UNIVERSIDADE FEDERAL DE PERNAMBUCO

CENTRO DE CIÊNCIAS BIOLÓGICAS

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOQUÍMICA E FISIOLOGIA

INFLUÊNCIA DE EXTRATO AQUOSO DE *Indigofera suffruticosa* NA MARCAÇÃO COM TECNÉCIO-99m i*n vivo* E *in vitro.*

Dewson Rocha Pereira

Recife

2009

UNIVERSIDADE FEDERAL DE PERNAMBUCO

CENTRO DE CIÊNCIAS BIOLÓGICAS

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOQUÍMICA E FISIOLOGIA

INFLUÊNCIA DE EXTRATO AQUOSO DE *Indigofera suffruticosa* NA MARCAÇÃO COM TECNÉCIO-99m i*n vivo* E *in vitro.*

Dissertação apresentada para o cumprimento das exigências para obtenção do título de Mestre em Bioquímica pela Universidade Federal de Pernambuco.

Dewson Rocha Pereira

Orientadora: Profa. Dra. Vera Lúcia de Menezes Lima

Co-Orientadora: Maria Teresa Jansem de Almeida Catanho

Recife-PE

Fevereiro – 2009

Pereira, Dewson Rocha

Influência de extrato aquoso de *Indogosfera suffruticosa* na marcação com Tecnécio-99m *in vivo* e *in vitro*. / Dewson Rocha Pereira – Recife: O Autor, 2009.

64 folhas: fig., tab.

Dissertação (mestrado) – Universidade Federal de Pernambuco. CCB. Departamento de Biofísica e Fisiologia, 2009.

Inclui bibliografia e anexo.

1. Indigofera suffruticosa. 2. Tecnécio-99m. 3. Plantas medicinais. I Título.

582.738	**CDU (2.ed.)**	**UFPE**
583.74	**CDD (22.ed.)**	**CCB – 2009- 60**

DEWSON ROCHA PEREIRA

"Influência de extrato aquoso de *Indigofera suffruticosa* na marcação com tecnécio-99m *in vivo* e *in vitro*"

Dissertação apresentada para o cumprimento parcial das exigências para obtenção do título de Mestre em Bioquímica e Fisiologia pela Universidade Federal de Pernambuco

Aprovado por:

Profa. Dra. Vera Lúcia de Menezes Lima - Presidente

Profa. Dra. Patrícia Maria Guedes Paiva – Examinador interno

Profa. Dra. Eulália Camelo Pessoa de A. Ximenes – Examinador Externo

Prof. Dr. César Augusto da Silva – Examinador Externo

“Deus é justo, injusto é o homem que não usa aquilo que ele nos dá.”

Autor desconhecido

Aos meus pais, Ataíde Pereira e Terezinha Rocha, aos meus irmãos e familiares, pela cumplicidade e compreensão em momentos importantes. Como também a minha esposa, Anália Fernandes e ao meu filho Lucas Fernandes, pelo amor, pela confiança, pela amizade e por existirem.

AGRADECIMENTOS

- Primeiramente a Deus por confortar minha mente e meu corpo nos momentos importantes da minha vida.
- Aos meus pais Ataíde e Terezinha pela vida, pelo incentivo aos estudos, pelas lições de moral, pelo afeto, pelo amor.
- Aos meus irmãos Klemerson, Jobson e às minhas irmãs Érika e Mônica pelo companherismo e apoio.
- A todos os meus familiares por sempre me incentivarem.
- A minha esposa Anália por me apoiar nas ocasiões mais decisivas da minha vida e pelo pequeno grande elo em nossas vidas, Lucas Fernandes, nosso filho.
- A Profa. Dra. Vera Lúcia de Menezes Lima pela brilhante orientação na minha vida acadêmica e por permitir a realização desse trabalho no laboratório de Lipídios e Lipoproteínas.
- A Profa. Teresa Jansem pela essencial orientação, teórica e prática, como para o desenvolvimento do projeto no Laboratório de Biologia Celular e aos alunos André e Jailson.
- A todos os professores do Programa de Pós-graduação em Bioquímica e Fisiologia.
- Aos alunos do laboratório de Lipídios Bianka Santana, Adenor Pimenta, Tiago Araújo, Gisele Rabelo, Aline Farias, Caíque, Ana, Luciana, Janaina, Tiago e Renato, pelo companheirismo.
- Ao nosso especial colaborador Albérico Leal pela ajuda científica, psicológica e espiritual,
- A todos os funcionários do departamento de Bioquímica, em especial a João Virginio, Miron, Neide, Djalma, Helena e Jorge.
- Aos amigos do mestrado, em especial Antonio Fernando, Leucio Filho, Douglas e Romero.
- A todos que participaram ativa ou passivamente durante este meu aprendizado.

Muito Obrigado!

ÍNDICE ANALÍTICO

LISTA DE FIGURAS

Introdução

Capítulo I

LISTA DE TABELAS

Capítulo II

LISTA DE ABREVIATURAS

OMS – Organização Mundial de Saúde

BC – Blood Cells

RBC – Red blood Cells

Hb – Hemoglobina

P – Plasma

IF-BC – Insoluble fraction of Blood Cells

IF-P – Insoluble fraction of plasma

Tc-99m – Tecnécio-99m

$Na^{99m}TcO_4$ – Pertecnetato de Sódio

TcO^{-4} – Íon Pertecnetato

$SnCl_2$ – Cloreto estanoso

Sn^{++} – Íon estanho

SPECT - Single photon emission computed tomography

PET - Positron emission tomography

keV – quiloeletron-volt

TCA - Ácido tricloroacético

%ATI – Porcentagem de radioatividade

MBq - megabecquerel

ANOVA - Análise de variância

RESUMO

Produtos naturais são amplamente utilizados como medicamento por varias populações. *Indigofera suffruticosa* é uma leguminosa encontrada na região Nordeste do Brasil e usada na medicina popular, porém suas propriedades biológicas ainda são desconhecidas. A marcação com tecnécio-99m (Tc-99m) de órgãos, tecidos e eritrócitos é utilizada no exame de imagem pela medicina nuclear. Este processo pode ser alterado pela ação de drogas, permitindo um possível erro ou inadequação na interpretação do exame. A marcação de eritrócitos depende de um agente redutor, o cloreto estanoso. Nós avaliamos o efeito de extrato aquoso de *I. suffruticosa* na biodistribuição do Pertecnetato de sódio ($Na^{99m}TcO_4$) em camundongos e na marcação, *in vitro*, com Tc-99m dos constituintes sanguíneos. Os dados mostram que o tratamento dos animais com extrato de *I. suffruticosa* alterou significativamente ($p<0,05$) a captação de $Na^{99m}TcO_4$ na tireóide, no intestino delgado e nos pulmões. O extrato de *I. suffruticosa* diminuiu ($p<0,05$) a marcação com Tc-99m nas células sanguíneas e nas frações insolúveis das células e do plasma. A morfologia dos eritrócitos alterou levemente contudo a fragilidade osmótica destas células aumentou na presença do extrato de *I. suffruticosa*. Nós sugerimos que o efeito do extrato de *I. suffruticosa* pode ser explicado por dano nas membranas plasmáticas envolvidas no transporte de íons Pertecnetato e estanho. Em conclusão, possíveis substâncias do extrato de *I. suffruticosa* pode alterar a marcação com Tc-99m, *in vitro* e *in vivo*. Assim, nossos resultados podem ajudar o entendimento da influência de drogas naturais em procedimentos de medicina nuclear. Além disso, permite novos conhecimentos sobre o extrato de *I. suffruticosa*, um produto natural utilizado na medicina popular.

Palavras chave: *Indigofera suffruticosa.* Planta medicinal. Tecnécio-99m.

ABSTRACT

Natural products are widely used as medicine by various populations. *Indigofera suffruticosa* is a legume found in the northeastern Brazil and it is used in the popular medicine, but its biological properties still are unknown. Labeling with technetium-99m (Tc-99m) of organs, tissues and erythrocytes is used in the examination by nuclear medicine imaging. This process may be altered by drugs leading to a possible error or inadequacy in the interpretation of the examination. The erythrocyte labeling depends of a reducing agent, the stannous chloride. We have evaluated the effect of the aqueous extract of *I. suffruticosa* in the biodistribution of sodium pertechnetate ($Na^{99m}TcO_4$) in mice and in the labeling with Tc-99m of blood constituents. The data showed that the treatment of animals with *I. suffruticosa* extract altered significantly ($p<0.05$) the uptake of $Na^{99m}TcO_4$ in the thyroid, small intestine and lungs. *I. suffruticosa* extract decreased significantly ($p<0.05$) the labeling in the blood cells and insoluble fractions of blood cells and plasma. The shape of the erythrocytes was slightly altered although the osmotic fragility of the this cells increased in the presence of the *I.suffruticosa* extract. We suggest that the effect of extract *I. suffruticosa* may be due to damages in the plasma membrane involved in the ions tranport. In conclusion, susbstances present in the *I. suffruticosa* extract can alter the labeling with Tc-99m, *in vitro* and *in vivo*. Thus, our results can help to understand the influence of natural products in nuclear medicine procedures. Moreover, introduce new knowledge about *I. suffruticosa* extract a natural product used in the popular medicine.

Keywords: Indigofera suffruticosa. Medicinal plant. Technetium-99m.

INTRODUÇÃO

O homem utiliza as riquezas da flora para o tratamento ou cura de diversas doenças há milhares de anos, existindo relatos do uso de plantas com finalidades terapêuticas desde o ano 3.000 a. C. (Ko, 1999; Tyler, 1996).

Contudo, era necessário estudar as propriedades biológicas destas plantas e como elas agem no organismo. Os estudos aumentaram e as tecnologias evoluíram no decorrer dos tempos e no início do século 19, o desenvolvimento das ciências naturais e dos métodos científicos na medicina, propiciou mais conhecimento sobre os fitomedicamentos. Nesta mesma época, o isolamento da morfina da *Papaver sommiferum* (1803-1806), pelo farmacêutico Friedrich Sertürner, marcou o início do processo de extração de princípios ativos de vegetais através de métodos químicos e analíticos (Schulz, Hänsel, Tyler, 2001).

A fitoterapia foi reconhecida pela Organização Mundial de Saúde (OMS), na Conferência de Alma Ata em 1978, ressaltando as plantas medicinais como parte do Programa Saúde Para Todos no Ano 2000. Isso proporcionou a realização de mais estudos e a propagação do uso das plantas medicinais regionais como uma maneira de diminuir custos dos programas de saúde pública (Yamada, 1998).

Atualmente, os fitoterápicos são amplamente utilizados no tratamento de doenças em diversos países. Seu uso cresceu demasiadamente nas últimas décadas levando o mercado mundial de fitoterápicos a girar em torno de aproximadamente 22 bilhões de dólares (Kam e Liew, 2002). Na África, por exemplo, 80% da população depende do uso de medicamentos naturais, já que suas condições financeiras não suportam os altos valores dos medicamentos sintéticos (Aschwanden, 2001).

O Brasil deveria ser um país privilegiado no mercado de produtos naturais, devido a sua imensa e diversificada flora. Contudo, ainda existia uma falta de confiança na

qualidade do produto, o que dificulta a aplicação de altos investimentos neste tipo de mercado (Yamada, 1998).

