetics of radiochemicals can be altered by a variety of chemical agents, as is widely known (Sampson, 1996). However, many factors, as drug therapy, radiation therapy, dietary conditions, besides pathological process could affect the biodistribution of the different radiopharmaceuticals (Diré *et al.*, 2001; Gomes *et al.*, 2002; Aguiar *et al.*, 2002) or the labeling of blood constituents (Sampson, 1996; Vidal *et al.*, 1998; De Oliveira *et al.*, 2000, 2002; Braga *et al.*, 2000; Nigri *et al.*, 2002; Santos-Filho

et al., 2002). This also requires the repetition of the examination procedure resulting in the unnecessary irradiation to the patient (Saha, 1998). An increasing number of people in the world are using traditional herbs medicines. Natural medicines may contain potentially toxic ingredients and contaminants such as heavy metal. Traditional Chinese Herbal Medicines (TCHM) have been reported to cause serious hematological adverse effects (Azuno *et al.*, 1999). *Sechium edule* (chayotte) a subtropical vegetable with potent diuretic action, is a cucurbitaceus species which is used as food or as medication in popular medicine. It was reported a case of severe hypokalemia pregnancy and that a chayotte preparation was implicated, as the potassium level returned to normal, without recurrence of hypokalemia, once the ingestion of this vegetable was stopped. The medicinal use of chayotte enclose the relief of diseases related to the kidneys, circulatory system, intestinal and cutaneous inflammation and to the cauterize the sores. The infusion of the leaves which contains a substance with cardiovascular properties is indicated to the pulmonary ailment and intestinal inflammation (Jensen and Lai, 1986; Flores, 1989). Gordon (2000) described the hypotensor effect of chayotte. Diré *et al.* (2001) have noticed that chayotte extract (macerated) was capable of altering the morphology of red blood cells in a qualitative analysis. In a *in vitro/in vivo* study, Diré *et al.* (2002) observed that the extracts (decoct and macerated) of chayotte were not capable of altering the radiolabeling of blood elements although they were able to alter the labeling of blood constituents in the treated animals with the referred extracts. Moreno *et al.* (2002), demonstrated that an extract of *Ginkgo biloba* has been altered the radiolabeling of blood elements in an *in vitro* analysis as well as the biodistribution of $^{99m}\text{TcO}_4\text{Na}$. Recently new radiopharmaceuticals labelled with technetium-99m as cytokines (interleukin-IL-2, IL-6, IL-8); human polyclonal immunoglobulin (HIG); radiolabelled antigranulocyte monoclonal antibodies, radiolabelled peptides and antibiotic have been developed to improve specificity in diagnosis of inflammation and infection (Comin *et al.*, 2002). It is described that AGEs may induction the redox-sensitive transcription nuclear factor NF- κ B in response to oxidative stress in turn leads to a transcriptional activation of many genes, many of which are highly relevant for inflammation, immunity and atherosclerosis. These include tumor necrosis factors (TNF- α and TNF- β), interleukins 1,6 and 8 (IL-1, IL-6 and IL-8) interferon- γ (IFN- γ) and cell adhesion molecules. The effect of diabetes associated with natural products on the biodistribution of radiochemicals has not been fully evaluated. Type-I diabetes mellitus is the consequence of