Esse quadro começou a mudar com o avanço das pesquisas científicas na área, o que permitiu o desenvolvimento de medicamentos naturais reconhecidamente seguros e eficazes, mostrando ao mercado a credibilidade do produto. Este avanço junto com a necessidade de busca, pela população, por terapias de baixo custo e menos agressivas aumentou o uso deste tipo de medicamento (Yunes *et al*., 2001).

Plantas medicinais

As plantas sintetizam produtos químicos essenciais ao seu desenvolvimento, que são os compostos do metabolismo primário (açúcares, proteínas, purinas e pirimidinas – ácidos nucléicos e a clorofila). Os metabólitos secundários (alcalóides, terpenóides, substâncias fenólicas) são produzidos a partir das rotas do metabolismo primário, eles são característicos de uma única espécie ou grupo delas, relacionadas evolutivamente, e não possuem função geral conhecida (Martin e Demain, 1980; Lima *et al*., 2008).

Vários destes bioprodutos são extraídos de plantas em larga escala para comercialização e desempenham papel muito importante na medicina moderna. As plantas podem fornecer fármacos que dificilmente seriam obtidos por síntese química e ainda podem ser modificados, tornando-se mais eficazes ou menos tóxicos. Assim, os produtos naturais são utilizados como protótipos estruturais inspirando químicos para síntese ou semi-síntese de drogas com um perfil farmacológico semelhante a dos compostos originais (Robbers, Speedie, Tyler, 1996).

Na região Nordeste o uso de plantas medicinais é muito comum na preparação de remédios caseiros para tratar várias enfermidades. As mais utilizadas são a hortelã-da-folha-miúda (*Mentha x villosa* Huds), a romã (*Punica granatum* L.), o melão-de-São

Caetano (*Momordica charantia* L.), o capim-santo (*Cymbopogon citratus* Stapf.) e o alecrim-pimenta (*Lippia sidoides* Cham.) (Medeiros Filho et al., 1997; Diniz et al.,1997, Amorim, 1999).

A OMS reconhece a importância do uso tradicional de uma planta com finalidade terapêutica, em nível de saúde pública, mas ressalta a necessidade do estabelecimento da segurança, eficácia e garantia de qualidade das preparações fitoterápicas (Lapa *et al*., 2003; WHO, 2002; Rates, 2001). Para isso é necessária a realização de estudos científicos sobre o tema, principalmente no Brasil, onde existem poucas pesquisas sobre a maioria das plantas nativas e o uso delas é baseado na etnofarmacologia.

O uso inadequado destes recursos terapêuticos pode originar efeitos adversos crônicos e/ou assintomáticos. Alguns constituintes vegetais são potencialmente perigosos e podem apresentar, por exemplo, efeito genotóxico (Sisenando et al., 2008) e nefrotóxico (Mengs e Stotzem, 1993) o que representa um risco na sua utilização ou exposição.

Influência na Medicina.

Estudos com produtos vegetais visam obter um medicamento natural para o tratamento de doenças, ou capazes de potencializar ações terapêuticas de determinadas drogas, o que diminuiria as doses aplicadas e os efeitos adversos apresentados (Gomes, *et al*., 2002).

Outros avaliam os efeitos de produtos naturais sobre a farmacodinâmica de determinadas substâncias utilizadas para tratamento, impedindo uma interação medicamentosa indesejada (Cañigueral e Vila, 2003; Rates, 2001). Como também, estes produtos não devem intervir na distribuição pelo organismo (biodistribuição) de

compostos radioativos utilizados no diagnóstico de doenças por imagem em Medicina Nuclear (Moreno *et al*., 2007).

Medicina Nuclear

A descoberta das radiações ionizantes e dos radionuclídeos despertou o interesse nas suas aplicações na Biologia e nas Ciências Médicas pelo seu valor como meio para auxiliar o diagnóstico e o tratamento de doenças (Hesslewood, 1994).

A Medicina Nuclear é uma especialidade médica não invasiva que utiliza estes elementos radioativos no diagnóstico de doenças através da detecção de processos funcionais, bioquímicos e metabólicos nos órgãos ou tecidos antes mesmo do aparecimento de manifestações anatômicas (Hladik III et al., 1987). São aplicados para avaliação óssea e oncológica, na detecção de sangramento gastrintestinal, hemangiomas, tromboses e avaliação da função cardíaca (Sampson, 1996; Alberico, *et al*. 2004).

Este tipo de diagnóstico utiliza as tecnologias de tomografia computadorizada de emissão de único fóton (do inglês - *single photon emission computed tomography* SPECT), que emprega radionuclídeos que emitem radiação gama e a tomografia de emissão de positron (do inglês - *positron emission tomography* PET), que utiliza radiação beta (Carlsson, 1995).

As imagens nesse tipo de diagnóstico são produzidas com alta qualidade permitindo uma rápida e eficiente elucidação do diagnóstico (Alberico, *et al*. 2004). É, ainda, um procedimento que utiliza normalmente baixas doses de radiação quando comparado a procedimentos de diagnóstico com Raio X, o que reduz a exposição tanto para o paciente como para a equipe que esta envolvida (Perkins and Frier, 1999).

Tecnécio-99m

O tecnécio-99m (Tc-99m) é um isótopo amplamente utilizado no diagnóstico por imagem em SPECT, pois possui características atraentes, como: tempo curto de meia vida (6 horas) e emissão de radiação gama de 140keV, com consequente impacto ambiental insignificante (Saha, 2004). Essas características permitem a obtenção de imagens com alta qualidade e eficiência aplicando baixas doses deste elemento nos pacientes (Hesslewood, 1994; Early and Sodee, 1996).

Desde 1960 o Tc-99m é administrado no organismo na forma de Pertecnetato de sódio ($Na^{99m}TcO_4$), um radiobiocomplexo ou radiofármaco utilizado em procedimentos de diagnóstico na medicina nuclear (Saha, 1998).

O $Na^{99m}TcO_4$ depois de injetado por via intravenosa se liga reversivelmente às proteínas plasmáticas e celulares (70-80%) e a outra parte se difunde através de fluidos vasculares. Este radiofármaco se dissocia no íon Pertecnetato (TCO^{-4}), e se difundem livremente através das membranas capilares para o líquido intersticial de onde são captados por diferentes órgãos (Owunwanne *et al*., 1995).

O Pertecnetato de sódio é empregado, também, na marcação de eritrócitos para a obtenção de imagem do fluxo sanguíneo, medida de volume de eritrócitos, detecção e localização de sítios de hemorragia gastrintestinal, para localização de hemangioma intramuscular e de obstrução do sistema circulatório (Callahan and Rabito, 1990; Chandra, 1998).

Marcação *in vivo*

Além da utilização para a realização de diagnóstico por imagem, a administração do $Na^{99m}TcO_4$ é utilizada para avaliar experimentalmente o efeito de drogas naturais ou sintéticas sobre a biodistribuição do Pertecnetato em animais (Cañigueral e Vila, 2003).

Nesse experimento o animal é tratado com a droga, depois o Pertecnetato é administrado e seus órgãos retirados. Após, poderá ser analisado se a droga agiu na radiofarmacocinética ou na captação do radiofármaco pelo tecido alvo específico (Perkins and Frier, 1999; Harbert, 1996).

Mudanças na biodistribuição de radiofármacos *in vivo* podem ocorrer devido à alterações das características químicas do radiofármaco ou por modificações do estado fisiológico do tecido de interesse (Oliveira et al., 2002). Assim, cada órgão possui um mecanismo diferente para permitir a passagem do íon Pertecnetato pela membrana e sua fixação no órgão, é essa característica que determina a afinidade do órgão pelo radiofármaco utilizado.

Na tireóide, por exemplo, o íon TCO^{-4} ultrapassa a membrana plasmática por transporte ativo (bomba sódio-iodo) sendo captado numa porcentagem de 2 a 4%. Quando o Pertecnetato de sódio é administrado via oral ou intramuscular o TCO^{-4} é absorvido pelo trato digestivo por difusão simples. Em outros tecidos esse transporte é realizado, principalmente, por canais de cálcio (Narra, *et al*. 1994).

Marcação *in vitro*

A marcação de eritrócitos, também, é de interesse para estudos experimentais *in vitro*, pois permite o conhecimento de propriedades biológicas de produtos vegetais (Oliveira *et al*., 1996).

A marcação do eritrócito com Tc-99m depende de um agente redutor, o cloreto estanoso ($SnCl_2$), visto que o TcO^{-4} não se liga facilmente a outras espécies químicas (Arano, 2002). O $SnCl_2$ sofre dissociação gerando o íon estanho (Sn^{++}) este reduz a valência do Pertecnetato de +7 para valências mais baixas (+3, +4, +5) (Harbert, 1996; Arano, 2002).

A marcação de elementos sanguíneos pode ser alterada devido a ações oxidantes de componentes da planta sobre o íon Sn^{++} ou de espécies reativas de oxigênio produzidas pela planta, e ainda por modificação da forma ou da integridade da estrutura da membrana dos eritrócitos, o que levaria a lise da célula ou a diminuição da eficiência do transporte trans-membrana tanto dos íons Sn^{++} como do TcO^{-4} (Gomes et al., 2002).

Eritrócitos

Os eritrócitos são células sanguíneas anucleadas com vida média de 120 dias. Elas exercem as funções no organismo de transporte de oxigênio através da hemoglobina (Hb) e transporte de dióxido de carbono e de íons hidrogênio, que são vitais para o desenvolvimento da vida (Stryer, 2003).

A membrana plasmática dos eritrócitos é composta por dupla camada lipídica e várias proteínas globulares. Essas proteínas formam sistemas de transporte de íons e moléculas de baixo peso molecular (Stryer, 2003; Guyton e Hall, 2006). Quando colocadas em solução suficientemente hipotônica, os eritrócitos absorvem água, até que ocorra o equilíbrio osmótico ou ruptura da membrana (Junqueira, 2004).

Uma dessas proteínas, a banda-3, além de desempenhar um papel estrutural na fixação do citoesqueleto (actina e espectrina) à membrana, atua como um transportador de ânions nos eritrócitos (Figura 1). Elas permitem a difusão dos íons cloreto, que atuam durante o transporte de O_2 e CO_2, importantes para a respiração (Guyton e Hall, 2006). Estudos mostram que o íon Pertecnetato atravessa o espaço intracelular por um mecanismo de troca de íons cloreto e/ou bicarbonato, indicando uma relação com a proteína banda-3 (Callahan and Rabito, 1990). Já a passagem do íon estanho pela membrana dos eritrócitos pode estar relacionada com os canais de cálcio, segundo pesquisas realizadas (Gutfilen, *et al.* 1993).

A hemoglobina é uma proteína que possui grupamentos heme, sítio de ligação do oxigênio e compõe 95% das proteínas totais do eritrócito (Stryer, 2003). Estudos relatam que a fixação do Pertecnetato no eritrócito é principalmente na cadeia-β da molécula de hemoglobina (Srivastava, *et al*., 1984). Também ocorre ligação de uma fração deste radiofármaco às proteínas plasmáticas, que pode ser observado através da técnica de precipitação com ácido tricloroacético (TCA) (Ribeiro, *et al.* 2007).

Band-3 = Banda-3; Actin = Actina; Spectrin = Espectrina; Intracellular = Intracelular; Bilipid layer = Camada bilipídica.

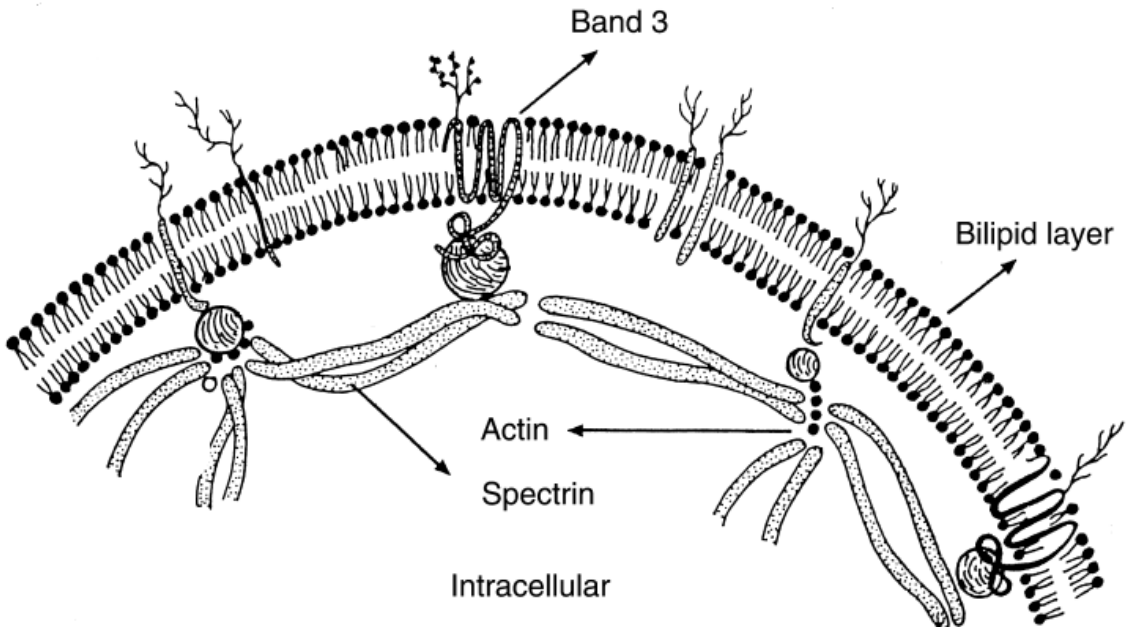

Figura 1- Modelo estrutura da membrana de eritrócito.
http://www.nature.com/ki/journal/v62/n1/images/4493070f2.gif

O mecanismo que ocorre na ligação do Tc-99m nos eritrócitos não esta completamente elucidado. Mesmo assim, são relatadas algumas possibilidades: primeiro o íon estanho pode se ligar a agentes como o citrato e se difunde para o interior das células; já o íon Pertecnetato se difunde livremente dentro e fora da célula; o íon Pertecnetato, dentro da célula, na presença do íon estanho é reduzido e se liga principalmente a fração globina na cadeia-β da hemoglobina (Gomes *et al*., 2002).

Análise estrutural do eritrócito

Eritrócitos incubados com drogas naturais ou sintéticas podem sofrer um distúrbio na sua estrutura e no seu citoesqueleto causado por alteração do coeficiente de partição na membrana destas células (Didelon, *et al*. 2000).

Para averiguar o possível mecanismo responsável por estes efeitos de drogas na marcação com radiobiocomplexos ou até mesmo para avaliar as propriedades químicas deste produto no organismo, diferentes técnicas experimentais podem ser utilizadas (Li et al., 1999).

Alguns autores pesquisam a ação de drogas sobre células sanguíneas analisando a morfologia destas células ao microscópio óptico (Vidal et al., 1998; Fernandes et al., 2005).

Outros utilizam o teste de fragilidade osmótica que avalia, através de uma curva, possíveis alterações na estrutura da membrana de eritrócitos que foram tratadas *in vitro* com alguma droga (Cavalcanti et al., 2003). Neste experimento é possível verificar a capacidade da droga em fragilizar a célula sanguínea e permitir a hemólise. A analise desta curva indicará a modificação estrutural através do percentual de hemólise promovido pela droga (Kempaiah e Sirinivasan 2002).

Fatores interferentes

Alguns fatores podem interferir na biodistribuição de diferentes radiobiocomplexos ou na marcação dos componentes sanguíneos, tais como, terapia com drogas, terapia com radiação, condições de dieta, processos patológicos e procedimentos cirúrgicos (Gomes *et al*., 2002; Sampsom, 1996; Brito, *et al*. 1998). Isto pode acarretar em possíveis erros ou a inadequação na interpretação do exame, e

consequentemente a repetição desnecessária do exame com nova exposição do paciente à radiação (Ko, 1999; Oliveira *et al*., 1997).

As plantas medicinais podem conter componentes naturais de ação tóxica ou contaminante como os metais pesados que podem influenciar neste processo diagnóstico (Kam e Liew, 2002; Ernest, 2002).

Estudos demonstram o efeito de drogas naturais na biodistribuição de radiobiocomplexos pelo organismo animal (Gomes *et al*., 2002; Moreno *et al*., 2007; Santos-Filho *et al.*, 2007). Na marcação *in vitro* de elementos sanguíneos, alguns produtos naturais também interferem, como: extratos de *Thuya occidentalis* (Oliveira et al., 1996), *Nicotiana tabacum* (Vidal et al., 1998), *Maytenus ilicifolia* (Oliveira et al., 2000), *Sechium edule* (chayotte) (Diré et al., 2001); existem aqueles que potencializam esta marcação: extrato de *Peumus boldus* (Reineger et al., 1999) e ainda os que não influenciam neste processo: extrato de *Brassica oleracea* (Lima et al., 2002).

Indigofera suffruticosa

A família Leguminosae, ordem Fabales, subclasse Rosidae, possui cerca de 670 gêneros e 17.615 espécies, subordinadas a três subfamílias: Mimosoideae, Caesalpinioideae e Papilionoideae (Barroso, 1984; Lewis, 1987). Alguns autores consideram-nas como famílias independentes, pertencentes à ordem Leguminales (Cronquist, 1981; Heywood, 1993; Judd *et al.,* 1999).

A subfamília Papilionoideae é um táxon bem representado no semi-árido nordestino (Emperaire, 1989; Rodal, 1992; Alcoforado Filho, 1993; Araújo *et al.,* 1995; Ferraz *et al.,* 1998; Araújo *et al.,* 1998; Rodal *et al.,* 1999; Lemos, 1999) o qual apresenta um grande número de espécies distribuídas em diversos gêneros, incluindo o gênero *Indigofera.* Para este gênero existem mais de 700 espécies, existido a

predominância da espécie *Indigofera suffruticosa* Mill (Indigofera anil Linn) no Brasil (Figura 2) (Moreira & Tozzi 1997) e em outros países da América, desde o sul dos Estados Unidos até a Argentina, sendo aclimatada na Ásia, África e Austrália (White, 1980).

A planta *Indigofera suffruticosa* é largamente encontrada na região do Semi-Árido Pernambucano e está incluída na lista de Leguminosas forrageiras da bacia leiteira do Estado de Alagoas (Ribeiro e *et al*, 1991). A *I. suffruticosa* é um arbusto que mede de 1 a 2 m de altura, possui ramos pubescentes, folhas pínadas contendo de 7 a 15 folíolos oblongos ou ovais, glabros na face e no verso, e apresenta flores miúdas, numerosas, albo-róseas ou amareladas, em racemos axilares. Ela também apresenta pequena vagem falciforme com 6-10 sementes, não comprimida entre estas (Garcez *et al*., 1989). As inflorescências são menores que as folhas e os frutos são numerosos desde a base do eixo da inflorescência (Bortoluzzi *et al*., 2003) (Figura 3).

Esta planta possui algumas propriedades medicinais contra: dores articulares, nefralgias, distúrbios circulatórios e afecções respiratórias. Assim como, suas folhas são usadas na entnomedicina como um composto antiespasmódico, sedativo, diuréticos, purgativos, estomáquico, emenagôgo e antídoto contra o mercúrio e o arsênio (Ribeiro e *et al*, 1991; Martins, *et al*., 2000; Domingues, 1978). A raiz é utilizada como febrífuga, diurética, purgativa, odontálgica e útil na cura da icterícia (Vieira, 1992).

Mesmo possuindo propriedades medicinais, a *I. suffruticosa* pode ser constituída por algum composto que cause dano ao organismo. Existem registros no estado do Rio de Janeiro, por exemplo, de anemia hemolítica e intoxicação no fígado e rim de bovinos, pelo consumo da *I. suffruticosa* (Neto *et al*., 2001), causando a morte destes animais sendo prejudicial à pecuária da região.

Trabalhos preliminares realizados pelo nosso laboratório (Laboratório de Química e Metabolismo de Lipídeos e Lipoproteínas) demonstraram que extrato aquoso bruto de folhas *I. suffruticosa* possui atividade antiinflamatória (Leite *et al.*, 2003), atividade citotóxica em células embrionárias em crescimento (Leite *et al.*, 2004), atividade antimicrobiana contra *Staphylococcus aureus* e contra os fungos dermatófitos *Microsporium canis* e *Trichophyton rubrum* (Leite *et al*., 2006).

Figura 2 – *Indigofera suffruticosa*: vista parcial de um indivíduo adulto.
http://flickr.com/photos/dinesh_valke/2682554864/

Figura 3 – *Indigofera suffruticosa*: ramos com folhas e inflorescência.
http://flickr.com/photos/dinesh_valke/2681740889/

OBJETIVOS

Geral

Avaliar o efeito do extrato aquoso de folhas de *Indigofera suffruticosa* na biodistribuição do pertecnetato de sódio nos tecidos de camundongos e na marcação de eritrócitos com tecnécio-99m.

Específicos

1- Preparar extrato aquoso de folhas da *I. suffruticosa*;

2- Obter sangue de animais para experimentação *in vitro*;

3- Estudar os efeitos do extrato de folhas de *I. suffruticosa* na marcação *in vitro* de eritrócitos com tecnécio-99m;

4- Estudar os efeitos do extrato de folhas de *I. suffruticosa* na marcação com tecnécio-99m, *in vitro*, de proteínas plasmáticas e celulares do sangue;

5- Averiguar alterações morfológicas nas hemácias devido à ação do extrato de *I. suffruticosa*;

6- Avaliar *in vivo* o efeito do tratamento com o extrato de folhas de *I. suffruticosa* sobre a biodistribuição do pertecnetato de sódio em diversos tecidos de camundongos;

7- Verificar a estabilidade da membrana de hemácias incubadas com extrato de *I. suffruticosa* pelo teste de fragilidade osmótica;

REFERENCIAS BIBLIOGRÁFICAS

ALBERICO, R. A.; HUSAIN, S. H. e SIROTKIN, I. Imaging in head ad neck oncology. *Journal Oncology*, v.13, pp.13-35, 2004.

ALCOFORADO-FILHO, F.G. Composição floristica e fitossociologia de uma área de caatinga arbórea no município de Caruaru-PE, Recife: UFPE, 220 p. Dissertação de Mestrado. 1993.

AMORIM, J. A. Fitoterapia popular e saúde da comunidade: diagnóstico para proposta de integração nos serviços de saúde, em Campina Grande, Paraíba. São Paulo, 206p. Tese de Doutorado − Faculdade de Saúde Pública, Universidade de São Paulo, 1999.

ARANO, Y. Recent advances in 99mTc radiopharmaceuticals. *Annals of Nuclear Medicine* ,v.16, p.79-93, 2002.