pancreatic beta-cell destruction mediated by mononuclear cells (insulinitis). This insulinitis process starts since many years before the onset of clinical manifestations of the disease. The presence of insulinitis could be only suspected in subjects at risk to develop autoimmune diabetes (relatives of patients affected by type I diabetes, who present positivity to disease-specific autoantibodies). Nevertheless, no tool is currently available to detect such pancreatic inflammation. This could be particularly useful in order to select patients who can benefit of specific preventive immunotherapies. In a pilot study, ^{123}I -IL2 was able to correctly identify the insulinitis process in 5 subjects at risk to develop type I diabetes. All these subjects developed diabetes within 4 years from the scintigraphy. At time of diagnosis, pancreatic beta-cell inflammation is present only in a subgroup of type I diabetic patients (approximately 50%). In recent study $^{99\text{m}}\text{Tc}$ -IL2 scintigraphy correctly identified this subgroup of patients. Indeed, those patients positive to IL2 scan, presented a better metabolic control after 1-year treatment with nicotinamide. These results suggest that treatment with specific immunotherapies can be useful only if a lymphocyte infiltration is still active in endocrine pancreas although it is known that some situations as oxidative stress or drugs may alter the radiolabeling efficiency and in this case a misdiagnosis would be attributed. Pharmacologic agents that specifically inhibit AGE formation have allowed the investigation of the role of AGEs in the development of diabetic complications in animal models. AGEs linked to the vascular matrix can chemically interfere with the bioavailability of NO, an important regulator of vascular tone inducing smooth muscle cells relaxation (Signore *et al.*, 2002).

The aim of this study was to evaluate the effect of a chayotte extract and the diabetes status induced by streptozotocin in the biodistribution of sodium pertechnetate radiopharmaceutical ($\text{Na}^{99\text{m}}\text{TcO}_4$) as well as the influence in the mass of the organs.

MATERIALS AND METHODS

This study was developed in the laboratories of Experimental Radiopharmacy and Experimental Pathology of the Biology Institute of the Universidade do Estado do Rio Janeiro, Brazil

Characterization of the chayotte sample: Chayotte was purchased from a local market in Rio de Janeiro city, RJ, Brazil. To prepare the extract, 50 g of skin of chayotte were mixed with 500 mL of water in an electric extractor. This preparation was filtered and this extract was considered 100%.

The presence of toxic compounds was evaluated and we did not find them in the extracts of chayotte used in our experiments. The method to verify the presence of these toxic products is based on inhibition of acetylcholinesterase in the presence of the pesticides (Bastos *et al.*, 1991). In this method, brain acetylcholinesterase is utilized as an *in vitro* detector of organophosphorus and carbamate insecticides. Briefly, a preparation of acetylcholinesterase was obtained after extraction of a rat brain microsomal fraction with Triton X-100 and was incubated with the extract of chayotte. Enzyme assay was performed by a potentiometric method based on the formation of acetic acid in the incubation mixture (preparation of acetylcholinesterase and extract of chayotte).

Preparing of the extract: To prepare the decoct of chayotte, this vegetable (50 g) was put in an Erlenmeyer with 500 mL of water and it was boiled on slow heat for ten minutes. After that, the solution was filtered and the watery extract was obtained. The same procedure was taken with the preparing of the macerated extract. It was used the skin (50 g) of the chayotte skin which were triturated in a liquidizer with 500 mL of water. The animals were treated during 7 days. In the control the animals just have received water backwards chayotte extracts.

Radiolabeling process

Biodistribution procedures: It was performed 4 groups in the experimental, each group with 4 animals. The chayotte extract (macerated) was administered (replaced by water in the treated group with chayotte and in the diabetic group treated with chayotte) during 7 days. The control group has received water like to the diabetic group. After that, $^{99\text{m}}\text{TcO}_4\text{Na}$ (0.3 mL, 3.7 MBq) was injected by ocular plexus. The animals were sacrificed and their organs were isolated (thyroid, brain, muscle, lung, heart, spleen, kidney, stomach, intestine, liver, bone, ovary, uterus and blood) and counted in a well counter. The %ATI in each organ was determined. The statistical analysis were performed by Tukey test ($p < 0.05$).

Diabetes induction: The injection of streptozotocin was realized in the ventral region next to the alba line with a unique dose of $30 \mu\text{g kg}^{-1}$ by body weight dissolved in saline solution or in a same volume of citrate (control group). In a period of 2 h after the injection the rats were maintained without water and after that it was added sugar high quantities in their drinking during 1.5 h. After 48 h of the induction it was performed the rate of sugar tests by tail punction. It was considered diabetic the rats with rate of sugar rates above 180 dg dL^{-1} .

Fig. 1: Effect of diabetes and chayotte extract in the mass of the organs isolated from Wistar rats. Female Wistar rats had drunk (treated group: diabetic and not diabetic) or not the extract (control group) during 7 days. The animals were sacrificed, the organs were isolated and weighed. The mass/g was determined. A statistical analysis (Tukey test, n = 4) was performed to compare the results

Table 1: Effect of chayotte extract and diabetes in the bioavailability of sodium pertechnetate in female Wistar Rats

%ATI/g	Control	Chayotte	Diabetes	Diabetes+Chayotte
Brain	0.003±0.001	0.004± 0.001	0.002±0.001	0.002±0.001
Liver	0.017±0.008	0.013±0.006	0.011±0.003	0.012±0.004
Intestine	0.131±0.019	0.021±0.106	0.192±0.183	0.192±0.083
Heart	0.029±0.016	0.066±0.040	0.029±0.014	0.049±0.054
Kidney	0.195±0.031	0.172±0.173	0.148±0.031	0.189±0.045
Stomach	0.892±0.482	0.922±0.318	1.006±0.210	1.247±0.377
Pancreas	0.027±0.012	0.102±0.016	0.128±0.049	0.117±0.048
Lung	0.056±0.034	0.072±0.012	0.032±0.013	0.059±0.037
Over	0.13±0.047	0.119±0.017	0.148±0.083	0.164±0.056
Uterus	0.117±0.011	0.126±0.006	0.435±0.121	0.164±0.112
Bone	0.082±0.010	0.082±0.031	0.085±0.013	0.076±0.012
Muscle	0.006±0.002	0.029±0.002	0.011±0.007	0.018±0.020
Thyroid	0.786±0.123	1.841±1.156	8.438±2.021	0.429±0.149
Spleen	0.098±0.016	0.115±0.015	0.105±0.024	0.110±0.039
Blood	0.202±0.053	0.152±0.035	0.192±0.041	0.262±0.087

Female Wistar rats had drunk (treated group: diabetic and not diabetic) or not the extract (control group) during 7 days and after that ^{99m}TcO₄Na was injected by ocular plexus. The animals were sacrificed, the organs were isolated and the % ATI/g was determined. For blood 1 mL was considered to be equivalent to 1 g. A statistical analysis (Tukey test, n = 4) was performed to compare the results

RESULTS

Due to the analysis of the results it was notice that there was a decrease in the mass of the spleen by the comparison of the control group to the group treated with chayotte extract (from 0.37±0.08 to 0.25±0.02) (Fig. 1). Comparing the group treated with chayotte extract with the diabetes group it was verified an increase of the mass (from 0.25±0.02 to 0.38±0.06) the same was noticed between the diabetes groups and the diabetes group treated with chayotte extract (from 0.38±0.06 to 0.44±0.02) (Fig. 1). Towards to the analysis of the stomach it was seen that there was a decrease in the mass in the mass in the group treated with chayotte when compared with the control group (from 1.10±0.19 to 0.87±0.21) (Fig. 1). In the pancreas there was a decrease in all groups (chayotte, diabetes and diabetes treated with chayotte) when compared to the control group (from 1.21±0.19 to 0.47±0.19; to 0.37±0.10; to 0.46±0.15) (Fig. 1). In the heart was perceived a decrease in the mass in the chayotte group compared to the control (from 0.70±0.11 to

0.52±0.02) (Fig. 1). To the analysis of the kidney it was realized an increase in the mass comparing the diabetes treated with chayotte to the control (from 0.58±0.05 to 0.75±0.04) and confronting the group treated with chayotte to the diabetes group treated with chayotte (from 0.58±0.08 to 0.75±0.04) (Fig. 1). In Table 1 has shown the effect of a chayotte extract and diabetes in the uptake of radioactivity in the organs. The analysis of the results has indicated that in the diabetes group had been an increase in the uptake of the radiopharmaceutical in pancreas (from 0.03±0.01 to 0.13±0.05) as well as in the diabetes groups treated with chayotte extract (from 0.03±0.01 to 0.11±0.05). Due to the analysis to the other organs it was not noticed any alterations in the bioavailability of sodium pertechnetate.