ARAUJO, E. L.; SAMPAIO, E.V.S.B. & RODAL, M. J. N. Composição floristica e fitossociologia de três áreas de caatinga de Pernambuco. *Revista Brasileira de Biologia*, v.55, p. 594-607, 1995.

ARAUJO, F. S. DE & MARTINS, F. R. Fisionomia e organização da vegetação do carrasco no planalto da Ibiapaba, Estado do Ceará. *Acta Botânica Brasílica* 13: 1-14. 1999.

ASCHWANDEN, C. Herbs for health, but how safe are they? *Bull. W. H. O.*, Geneva, v.79, n.7, p.691-692, 2001. Disponível em: www.who.int/entity/bulletin/archives/79(7)691.pdf . Acesso em 13 jan 09.

BARROSO, G. M. Sistemática das Angiospermas do Brasil. São Paulo: EDUSP, Vol. 2. 1984.

BORTOLUZZI, R. L. C.; GARCIA, F. C. P.; CARVALHO-OKANO, R. M.; TOZZI, A. M. G. A. Leguminosae-Papilionoideae no Parque Estadual do Rio Doce, Minas Gerais, Brasil. I: trepadeiras e subarbustos. Iheringia, Série Botânica v.58, p.25-60, 2003.

BRITO, D. M. M; GOMES, M. L.; RODRIGUES, P. C.; PAULA, E. F.; GUTFILEN, B.; BERNARDO-FILHO, M. Effect of the chemotherapeutic drugs on the biodistribution of 99mTc-DTPA in Balb/c mice. Journal of Experimental & Clinical Cancer Research, v.17, p.313–316, 1998.

CALLAHAN, R.J., RABITO, C.A. Radiolabeling of erythrocytes with technetium-99m: role of Band-3 protein in the transport of pertechnetate across the cell membrane. Journal of Nuclear Medicine v.31, p.2004–2008,1990.

CAÑIGUERAL, S.; VILA, R.;. La fitoterapia racional. In: VANACLOCHA, B. V. e FOLCARÁ, S. C (org.) Fitoterapia. Vademécum de prescripción.4.ed. Barcelona: Masson, p.15-27, 2003.

CARLSSON, S. A glance at the history of nuclear medicine. *Acta. Oncology*, v.34, p.1095-1102, 1995.

CAVALCANTI, T.C.; C.C. GREGORINI, F. GUIMARÃES; O. RETTORI e A.N. VIEIRA-MATOS. Changes in red blood cell osmotic fragility induced by total plasma and plasma fractions obtained from rats bearing progressive and regressive variants of the Walker 256 tumor. *Brazilian Journal Medicinal Biology Research,* v. 36, n.7, p. 887-95, 2003.

CHANDRA, R. Nuclear Medicine Physics the Basics 5th edition. London: Williams & Wilkins 1998.

CRONQUIST, A. Na integrated system of classification of florewing plants. New York: Columbia University Press, p.1262, 1981.

DIDELON, J., P. MAZERON, S. MULLER e J.F. STOLTZ. Osmotic fragility of the erythrocyte membrane: characterization by modeling of the transmittance curve as a function of NaCl concentration. *Biorheology,* v. 37, n. 5-6, p. 409-16, 2000.

DINIZ, M. F. F. M.; OLIVEIRA, R. A. G.; MEDEIROS, A. C. D. e MALTA JÚNIOR, A. Memento fitoterápico: as plantas como alternativa terapêutica, conhecimentos populares e científicos**.** João Pessoa: Ed. Universitária, 1997.

DIRÉ, G.; LIMA, E, GOMES M, BERNARDO-FILHO M. The effect of a chayotte (*Sechium edule*) extracts (decoct and macerated) on the labeling of blood elements with Technetium-99m and on the biodistribution of the radiopharmaceutical sodium pertechnetate in mice: an in vitro and in vivo analysis. *Pakistan Journal Nutrition*, v.2, p.221–227, 2003.

DOMINGUEZ, X. A., MARTINEZ, C., CALERO, A. HINOJOSA, M., ZAMUDIO, A. WATSON, W. H. & ZABEL, V. Chemical components from Jiquelite Indigofera *suffruticosa* Mill. *Planta Medica*, v.34, p.172-175, 1978.

EARLY P J, SODEE D B Principles and Practice of Nuclear Medicine, New York: Mosby Year Book, 1996.

EMPERAIRE, L. Vegetation et gestion des ressources naturelles dans la caatinga du sud-est du Piauí (Brésil). Doctorat d'Etat ès Sciences Naturelles: Université Pierre et Marie Curie, Paris. 1989.

ERNEST, E. Toxic heavy metals and undecleared drugs in Asian herbal medicines. *Trends in Pharmacological Sciences*, v.23, n.3, 2002.

FERNANDES, J. F. O.; BRITO, L.C.; FRYDMAN, J. N.G.; SANTOS, S.D.F.; BERNARDO-FILHO, M. An aqueous extract of *Pfaffi a* sp**.** does not alter the labeling of blood constituents with technetium-99m and the morphology of the red blood cells, v. 15, n.2, p. 126-132, 2005.

FERRAZ, E. M. N.; RODAL, M. J. N.; SAMPAIO, E. V. B. & PEREIRA, R. DE C. A. Composição floristica em trechos de vegetação de caatinga e brejo de altitude na região do vale do Pajeú, Pernambuco. *Revista Brasileira de Botânica*, v.21, p. 7-15, 1998.

GARCEZ, W. S. GAECEZ, F.R. HONDA, N.K. & DASILVA, A. J. R. A nitropropanoyl – glucopyranoside from *Indigofera suffruticosa* . *Phytomistry*, v. 28, p. 1251-1252, 1989.

GOMES, L. M.; OLIVEIRA, de N. B. M. and BERNARDO-FILHO, M. B. Drug interaction with radiopharmaceuticals: Effect on the Labeling of Red Blood Cells with Technetium-99m and on the Bioavailability of Radiopharmaceuticals. *Brazilian Archives of Biology and Techonology*, vol.45, pp.143-9, 2002.

GUTFILEN, B.; BOASQUEVISQUE, E.M. e BERNARDO-FILHO, M. Calcium channel blockers: interference on red blood cell and plasma protein labeling with Tc-99m. *Revista Espanola de Medicina Nuclear,* v.11, p.195–199, 1993.

GUYTON, A. C. e HALL, J. E. Tratado de Fisiologia Médica. Guanabara Koogan, Rio de Janeiro.2006.

HARBERT, J. C.; ECKELMAN, W. C. e NEUMANN, R. D. Nuclear Medicine Diagnosis and therapy. Thieme Medical, New York , 1996.

HESSLEWOOD, S., E. LEUNG. Drug interaci]tions with radiopharmaceuticals. *European Journal of Nuclear Medicine* v.21, p.348, 1994.

HEYWOOD, V. H. Florewing Plant of World. Oxford University Press. New York., p. 335, 1993.

HLADIK III W.B; SAHA G.B; e STUDY K.T. Essentials of Nuclear Medicine Science. William and Wilkins, London ,1987

JUDD, W.S.; AMPBELL, C.S.; KELLOGG, E.A. & STEVENS, P.F. Plant Systematics: A Phylogenetic. Approach. Sunderland: Copyright, p.464, 1999.

JUNQUEIRA, L. C. e CARNEIRO, J. Histologia Básica. Guanabara Koogan, Rio de Janeiro, 2004.

KAM, P. C. A. e LIEW, S. Traditional Chinese herbal medicine and anaesthesia. *Anaesthesia*, v.57, p.1083-1089, 2002.

KEMPAIAH, R. K. e SRINIVASAN, K. Integrity of erythrocytes of hypercholesterolemic rats during spices treatment. *Molecular And Cellular Biochemistry*, v.236, p.155-161, 2002.

KO, R.J. Causes, epidemiology, and clinical evaluation of suspected herbal poisoning. *Clinical Toxicology*, New York, v.37, n.6, p.697-708, 1999.

LAPA, A. J.; SOUCCAR, C.; LIMA-LANDMAN, M. T. R.; GODINHO, R.O. e NOGUEIRA, T. C. M. L. Farmacologia e toxicologia de produtos naturais. In: SIMÕES, C. M. O.; SHENKEL, E. P.; GOSMANN, G.; MELLO, J. C. P.; MENTZ, L. A. e PETROVICK, P. R. (org.) Farmacognosia: da planta ao medicamento. 5.ed. Porto Alegre/Florianópolis: Editora da UFRGS/Editora da UFSC, p.247-262, 2003.

LEITE, S. P., MEDEIROS, P. L., SILVA, E. C., MAIA, M. B. S., LIMA, V. L. M., SAUL, D. E. Embryotoxicity in vitro with extract of Indigofera suffruticosa leaves. Reproductive. *Toxicology*, v.18, p.701-705. 2004.

LEITE, S. P., SILVA, L. L. S., CATANHO, M. T. J. A., LIMA, E. O., LIMA, V. L. M. Atividade antiinflamatória do extrato de Indigofera suffruticosa. *Revista Brasileira de Ciências da Saúde*, v.7, p. 47 – 52, 2003.

LEITE, S. P., VIEIRA, J. R. C., MEDEIROS, P. L., LEITE, R. M. P., LIMA, V. L. M., XAVIER, H. S., LIMA, E. O. Antimicrobial activity of *Indigofera suffruticosa*. *Evidence-Based Complementary and Alternative Medicine*, v.3, n. 2, p.261-265, 2006.

LEMOS , J. R. Fitossociologia do componente lenhoso de um trecho de vegetação arbustiva caducifólia espinhosa no parque Nacional Serra da Capivara, Piauí, Brasil. Recife: UFPE, 55 p. Dissertação de Mestrado. 1999.

LEWIS, J. P. Legumes of Bahia. Kew: Royal Botanic Garden.1987.

LI, A; SEIPELT, H; MULLER, C; SHI ,Y e ARTMANN, M. Effects of salicylic acid derivatives on red blood cell membranes. *Pharmacology & Toxicology*, v.85, p. 206-211, 1999.

LIMA, E. A. C.; DIRÉ, G.; MATTOSA, D. M. M.; FREITASA, R. S.; GOMES, M. L.; OLIVEIRA, M. B. N. DE; FARIA, M. V. C.; JALES, R. L. e BERNARDO-FILHO, M. Effect of an extract of cauliflower (leaf) on the labeling of blood elements with technetium-99m and on the survival of *Escherichia coli* AB1157 submitted to the treatment with stannous chloride. *Food and Chemical Toxicology*, v. 40, p. 919–923, 2002.

LIMA, R. B. S.; GONÇALVES, J. F. de C.; PANDO, S. C.; FERNANDES, A. V. e SANTOS, A. L. W. dos. Primary metabolite mobilization during germination in Rosewood (*Aniba rosaeodora* Ducke) seeds. *Revista Árvore*, v.32, n.1, p.19-25, 2008.

MARTIN, J. F. e DEMAIN, A. L. Control of antibiotic biosynthesis. *Microbiological Reviews*, vol. 44, n. 2, pp. 230-251, 1980.

MARTINS, E. R.; CASTRO, D. M.; CASTELLANI, D. C.; DIAS, J. E. Plantas medicinais. Viçosa: Editora UFV, 2000.

MEDEIROS FILHO, J. G.; PIRES, M. P. C. e FREIRE, A. C. M. Toxicidade de plantas medicinais na terapêutica infantil. *Revista Brasileira de Ciências da Saúde 1*: 45-52, 1997.

MENGS, U., STOTZEM, C.D. Renal toxicity of aristolochic acid in rats as an example of nephrotoxicity testing in routine toxicology. *Archives of Toxicology*, v.67, p.307–311, 1993.

MOREIRA J. L. A, TOZZI, A. M. G. A. *Indigofera* L. (Leguminosae, Papilinoideae) no estado de São Paulo, Brasil. *Revista Brasileira Botanica*, v. 20, p. 97-117, 1997.

MORENO, S. R. F.; ARNOBIO, A.; CARVALHO, J. J. de; NASCIMENTO, A. L.; TIMOTEO, M. O.; OLEJ, B.; ROCHA, E. K. e CALDAS, L. Q. A. The ingestion of a

Nectandra membranacea extract changes the bioavailability of technetium-99m radiobiocomplex in rat organs. *Biology research*, v.40, p.131-135, 2007.

NARRA, K.R., K.S.R. SASTRY, S.M. GODDU, R.W. HOWELL, S.E. STRAND e D.V. RAO. Relative biological effectiveness of 99mTc-radiopharmaceuticals. *Med.Phys*., v.21, p.1921-1926. 1994.

NETO, J. D. B.; OLIVEIRA, C. M. C.; PEIXOTO, P. V.; BARBOSA, I. B. P.; ÁVILA, S. C. e TOKARNIA, C. H. Anemia hemolítica causada por *Indigofera suffruticosa* (Leg. Papilionoideae) em bovinos. *Pesquisa Veterinária Brasileira*, v. 21, n.1, p.18-22, 2001.

OLIVEIRA, J,F.; BRAGA, A.N.S.; OLIVEIRA, M.B.N.; ÁVILA A,S.; ARAUJO, A.C.; CARDOSO,V.N.; BEZERRA J.A.C e BERNARDO-FILHO, M. Assessment of the effect of *Maytenus ilicifolia* (espinheira santa) extract on the labeling of red blood cells and plasma proteins with technetium-99m. *Journal of Ethnopharmacology* v.72, p. 179–184, 2000.

OLIVEIRA, J. F. DE; AVILA, A. S.; BRAGA, A. C. S; OLIVEIRA, M. B. N. DE; BOASQUEVISQUE, E. M.; JALES, R. L.; CARDOSO, V. N.; BERNARDO-FILHO, M. 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*, v.73, p.305-312, 2002.

OLIVEIRA, J. F.; BRAGA, A. C. C.;ÁVILA, A. S., FONSECA, L. M. B.;GUTFILEN, B. e BERNARDO-FILHO, M.. Effects of *Thuya occidentalis* on the labeling of red blood cells and plasma proteins with technetium-99m. *Yale journal of biology and Medicine*, v.69, p.489-494, 1997.

OLIVEIRA, J. F.; BRAGA, A. C. S.; AVILA, A. S.: FONSECA, L. M. B.; GUTFILEN, B. e BERNARDO-FILHO, M. Effect of Thyua occidentalis on the labeling of red blood cells and plasma proteins with technetium-99m. Yale Journal of Biology Medicine. v.69, p-489-494, 1996.

OWUNWANNE A.; PATEL, M. e SADEK S. Preparation of Radiopharmaceuticals. The Handbook of Radiopharmaceuticals. Chapman & Hall: London, 1995.

PERKINS, A. e FRIER, M. Nuclear Medicine in Pharmaceutical Research. London: Taylor & Francis, 1999.

RATES, S. M. K. Promoção do uso racional de fitoterápicos: uma abordagem no ensino de Farmacognosia. Revista Brasileira de Farmacognosia. v.11, p. 57-69, 2001.

REINIGER, I. W.; SILVA, C. R.; FELZENSZWALB,I.; MATTOS, J.C.P. ; OLIVEIRA J.F.; DANTAS, F.J.S.;R. J. A.C. BEZERRA, ARAUJO, A.D. e BERNARDO-FILHO, M.. Boldine action against the stannous chloride effect. *Journal of Ethnopharmacology*, v. 68, p. 345–348, 1999.

RIBEIRO, C. G.; REBELLO, B. M.; NEVES, R. F.; SANTOS-FILHO, S. D.; FONSECA, A. de S. da.; MEDEIROS, A. da C.; BERNARDO-FILHO, M. e CATANHO, M. T. de A. Evaluation of the effect of an extract of Sabugueiro (*Sambucus australis)* on the labeling of blood constituents with technetium-99m. *Brazilian archives of biology and technology*, v.50, pp. 161-166, 2007.

RIBEIRO, L. R.; BATISTA, A .R.P.L.; SILVA, A .R.; SALES, L. A ; SALVADORI, D. M. F. e MAIA, P. C. Toxicological and toxicogenetic effects of plants used in popular medicine and in cattle food. *Memórias do Instituto Oswaldo Cruz*, v.86, p.89-91, 1991.

ROBBERS, J. E.; SPEEDIE, M. K. e TYLER, V. E. Pharmacognosy and pharmacobiotechnology. Baltimore: Willians & Wilkins, p.1-14, 1996.

RODAL, M. J. N. Fitossociologia da vegetação arbustiva-arborea em quatros áreas de caatinga em Pernambuco. Campinas: UNICAMP, 224 p. Tese de Doutorado. 1992.

RODAL, M. J.; NASCIMENTO, L. M. & MELO, A. L. Composçao floristica de um trecho de vegetação arbustiva caducifólia no Município de Ibimirim, PE, Brasil. Acta Botânica Brasílica, v. 13, p. 1-14, 1999.

SAHA, G. B. Characteristic of Specific Radiopharmaceuticals: *Fundamentals of nuclear pharmacy*. New York : Springer-Verlag.ed, 2004

SAHA, G.B. Fundamentals of Nuclear Pharmacy. Springer-Verlag, New York, 1998.

SAMPSON, C.B Complications and difficulties in radiolabelling blood cells: A review. *Nuclear Medicine Commun* v.17, p.648–658, 1996.

SANTOS-FILHO, S. D.; MAIWORM, A. I.; PRESTA, G. A.; PAOLI, S. DE P.; GIANI, T. S. e BERNARDO-FILHO, M. Aqueous extract of the medicinal plant *Mentha crispa* alters the biodistribution of the radiopharmaceutical sodium pertechnetate in Wistar rats. *Medicinal Chemistry Research*, v.16, p.230–237, 2007.

SCHULZ, V.; HÄNSEL, R.; TYLER, V. E. Rational Phytotherapy – A physician´s guide to herbal medicine. *Springer*, 4 edition, 2001.

SISENANDO, H. A. A. A. C. N.; MACEDO, M. F. S.; SATURNINO, A. C. R. D.; COELHO, L. C. B. B.; MEDEIROS, S. R. B. Evaluation of the genotoxic potential of *Bauhinia Monandra* Leaf lectin (BMOLL). *Food and Chemical Toxicology*, p. 01-26, 2008.

SRIVASTAVA, S. C.; STRAUB, R. F. e RICHARDS, P. Mechanistic aspects of the technetium-99m-tin-RBC labeling reactions. *Journal* of Labelled Compounds and. Radiopharmaceutical., v. 21, p.1055, 1984.

STRYER, L. Bioquímica. Guanabara Koogan. Rio de Janeiro. 2003.

TYLER, V.E. Natural products and medicine: an overview. In: BALICK, M.J.; ELISABETSKY, E.; LAIRD, S.A., eds. Medicinal resources of the tropical forest, biodiversity and its importance to human health. New York: Columbia University Press, *Biology and resource management series*. p.3-10, 1996.

VIDAL, M.V.; GUTFILEN, B.; FONSECA, L.M.B. e BERNARDO-FILHO, M. Influence of tobacco on the labeling of red blood cells and plasma proteins with technetium-99m. *Journal of Experimental and Clinical Cancer Research*, v.17, p.1–6, 1998.

VIEIRA, L. S. Fitoterapia da Amazônia. Manual de plantas medicinais. A farmácia de Deus. 2ª ed. São Paulo: Agronômica Ceres, 1992.

WHITE, P. S. Indigofera. Annals *of the Missouri* Botanical Garden, v.67, p.706-714, 1980.

World Health Organization (WHO). Traditional medicine strategy 2002-2005. 2002. Genova: World Health Organization.

YAMADA, C. S. B. Fitoterapia sua história e importância. *Racine* 50-51, 1998.

YUNES, R. A.; PEDROSA, R. C. e CECHINEL FILHO, V. Fármacos e fitoterápicos: a necessidade do desenvolvimento da indústria fitoterápicos e fitofármacos no Brasil. *Quimica Nova,* Vol. 24, No. 1, 147-152, 2001.

Capítulo I

O presente trabalho será submetido à publicação na revista Natural Products

Influence of the aqueous *Indigofera suffruticosa* extract on the labeling with technetium-99m of blood cosntituents and on the membrane integrity of erythrocytes.

Dewson Rocha Pereira[1], Jailson Oliveira da Silva[2], Maria Teresa Jansem de Almeida Catanho[2] and Vera Lúcia de Menezes Lima[1,*].

[1]Departament of Biochemistry, [2]Departament of Biophysics and Radiobiology, Federal University of Pernambuco, Recife. PE. Brazil.

***Corresponding author**
Vera Lúcia de Menezes Lima
Avenida Professor Moraes Rego, S/N.
Cidade Universitária, Recife, Pernambuco, Brazil.
Cep: 50670-420. Fone: (+55 81) 2126-8540
E-mail adress: lima.vera.ufpe@gmail.com

Influence of the aqueous *Indigofera suffruticosa* extract on the labeling with technetium-99m of blood cosntituents and on the membrane integrity of erythrocytes.

Dewson Rocha Pereira[1], Jailson Oliveira da Silva[2], Maria Teresa Jansem de Almeida Catanho[2] and Vera Lúcia de Menezes Lima[1,*].

[1]Departament of Biochemistry, [2]Departament of Biophysics and Radiobiology, Federal University of Pernambuco, Recife. PE. Brazil.

Abstract

Natural products are widely used as medicine by various populations. *Indigofera suffruticosa* is a legume used in popular medicine but its biological properties still are unknown. Erythrocytes labeled with technetium-99m (Tc-99m) are utilized in nuclear medicine imaging. This labeling depends of a reducing agent and may be altered by drugs. We have evaluated the effect of the extract of *I. suffruticosa* in the labeling with Tc-99m of blood constituents. Blood was incubated with *I. suffruticosa*, then stannous chloride and Tc-99m were added. Samples were centrifuged and blood cells (BC) and plasma (P) were separated and precipitated with trichloroacetic acid isolating insoluble fractions of BC and P. The radioactivity in each fraction was counted and the percentage of radioactivity (%ATI) was calculated. The morphology of erytrocytes was evaluated under an optical microscope and the osmotic fragility was determined by optical density in a spectrophotometer. The data showed that the *I. suffruticosa* decreased significantly ($p<0.05$) the %ATI on BC and insoluble fractions. Light alterations were verified on shape of RBC but it showed increased osmotic fragility. We suggest that the *I. suffruticosa* effect could be due to the damages in the plasma membrane or by chelant and oxidative action on the stannous and pertechnetate ions.

Keywords: *Indigofera suffruticosa*; medicinal plant; technetium-99m; osmotic fragility

Introduction

Plants are intended not only to the treatment of diseases, but also, to enhance the actions of certain therapeutic drugs, thus reducing the doses used and the adverse effects shown[1].