DISCUSSION

The distribution, uptake, retention and the elimination of radiopharmaceuticals depend on several factors, such as regional blood flow tissue metabolism

and the binding to the blood elements (Hladik III *et al.*, 1987; Sampson, 1996). The labeling of blood elements with ^{99m}Tc has many applications. It is known that extracts obtained from medical plants can alter the labeling of blood elements with ^{99m}Tc as well as the morphology of red blood cells (De Oliveira *et al.*, 2000, 2002, Oliveira, 1996; Vidal *et al.*, 1998; Reiniger *et al.*, 1999; Braga *et al.*, 2000; Lima *et al.*, 2002 Capriles *et al.*, 2002). The evidence that drugs can affect either the radiolabeling as the biodistribution of red blood cells or the morphology of them in the context of nuclear medicine clinic has come to light only comparatively recently and it is an important factor in the interpretation of scintigraphic images. In this work it was noticed that chayotte extract was capable of normalizing the uptake of $^{99m}\text{TcO}_4\text{Na}$ in the pancreas in the animals treated with the referred extract. In comparison to that in the diabetes animals was noticed that the disease status was capable to induce a decrease in uptake of the radiopharmaceutical in pancreas. A great number of workers have turned their attention to *in vitro* and *in vivo* evaluation of drugs in the process to label blood cells and in the biodistribution of radiopharceutical (Hladik III *et al.*, 1987; Hesselewood and Leug, 1994; Owunwanne *et al.*, 1995; Sampson, 1996). Nigri *et al.* (2002), analyzing concentrations levels higher than the therapeutic levels in humans it was demonstrated that antiseizure drugs like phenobarbital, clonazepam and phenytoin have the capacity of altering the radiolabeling of blood elements. Gomes *et al.* (2002), have demonstrated that a component of many chemotherapeutic regimens, mitomycin-C, has altered the bioavailability of technetium-99m-labelled sodium pyrophosphate in mice.

In the labeling process of blood constituents with ^{99m}Tc is needed a reducing agent and probably the stannous ion would be oxidized *in vitro* studies was verified that extracts of *Thuya occidentalis* (Oliveira *et al.*, 1997), *Nicotiana tabacum* (Vidal *et al.*, 1998), *Maytenus ilicifolia* (De Oliveira *et al.*, 2000), *Syzygium jambolanum* (Santos *et al.*, 2002), *Stryphnodendron adstringens* (Mart.) Coville (Costa *et al.*, 2002) and *Ginkgo biloba* (Moreno *et al.*, 2002), possibly, would have oxidants compounds and the labeling of blood elements decrease in the presence of these extracts. In a research was verified that *Paullinia cupana* extract was capable of altering the radiolabeling of blood elements as well as to alter quantitatively the shape of red blood cells (Oliveira *et al.*, 2002). In other *in vitro* study with *Fucus vesiculosus* extract was noticed that the referred extract has induced a qualitative alterations on the morphology of red blood cells together with alterations on the labeling of blood elements with ^{99m}Tc (Oliveira *et al.*, 2003). In a *in vivo* studies Diré *et al.* (2002), have