Indigofera suffruticosa Mill (Leg. Papilionoideae) is a legume popularly known as “anil”, widely found in the range of semi-arid region of northeastern Brazil. The leaves are used in the popular medicine as antispasmodic, sedative, diuretic and purgative[2, 3, 4]. However, medicinal plants may contain natural components with potential toxic or contaminants such as heavy metals[5], which represents a risk in their use or exposure.

Nuclear medicine is a noninvasive medical specialty that uses radioactive elements for the diagnosis of diseases[6]. Technetium-99m is the (Tc-99m) radionuclide widely used in image diagnosis due to their attractive characteristics, such as short half-life time and low environmental impact, which gives images with high efficiency using low doses [6, 7].

Since 1960, in the nuclear medicine exam, the Tc-99m is administered to the body as sodium pertechnetate ($Na^{99m}TcO_4$), a radiopharmaceutical and this is dissociated in the pertechnetate ion (TcO^{-4}) [8].

Blood cells labeled with Tc-99m have been applied widely in the nuclear medicine for obtaining of blood flow images, red blood cells volume measurement, detection and location of sites of gastrointestinal bleeding, for location of haemangiome and obstruction of the circulatory system [9, 10, 8]. This labeling process depends on the presence of a reducing agent and the stannous chloride ($SnCl_2$) is used as the stannous ion [11, 12].

There are studies have demonstrated the effect of natural and synthetic drugs in the radiolabeling of blood elements with Tc-99m *in vitro* [13, 14, 15]. These drugs may

contain compounds with oxidant and chelant properties or that alter the structure of blood cells decreasing erythrocytes labeling[16, 17].

This fact is significant for diagnostic because can cause error or inadequacy in the interpretation of the examination, causing thus the unnecessary repetition of the test with new exposure to radiation of the pacient [6, 18, 19].

Moreover, the labeling of these blood components is also of interest for experimental studies for the knowledge of biological properties of extracts and natural products[20].

Morphological analysis of the blood cell can be performed as a qualitative method to evaluate the effects of drugs in this process through changes in shape of erythrocytes[21].

Other method widely used is the capability of the erythrocytes to resist hemolysis this technique characterizes the called osmotic fragility of the plasma membrane in different hypotonic solution[22]. The effect hemolytic of these solutions may results in structural perturbation of the membrane and of the cytoskeleton caused by high distribution of the partition coefficient in the membrane[23]. The osmotic fragility reflects thus the structural and geometrical changes in blood cells[24].

In relation to the effects of medicinal plants on the body, little knowledge exists, requiring more studies about theme. This study aimed to evaluate the effect of the aqueous extract of *I. suffruticosa* in the labeling with Tc-99m of blood constituents and in the membrane structure from erythrocytes.

Results and Discussion

Natural products are largely used in several populations, due to the low cost of these drugs. Leaves of *I. suffruticosa* are used in popular medicine and important biological activities had been reported [25, 26, 27].

The labelling of blood constituents with Tc-99m can be a useful method to study of the labelling process and important findings have already been published [28, 17]. The development and use of this *in vitro* test generates importants information about the possible drug/radiopharmaceutical interactions, besides evaluating properties of various drugs used by the human beings.

However, works that study the effects of naturals drugs in labeling of blood elements with radiopharmaceuticals and your responsible mechanisms are scarce. To answer these questions, some authors have analyzed the morphology and integrity of the membrane from erythrocytes [29, 30]. Others authors have examined the presence of redox properties of the compounds from naturals products that may reduce the stannous ion and prevent the radiolabeling[31].

In the present study, we have demonstrated that aqueous extract of *Indigofera suffruticosa* altered the percentage of radioactivity (ATI%) in blood cells (BC) and plasma (P) from Wistar rats blood. The ATI% in BC decreased significantly ($p<0.05$) in different concentrations of extract (37.5; 75 and 150 mg/mL), from 98.17 ± 0.29 to 59.58 ± 3.51 (Figure 1).

The insoluble fractions of blood cells (IF-BC) and of plasma (IF-P) obtained from whole blood treated with different concentrations of *I. suffruticosa,* showed also a significant ($p<0.05$) decrease of ATI% in IF-BC (95.18 ± 1.27 to 80.08 ± 0.23) and IF-P (88.70 ± 1.28 to 43.77 ± 0.23), indicating a fall in uptake of Tc-99m in protein samples treated with most of the concentrations (Figure 2).

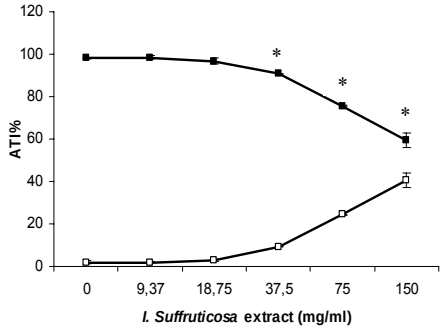

Figure 1. Effects of I. Suffruticosa extract (9.37, 18.75, 37.5, 75, 150 mg/mL) on the uptake of the Tc-99m in the BC (■) and in the P (□). Data are represented as mean ± standard deviation of the mean for ten individual experiments. (*p<0.05), when compared to control group of BC.

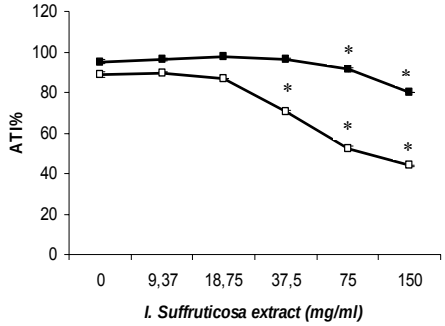

Figure 2. Effects of I. Suffruticosa extract (9.37, 18.75, 37.5, 75, 150 mg/mL) on the uptake of the Tc-99m in the IF-BC (■) and in the IF-P (□). Data are represented as mean ± standard deviation of the mean for ten individual experiments. (*p<0.05), when compared to control group of BC.

The morphological analysis of erythrocyte treated with aqueous *I. suffruticosa* extract under optical microscopy has revealed alterations in its shape. The samples of blood from rats were incubated with saline solution (Figure 3) and with the highest concentration of extract (150 mg/mL) (Figure 4).

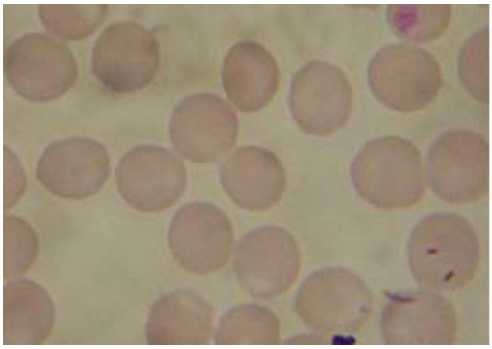

Figure 3. Photomicrography of blood smears from blood samples treated with NaCl 0.9% solution (control group). Blood smears were prepared, dried, fixed and stained. The morphology of erythrocytes was evaluated under optical microscopy (x100).

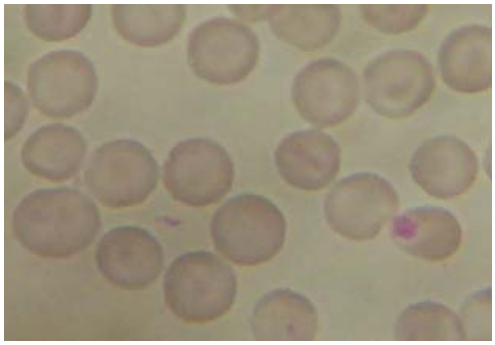

Figura 4. Photomicrography of blood smears from blood samples treated with aqueous of *I. Suffruticosa* extract (150 mg/mL). Blood smears were prepared, dried, fixed and stained. The morphology of erythrocytes was evaluated under optical microscopy (x100).

The assessment of osmotic fragility of erythrocyte treated with aqueous *I. suffruticosa* extract through of hemolysis percentage curve versus different saline concentration is showed in Figure 5. The results indicate that the extract concentrations (150 mg/mL) altered the profile of osmotic fragility curves when compared with the

control group. The comparison of the means of hemolysis between treated and control group was significant ($p<0.05$) to the interval II (Figure 6), increasing in 70% the hemolysis of erythrocytes in presence of the extract.

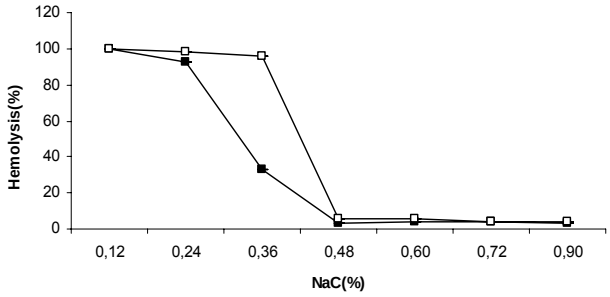

Figure 5. Osmotic fragility of blood samples treated with 150 mg/ml *I. suffruticosa* extract (□) or with sodium chloride solution (0.9% NaCl) (■), as control. (*) denotes significant difference ($p<0.05$), when compared to control group.

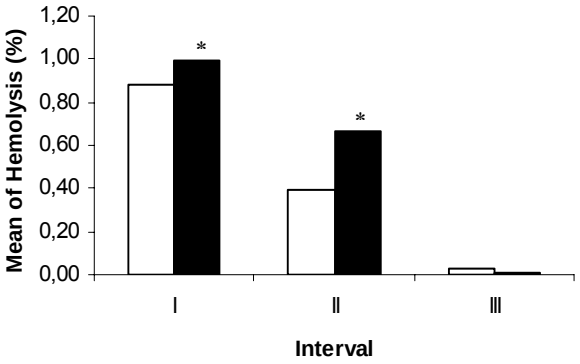

Figure 6. Means of hemolysis of the blood samples treated (■) or not treated (□) with *I. suffruticosa* extract (150mg/mL). Three intervals were determined in fragility curves: interval I (from 0.12 to 0.24% NaCl), interval II (from 0.24 to 0.48% NaCl), and interval III (from 0.48 to 0.9% NaCl) in according with the curve tendency. The means and standard deviations of each interval were determined and the statistical analysis was performed. (*) $p<0.05$.

The radiolabeling of erythrocyte with 99mTc is used in the nuclear medicine. Therefore, is important to study possible interference in this process, to promote a correct diagnosis and consistent result of the examination

Our data show a decrease of Tc-99m marking in blood cells caused by incubation with different concentrations of aqueous extract of *I. suffruticosa*. Similar results were found using extracts from *Vitex agnus* castus, *Cinnamomum zeylanicum*, *Terminalia chebula*, a medicinal plant Chinese and from barbatimão (*Stryphnodendron*

adstringens), a tree used on popular medicine in the treatment of gastric lesions[15, 32, 33, 34].

Some reasons are assigned to this influence: oxidation and/or chelating action on the stannous ion and changes in the structure of the plasma membrane from erythrocyte preventing the stannous and pertechnetate ion transport[16].

Thus, to understand these effects different techniques are used to study the interaction between blood cells and drugs[21]. Some authors had analyzed the structure of its plasma membrane by evaluation of its morphology and/or of its osmotic fragility [35].

In this study, qualitative analysis of the erythrocytes showed that the labeling was reduced even not observing significant alteration in the shape of the erythrocyte. Oliveira and collaborators (2000) found similar results studying the effect in radiolabeling in blood elements treated with *Maytenus ilicifolia* (espinheira santa) and by Benarozz and collaborators (2008) utilizing extract of *Cinnamomum genus* (cinnamon)[15, 20].

The extract of *I. suffruticosa* increased blood cells osmotic fragility, suggesting possible modifications in membrane structure. Similar work, too, has verified modification in the osmotic profile in erytrocytes, due to action of a Chinese plant (*Buzhong Yi Qi Wan)* and a extract of *Ricinus communis L.* [36, 37]. Other reported histological alterations of the red blood cells by tobacco extract which could be the responsible by the modifications on the labeling of the blood cells with Tc-99m[17].

This fact may justify the reduction of the radiolabeling of blood cells caused by the plant extract. Since that this process is dependent of an appropriate transport of stannous and pertechnetate ions by membrane of erythrocyte[38].

Studies suggest that the entry of the pertechnetate and stannous ion by membrane from erythrocyte is performed respectively by the band-3 system and by the calcium channels[39, 40].

In the blood cell the stannous ions reduce the pertechnetate ion permitting its fixation in the cellular proteins of the erythrocyte [12, 41, 42].

Thus, actions in these cells membranes may modify the transport system of these ions in the membrane, preventing the labeling of the erythrocyte [15, 43].

The action of the aqueous extract of *I. suffruticosa* in the structure of the membrane was related with antimicrobial effect of this plant against *Staphylococcus aureus*, dermatophytes fungi *Microsporium canis* and *Trichophyton rubrum*[27], indicating that the modification of membrane integrity may result in damage to living cells.

Our results showed, still, the reduction in the uptake of Tc-99m by action of the extract of *I. suffruticosa* in the protein fractions isolated from blood cells (IF-BC) and plasma (IF-P) in (Figure 2). In study with extract from *Paullinia cupana* (guarana) the labeling of plasmatics and cells protein also was reduced [29].

This suggests that the components of the extract can change the connection site from Tc-99m in erythrocytes and plasma proteins, and/or it can even if connect in these sites and promoting a competition with Tc-99m [19].

Moreover, the ability of fixation of Tc-99m in the IF-BC was maintained even at high dose of extract of *I. suffruticosa* in relation to the IF-BC (Figure 2). Thus, the effect of the extract in IF-BC was lower than observed in BC (Figure 1) suggesting that the components from extract can act more specifically in the blood cell.

In conclusion, ours experimental data showed the decrease in the labeling with Tc-99m of blood cells (BC), insoluble fraction of blood cells (IF-BC) and of plasma (IF-P) due to the presence of the aqueous extract of *Indigofera suffruticosa*. We suggest that this effect can be due alterations in the structure of the plasmatic membrane of the erythrocyte causing change in the ions transport. Also exist the possibility of the extract act directly in the stannous ion as oxidant agent preventing the radiolabeling. Therefore, in order to elucidate this effect of the *I. suffruticosa* experiments that available its properties oxidant and the generation of the reactive oxygen species (ROS).

Anyway, our results can help the understanding of the natural drug interaction in nuclear medicine procedures getting quality images and a correct interpretation of the test. Moreover, permit the knowledge about a natural product used by populations in the popular medicine for actions antispasmodic, sedative, diuretic and purgative.

Experimental Section

Plant Material. The leaves of *Indigofera suffruticosa* were collected in March 2008 in the city of Sao Caetano - PE, semi-arid region of northeastern Brazil. The plant was taxonomically identified by Dr. Marlene Carvalho de Alencar Barbosa from the Departament of Botany, Federal University of Pernambuco (UFPE), Brazil, institution where a Voucher specimen has been deposited in the Herbarium UFP Geraldo Mariz (number 45.217).

Extraction and Isolation. The powdered (50g) from the leaves of *I.suffruticosa* was extracted at room temperature with distilled water, for 14-16 hours in shaker. The liquid phase was removed by decantation and the residue was submitted to a new extraction with water. At the end, the extracts were united, filtered and lyophilized, and the aqueous extract of this plant was obtained.

Animals. Adult male Wistar rats (3-4 months of age, body mass 250-300g) were kept in a controlled environment, with ideal conditions of temperature (22 C ± 1), light and free access to food and water. The project was approved by the Ethics Committee on animal experimentation, UFPE, Case Number 014413/2007-78.

Labeling of blood elements. The experiment of radiolabelling followed the method described in other works[28, 44, 45]. The tubes used were sealed with rubber cap and a syringe was used to reduce the air (vacuum) inside the bottles. Heparinized blood was

collected from rats (n = 6), to obtain a pool of blood. Blood samples of 0.5 mL of Wistar rats were incubated in 100µL of different concentrations (9.37, 18.75, 37.5, 75, 150mg/ml) of aqueous extract of *I. suffruticosa* or with saline (0.9% NaCl), as control, for 60 minutes (room temperature). After, 0.5 mL of stannous chloride (1.2 g / mL) prepared at the same time to prevent its oxidation, was added and incubated for 60 minutes. After this period, 100 µL of 99mTc (3.7 MBq) as sodium pertechnetate, recently collected from a generator 99Molibdenio/99Tecnécio (source) was added and incubated for 10 minutes. These samples were centrifuged in a clinical centrifuge (1500xg, 5min) and aliquots (20 µl) of plasma (P) and blood cells (BC) were separated and precipitated with 1ml of trichloroacetic acid (TCA) 5% separating the soluble fraction (SF) and insoluble (IF). The radioactivity in P, BC, SF-P, IF-P, SF-BC and IF-BC was determined in a gamma counter (Cobra II Auto-Gamma, Packard BioScience, CT, USA). Thus, the percentage of radioactivity (% ATI) was determined as described in previous work [11, 44]. A statistical analysis (ANOVA test, with significance level $p < 0.05$, n = 10) was utilized to compare the values found.

Histological preparations. Blood samples were carried with (0,5ml) of Wistar rats treated for 60 minutes with different concentrations (37.5, 75, 150mg/mL) of aqueous extract of *I. suffruticosa* or with saline (0.9% NaCl), as control. Blood sample was prepared, dried, fixed and stained [46, 47]. Then morphological analysis of erythrocytes was realized under optical microscope (x1000, Olympus, BX model, Japan) and blood cells images were taken.

Osmotic fragility assay. The blood samples of Wistar rats were incubated in *I. suffruticosa* extract (150mg/mL) or in saline, as a control, for 60 minutes at room temperature. Samples of these whole blood (control and treated) were centrifuged (1500xg, 15min) and aliquots of RBC were separated. RBC samples (100 µl) of control and treated were gently mixed with different hypotonic NaCl (from 0.12 to 0.72%) solutions[48]. After 60 min, at room temperature, the preparations were centrifuged (1500

rpm, 15 minutes). The supernatants were isolated and the optical density (OD) to each NaCl concentration was observed in a spectrophotometer (ANALYSER 800M, *Analyser Comércio Indústria LTDA,* São Paulo) at 540 nm. The supernatant at 0.9% NaCl was considered for the blank of the preparation, because it has no hemolysis. The percentage of hemolysis (%Hemolysis) of each sample was obtained by the ratio of the OD of each supernatant by the OD of 0.12% NaCl solution (100% lysis). According to fragility curve tendency, three intervals were determinated: interval I between 0.12 and 0.36% NaCl, interval II between 0.36 and 0.60% NaCl and interval III between 0.60 and 0.90% NaCl[24]. The experiments were analyzed with paired t-test to verify potential differences between hypotonic and isotonic solutions (% concentrations of NaCl) versus relative hemolysis (% hemolysis).

Acknowledgments

This work was financially supported by CNPq

References

(1) Gomes, L. M.; Oliveira, de N. B. M. and Bernardo-Filho, M. B. Drug interaction with radiopharmaceuticals: Effect on the Labeling of Red Blood Cells with Technetium-99m and on the Bioavailability of Radiopharmaceuticals. *Brazilian Archives of Biology and Techonology*, 2002, 45, 143-9.

(2) Dominguez, X. A., Martinez, C., Calero, A. Hinojosa, M., Zamudio, A. Watson, W. H. and Zabel, V. Chemical components from Jiquelite *Indigofera suffruticosa Mill*. *Planta Medica*, 1978, 34, 172-175.

(3) Garcez, W. S. Gaecez, F.R. Honda, N.K. and Dasilva, A. J. R. A nitropropanoyl – glucopyranoside from *Indigofera suffruticosa* . *Phytomistry*, 1989, 28: 1251-1252.

(4) Ribeiro, L. R., Batista, A .R.P.L., Silva, A .R., Sales, L. A . Salvadori, D. M. F. and Maia, P. C. Toxicological and toxicogenetic effects of plants used in popular medicine and in cattle food. Memórias do Instituto Oswaldo Cruz, 1991, 86: 89-91.

(5) Kam, P. C. A. and Liew, S.. Traditional Chinese herbal medicine and anaesthesia. Anaesthesia, 2002, 57(11), 1083 – 1089.

(6) Hladik III W. B; Saha G. B and Study, K. T. Essentials of Nuclear Medicine Science. William and Wilkins, London, 1987.

(7) Early P J, Sodee D B. Principles and Practice of Nuclear Medicine, New York: Mosby Year Book, 1996.

(8) Saha, G. B. *Fundamentals of nuclear pharmacy*. New York : Springer-Verlag, 1998.

(9) Tsai CC, Yen TC, Tzen KY. The value of Tc-99m red blood cell SPECT in differentiating giant cavernous hemangioma of the liver from other liver solid masses. Clin Nucl Méd, 2002; 27: 578-81.

(10) Shih WJ, Bognar B. Early appearance of the inferior vena cava in a Tc-99m red blood cell first-pass study: a sign of superior vena cava obstruction. Clin Nucl Méd, 2000; 25:679-81.

(11) Bernardo-Filho, M., Gutfilen, B., Maciel, O.S.,. Effect of different anticoagulants on the labeling of red blood cells and plasma proteins with technetium 99m. Nuclear Medicine Comm., 1994, 15, 730–734.

(12) Harbert, J. C; Eckelman, W. C, Neumann, R. D. Nuclear Medicine Diagnosis and therapy. Thieme Medical, New York, 1996.

(13) Frydman, J. N. G., Oliveira, M. B. N., Santos, A. E. O., Fonseca, A. S., Santos, R., Bernardo-Filho, M. Inuence of methyl xanthines on 99m the labeling of blood elements with Technetium.Pak. J. Biol.Sci. 2004, 4, 521-524.

(14) Fonseca AS, Frydman JN, Santos R, Bernardo-Filho M. Influence of antipyretic drugs on the labeling of blood elements with technetium-99m. Acta Biol Hung, 2005, 56:275–282.

(15) Benarroz, M. O.; Fonseca, A. S.; Rocha; G. S. ; Frydmana, J. N. G.; Rocha; V. C.; Pereira, M. O.; Bernardo-Filho, M. Cinnamomum zeylanicum extract on the radiolabelling of blood constituents and the morphometry of red blood cells: In vitro assay. Applied Radiation and Isotopes, 2008, 66, 139–146.

(16) Oliveira, J. F., Braga, A. C. C., Ávila, A. S., Fonseca, L. M. B., Gutfilen, B., Bernardo-Filho, M.,. Effects of Thuya occidentalis on the labeling of red blood cells and plasma proteins with technetium-99m. Yale journal of biology and Medicine 1997, 69,489-494.