demonstrated that the chayotte extracts (macerated and decoct) were capable of altering the radiolabeling of blood elements. Similar results were observed with an extract of *Solanun melongena* (eggplant) which was capable of altering radiolabeling of blood elements with ^{99m}Tc as well as the bioavailability of NaTcO_4 (Capriles *et al.*, 2002). Moreno *et al.* (2002), eyed that in a *in vitro* study the extract of *Ginkgo biloba* altered the morphology of red blood cells together with the radiolabeling of blood elements, the opposite, was observed in a *in vivo* study which this fact may be explained by the generate of metabolites *in vivo* without direct action on the morphology of red blood cells despite the referred extract had been altered the biodistribution of $^{99m}\text{TcO}_4\text{Na}$. Santos-Filho *et al.* (2002), reported that the extracts of *Mentha crispera* L. (mint) and *Piper methysticum* (Kava Kava) were capable of altering the morphology of red blood cells notwithstanding mint extract has also altered the radiolabeling process. Braga *et al.* (2000), in a *in vitro* study demonstrated that *Peumus boldus* did not alter the labeling of blood elements with ^{99m}Tc , in this same study it was observed that the extracts of *T. occidentalis* and *N. tabacum* have altered the radiolabeling of blood elements as well as the morphology of red blood cells. Lima *et al.* (2002) in a *in vivo* study have shown that an extract of cauliflower (leaf) was not capable of altering the labeling of blood elements with technetium-99m. the referred author has demonstrated that cauliflower extract was not able to alter the biodistribution of $^{99m}\text{TcO}_4\text{Na}$. Diré *et al.* (2001), in a qualitative analysis *in vivo*, have eyed that a chayotte extract (macerated) has induced alteration on the shape of red blood cells. We can speculate like observed by Mongelli *et al.* (1997), in a study with *Bolax gummifera* extract, that the chayotte extract when administrated to the animals due to their possible metabolization may generate reactive metabolites with oxidant properties which may be able to alter the active of cell membrane which can modify the uptake of $^{99m}\text{TcO}_4\text{Na}$ in pancreas as it was noticed together with the decrease of mass. Although the non alteration of the bioavailability in the other organs it was verified a variety in the mass to spleen, stomach, heart and kidney comparing the groups to the control and between them. Maybe the variation in the mass could be due to different mechanisms of action of the compounds present in the extract, by the effect of the many circumstances related to the diabetes status or by the effects of the extract associated with the ones of the diabetes which would be related to molecular and cellular pathways which would be linked to the alteration of the %ATI/g as well as to the mass by the alterations of the structure of the extra

cellular matrix and the morphophysiological status of the cell. The results may be supported by the study in which to dissect the contribution of RAGE-ligand interaction in the pathogenesis of diabetic vasculopathy, an acute animal model of diabetes-associated hyperpermeability was tested first, using reagents blocking the receptor itself or blocking the access of ligands to RAGE, by administering the decoy protein soluble RAGEs. Rats rendered diabetic with streptozotocin, after 9-11 weeks of diabetes showed increased vascular permeability in multiple organs, especially the intestine, the skin and the kidney (Basta *et al.*, 2004).

CONCLUSIONS

Due to the results obtained in this study we can speculate that *Sechium edule* extract and diabetes status were capable of altering the biodistribution of $^{99m}\text{TcO}_4\text{Na}$ in pancreas as well as the mass in many organs. This fact could be related to the presence of compounds with oxidant properties which could be produced by the metabolization of the extract and by the generation of AGEs in diabetes. Moreover, although our results were obtained with animals, we suggest paying attention with examination in nuclear medicine in patients under the treatment referred to popular medicine who is drinking chayotte extract therapeutically.

REFERENCES

- Aguiar, R.O., J.K. Kwee, E.F. De Paula, M.L. Gomes and M. Bernardo-Filho, 2002. The effect of murine B16F10 melanoma on the biodistribution of ^{99m}Tc -MDP in male C57BL/6J mice. *Cell. Mol. Biol.*, 48: 747-750.
- Azuno, Y., K. Yaga, T. Sasayama and K. Kimoto, 1999. Thrombocytopenia induced by Jui, a traditional Chinese herbal medicine. *Lancet.*, 354: 304-305.
- Basta, G., A.M. Schmidt and R.D. Caterina, 2004. Advanced glycation end products and vascular inflammation: Implications for accelerated atherosclerosis in diabetes. *Cardiovascular Res.*, 63: 582-592.
- Bastos, V.L.F.C., J.C. Bastos, J.S. Lima and M.V.C. Faria, 1991. Brain acetylcholinesterase as an *in vitro* detector of organophosphorus and carbamate insecticides in water. *Water Res.*, 25: 835-840.
- Braga, A.C.S., M.B.N. Oliveira, G.D. Feliciano, I.W. Reiniger, J.F. Oliveira, C.R. Silva and M. Bernardo-Filho, 2000. The effect of drugs on the labeling of blood elements with Technetium-99m. *Curr. Pharm. Design*, 6: 1179-1191.
- Capriles, P.V.S.Z., A.P.M. Dias, T.E.M.M. Costa, M.B.N. Oliveira, M.V.C. Faria, E.G. Moura, B.A.L. Abreu and M. Bernardo-Filho, 2002. Effect of eggplant (*Solanum melongena*) extract on the *in vitro* labeling of blood elements with technetium-99m and on the biodistribution of sodium pertechnetate in rats. *Cell. Mol. Biol.*, 48: 771-776.
- Costa, T.E.M.M., A.P.M. Dias, P.V.S.Z. Capriles, M.B.N. Oliveira, E.L.C. de Amorim, C.S. de Andrade Lima and M. Bernardo-Filho, 2002. Effect of barbatimao [*Stryphnodendron adstringens* (Mart.) Coville] infusion on the labeling of blood elements with technetium-99m. *Rev. Bras. Farmacogn.*, 12: 7-9.
- Comin, J.M., V.N. Cardoso, P. Plaza and M. Roca, 2002. Hank's balanced salt solution: An alternative resuspension medium to label autologous leukocytes. Experience in inflammatory bowel disease. *Braz. Arch. Biol. Tech.*, 45: 39-44.
- De Oliveira, J.F., A.C.S. Braga, M.B.N. de Oliveira, A.S. Avila and A. Caldeira-de-Araujo *et al.*, 2000. Assessment of the effect of Maytenus ilicifolia (espinheira santa) extract on the labeling of red blood cells and plasma proteins with technetium-99m. *J. Ethnopharmacol.*, 72: 179-184.
- De Oliveira, J.F., A.S. Avila, A.C.S. Braga, M.B.N. de Oliveira and E.M. Boasquevisque *et al.*, 2002. Effect of extract of medicinal plants on the labeling of blood elements with Technetium-99m and on the morphology of red blood cells: I-a study with Paullinia cupana. *Fitoterapia*, 73: 305-312.
- Diré, G., E. Lima, D. Mattos, M.B. Oliveira, M.J. Pereira, S. Moreno, R. Freitas, M.L. Gomes and M. Bernardo-Filho, 2001. Effect of chayotte (*Sechium edule*) extract on the biodistribution of technetium-99m and on the morphometry of red blood cells. *J. Labelled Cpd Radiopharm.*, 44: 648-650.
- Diré, G.D., E.A.C. Lima, M.J.S. Pereira, M.B.N. Oliveira, S.R.F. Moreno, D.M.M. Mattos, R.L. Jales and M. Bernardo-Filho, 2002. Effect of a chayotte (*Sechium edule*) extract on the labeling of red blood cells and plasma proteins with technetium-99m: *in vitro* and *in vivo* studies. *Cell. Mol. Biol.*, 48: 751-755.
- Flores, E.M., 1989. El chayote, *Sechium edule* Swartz (Cucurbitaceae). *Rev. Biol. Trop.*, 1: 1-54.
- Gomes, M.L., D.M. Mattos, R.S. Freitas, G.F. Diré, E.A.C. Lima, S.M.S. Souza and M. Bernardo-Filho, 2002. Evaluation of the effect of mitomycin-C on the bioavailability of technetium-99m-labelled sodium pyrophosphate in mice. *Cell. Mol. Biol.*, 48: 757-759.
- Gordon, E.A., 2000. The antihypertensive effects of the Jamaican Cho-cho. *West. Indian Med. J.*, 1: 27-31.

- Hladik III, W.B., G.B. Saha and K.T. Study, 1987. Essentials of Nuclear Medicine Science. Willians and Wilkings, Baltimore-London.
- Hesslewood, S. and E. Leung, 1994. Drug interactions with radiopharmaceuticals. Eur. J. Nucl. Med., 21: 348-356.
- Jensen, L.P. and A.R. Lai, 1986. Chayote (*Sechium edule*) causing hypokalemia in pregnancy. Am. J. Obst. Gynecol., 5: 1048-1049.
- Lima, E.A.C., G. Diré, D.M.M. Mattos, R. Freitas, M.L. Gomes, M.B.N. liveira, M.D.C. Faria, R.L. Jales and M. Bernardo-Filho, 2002. Effect of an extract of cauliflower (leaf) on the labeling of blood elements with technetium-99m and on the survival of *Escherichia coli* AB 1157 submitted to the treatment with stannous chloride. Food Chem. Toxicol., 40: 919-923.
- Mongelli, E., C. Desmarchelier, J. Coussio and G. Ciccia, 1997. Biological studies of Bolax gummifera, a plant of the Falkland Islands used as a treatment of wounds. J. Ethnopharmacol., 56: 117-121.
- Moreno, S.R.F., G.D. Feliciano, R.S. Freitas, M.B. Farah, G.L. Laurentino-Filho, E.K. Rocha, R.L.C. Jales and M. Bernardo-Filho, 2002. Effect of *Ginkgo biloba* on the labeling of blood elements with technetium-99m: *in vitro* study. Rev. Bras. Farmacogn., 12: 62-63.
- Nigri, F., M.B.N. Oliveira and M. Bernardo-Filho, 2002. Assessment of the effect of antiseizure drugs on the labeling process of red blood cells and plasma proteins With Technetium-99m. Cell. Mol. Biol., 48: 793-801.
- Oliveira, J.F., A.C. Braga, A.S. Avila, L.M. Fonseca, B. Gutfilen and M. Bernardo-Filho, 1996. Effect of *Thuya occidentalis* on the labeling of red blood cells and plasma proteins with technetium-99m. Yale J. Biol. Med., 69: 489-494.
- Owunwanne, A., M. Patel, S. Sadek, 1995. The Handbook of Radiopharmaceuticals. Chapman and Hall Medical, London.
- Reiniger, I.W., J.F. Oliveira, A.C. Araujo and M. Bernardo-Filho, 1999. Effect of *Peumus boldus* on the labeling of red blood cells and plasma proteins with technetium-99m. Applied Rad. Isotopes, 51:145-149.
- Saha, G.B., 1998. Fundamentals of Nuclear Pharmacy. Springer-Verlag, New York.
- Sampson, C.B., 1996. Complications and difficulties in radiolabelling blood cells: A review. Nucl. Med. Commun, 17: 648-658.
- Santos, A.E.O. dos, C. Moreira, S. Dos, M.B.N. Oliveira, G. Diré, R.L. Jales and M. Bernardo-Filho, 2002. Effect of a *Syzygium jambolanum* (jamelão) extract on the labeling of blood elements with sodium pertechnetate ($\text{Na}^{99\text{m}}\text{TcO}_4$). Rev. Bras. Pl. Med., 5: 63-67.
- Santos-Filho, S.D., C.K. Ribeiro, G.F. Diré, E. Lima, M. Pereira and M. Bernardo-Filho, 2002. Morphological Alterations on Red Blood Cells Labeled with Technetium-99m: The Effect of *Mentha crispa* L. (Hortelã) and *Piper methysticum* (Kava Kava) Extracts. Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine, SGEEditoriali, Padova, 6: 503- 505.
- Signore, A., C. D'Alessandria, A. Annovazzi and F. Scopinaro, 2002. Radiolabelled cytokine for imaging chronic inflammation. Braz. Arch. Biol. Technol., 45: 15-23.
- Tseng, K.C., B.S. Sheu, J.C. Lee, H.M. Tsai, N.T. Chiu and Y.C. Dai, 2005. Application of technetium-99m-labeled human serum albumin scan to assist surgical treatment of protein-losing enteropathy in Cronkhite-Canada syndrome: Report of a case. Dis Colon Rectum, 4: 870-873.
- Vidal, M.V., B. Gutfilen, L.M.B. Fonseca and M. Bernardo-Filho, 1998. Influence of tobacco on the labeling of red blood cells and plasma proteins with technetium-99m. J. Exp. Clin. Cancer. Res., 17: 1-16.