(17) Vidal, M.V., Gutfilen, B., Fonseca, L.M.B., Bernardo-Filho, M., 1998. Influence of tobacco on the labeling of red blood cells and plasma proteins with technetium-99m. Journal of Experimental and Clinical Cancer Research 1998, 17, 1–6.

(18) Hart, D and Wall B. F. UK nuclear medicine survey 2003–2004. Nucl Med Commun, 2005, 26:937–946.

(19) Hesslewood, S., E. Leung. Drug interaci]tions with radiopharmaceuticals. Eur. J. Nucl. Med. 1994, 21, 348.

(20) Oliveira, J. F. de; Braga, A. C. S.; Oliveira, M. B. N. de; Ávila, A. S.; Caldeira-de-Araujo, A. Cardoso, V. N.; Bezerra, R. J. A. C.; Bernardo-Filho, M. Assessment of the effect of *Maytenus ilicifolia* (espinheira santa) extract on the labeling of red blood cells and plasma proteins with technetium-99m Journal of Ethnopharmacology 2000, 72, 179–184.

(21) Li A, Seipelt H, Muller C, Shi Y, Artmann M. Effects of salicylic acid derivatives on red blood cell membranes. *Pharmacol Toxicol*, 1999, *85,* 206-211.

(22) Wang, X.; L. Wei, J.P. Ouyang, S. Muller, M. Gentils, G. Cauchois and J.F. Stoltz. Effects of an angelica extract on human erythrocyte aggregation, deformation and osmotic fragility. *Clin Hemorheol Microcirc*. 2001, 24(3): 201-5.

(23) Didelon, J., P. Mazeron, S. Muller and J.F. Stoltz. Osmotic fragility of the erythrocyte membrane: characterization by modeling of the transmittance curve as a function of NaCl concentration. *Biorheology.* 2000, 37(5-6): 409-16.

(24) Cavalcanti, T.C., C.C. Gregorini, F. Guimarães, O. Rettori and A.N. Vieira-Matos. Changes in red blood cell osmotic fragility induced by total plasma and plasma fractions obtained from rats bearing progressive and regressive variants of the Walker 256 tumor. *Braz J Med Biol Res.* 2003, 36(7): 887-95.

(25) Leite, S. P., Medeiros, P. L., Silva, E. C., Maia, M. B. S., Lima, V. L. M., Saul, D. E. Embryotoxicity in vitro with extract of *Indigofera suffruticosa* leaves. Reproductive Toxicology, 2004, 18, 701-705.

(26) Leite, S. P., Silva, L. L. S., Catanho, M. T. J. A., Lima, E. O., Lima, V. L. M. Atividade antiinflamatória do extrato de *Indigofera suffruticosa*. Revista Brasileira de Ciências da Saúde, 2003, 7, 47 – 52.

(27) Leite, S. P., Vieira, J. R. C., Medeiros, P. L., Leite, R. M. P., Lima, V. L. M., Xavier, H. S., Lima, E. O. Antimicrobial activity of *Indigofera suffruticosa*. Evidence- Based Complementary and Alternative Medicine, 2006, 3(2), 261-265.

(28) Bernardo-Filho, M.; Caniné, M. S.; Lopes, R. L. F. and Boasquevisque E. M., Effect of temperature on the "in vitro" labeling of red blood cells with technetium-99m. *Arq. Biol. Tecnol.* 1986, 29, 407-412.

(29) Oliveira, J.F. de , Avila, A.S. , Braga, A.C.S; Oliveira, M.B.N. de; Boasquevisque, E.. M.; Jales, R. L.; Cardoso, V. N.; Bernardo-Filho, M. 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, 2002, 73, 305-312.

(30) Maiworm, A. I.; Presta, G. A.; Santos-Filho, S. D.; Paoli, S. de; Giani, T. S.; Fonseca, A. S. and Bernardo-Filho, M. Osmotic and morphological effects on red blood cell membrane: action of an aqueous extract of *Lantana camara.* Brazilian Journal of Pharmacognosy 2008, 18(1), 42-46.

(31) Lima, E. A. C.; Diré, G.; Mattosa, D. M. M.; Freitasa, R. S.; Gomes, M. L.; Oliveira, M. B. N. de; Faria, M. V. C.; Jales, R. L. and Bernardo-Filho, M. Effect of an extract of cauliflower (leaf) on the labeling of blood elements with technetium-99m and on the survival of Escherichia coli AB1157 submitted to the treatment with stannous chloride Food and Chemical Toxicology, 2002, 40, 919–923

(32) Costa, M. R. de M.; Ribeiro, C. G.; Santos-Filho, S. D.; Neves, R. de F.; Fonseca, A. de S. da.; Bernardo-Filho, M. and Catanho, M. T. J. de A. An aqueous Extract of *Vitex agnus castus* Alters the Labeling of Blood Constituents with Technetium-99m. Brazilian Archives of Biology and Technology, 2007, v.50, pp.183-188.

(33) Soares, S. F.; Brito, L. C.; Souza, D. E.; Bernardo, L. C.; Oliveira, J. F.; Bernardo-Filho, M. Potential pitfalls in the nuclear medicine imaging: Experimental models to evaluate the effect of natural products on the radiolabeling of blood constituents, bioavailability of radiopharmaceutical and on the survival of Escherichia coli strains submitted to the treatment with stannous ion. Nuclear Instruments and Methods in Physics Research A, 2006, 569, 505–508

(34) Costa, Thadeu E. M. Maramaldo 1,2; Aline P. Muzy Dias1,2; Priscila V. S. Zabala Capriles1,2; Márcia Betânia N. Oliveira1; Elba Lúcia Cavalcanti de Amorim3; Cláudia Sampaio de Andrade Lima3; Mario Bernardo-Filho1* Effect of barbatimão [*Stryphnodendron adstringens* (Mart.) Coville] infusion on the labling of blood elements with technetium– 99m. Rev. Bras. Farmacogn., 2002, v. 12, supl., p. 07-09.

(35) Aquino, T. M; Amorim E. B. C; Lima C. S. A and Albuquerque U. P. Influence of bifl orin on the labelling of red blood cells, plasma protein, cell protein, and lymphocytes with technetium-99m: in vitro study. *Braz J Pharmacogn*, 2007, *17: 1*81-185.

(36) Giani, T. S.; Paoli1, S. de; Presta, G. A.; Maiworm, A. I.; Santos-Filho, S. D.; Fonseca, A. S. and Bernardo-Filho, M. Assessment of effects of a formula used in the traditional Chinese medicine (*Buzhong Yi Qi Wan*) on the morphologic and osmotic fragility of red blood cells. Brazilian Journal of Pharmacognosy, 2007, 17(4): 501-507.

(37) Mousinho, K. C.; Correia, M. B. L.; Silva, J. O. da; Magnata, S. de S. L. P.; Souza, I. A. de and Catanho, M. T. J. de A. Effect of the extract of Ricinus communis L. on the osmotic Fragility, labeling of red blood cells with Technetium-99m and morphology of the cells. Brazilian archives of Biology and Technology, 2008, 51(6), 1139-1146,.

(38) Gutfilen, B., Boasquevisque, E.M., Bernardo-Filho, M.,. Calcium channel blockers: interference on red blood cell and plasma protein labeling with Tc-99m. Revista Espanola de Medicina Nuclear, 1993, 11, 195–199.

(39) Callahan, R.J., Rabito, C.A., 1990. Radiolabeling of erythrocytes with technetium-99m: role of Band-3 protein in the transport of pertechnetate across the cell membrane. Journal of Nuclear Medicine, 2008, 31, 2004.

(40) Gutfilen, B., Ribeiro, B.L.A.R., Mattos, M.F., Ribeiro, C.R., Bernardo- Filho, M.,. Labeling of thymine with technetium-99m: suggestion of a chemical model. Arq. Biol. Tecnol. 1996, 39, 69–74.

(41) Rehani, M. M. and Sharma, S. K. Site of Tc-99m binding to the red blood cell: concise communication. *J. Nucl. Med.*, 1980, 21, 676-678.

(42) Kuehne, R. and Reuter, E. High RBC labeling efficiency by controlling pretinning with the modified in vivo, in vitro labeling method. J. Nucl. Med. Technol., 1999, 27: 222-226.

(43) Neves, R. de F.; Moreno, S. R. F.; Rebello, B. M.; Caldas, L. Q. de A.; fonseca, A. de S. da; Bernardo-Filho, M. e Medeiros, A. da C. Effect of na Arctium lappa (Burdock) Extract on the Labeling of Blood Constiuents with Technetium-99m and on the Morphology of the Red Blood Cells. Brazilian Archives of biology and technology, 2007, 50, 167-174.

(44) Bernardo-Filho, M.; Moura, I. N. S.; Boasquevisque, E. M . 0. 99m-Technetium-labeled red blood cells `in vitro'. Arq. Biol. Tecnol. 1983, 26, 455-461,.

(45) Bernardo-Filho, M.; NOGUEIRA, J. F.; STURM, J. A.; BOASQUEVISQUE, E. M. Plasma proteins labeling with 99m-Technetium. Arq. Biol. Tecnol. 1990, 33, 811-817.

(46) Junqueira, L. C, Carneiro J. Histologia basica. Rio de Janeiro: Guanabara Koogan Portuguese , 1992.

(47) Oliveira, L. A.; Soares, J. B. e Greco, J. B *et al*. Metodos de laboratorio aplicados a clınica: tecnica e interpretacão. Rio de Janeiro: Guanabara Koogan Portuguese , 1992.

(48) Dacie J and Lewis SM. Practical Haematology. London: Churchill Livingstone, 2001.

Capítulo II

O presente trabalho será submetido à publicação na revista Nuclear Medicine and Biology

EFFECT ON THE BIOAVAILABILITY OF THE SODIUM PERTECHNETATE IN MICE TREATED WITH *Indigofera suffruticosa*.

Dewson Rocha Pereira[1], André Luiz de Souza Barros[2], Maria Teresa Jansem de Almeida Catanho[2] and Vera Lúcia de Menezes Lima[1,*].

[1]Departament of Biochemistry, [2]Departament of Biophysics and Radiobiology, Federal University of Pernambuco, Recife. PE. Brazil.

***Corresponding author**
Vera Lúcia de Menezes Lima
Avenida Professor Moraes Rego, S/N.
Cidade Universitária, Recife, Pernambuco, Brazil.
Cep: 50670-420. Fone: (+55 81) 2126-8540
E-mail adress: lima.vera.ufpe@gmail.com

EFFECT ON THE BIOAVAILABILITY OF THE SODIUM PERTECHNETATE IN MICE TREATED WITH *Indigofera suffruticosa*.

Dewson Rocha Pereira[1], André Luiz de Souza Barros[2], Maria Teresa Jansem de Almeida Catanho[2] and Vera Lúcia de Menezes Lima[1,*].

[1]Departament of Biochemistry, [2]Departament of Biophysics and Radiobiology, Federal University of Pernambuco, Recife. PE. Brazil.

Abstract

The nuclear medicine is a specialty non invasive that used radioactive elements for image diagnostic. Technetium-99m is administered as sodium pertechnetate ($Na^{99m}TcO_4$) in nuclear medicine because has short half-life time and low emission of radioactive energy. This process can be altered by action of natural drugs. *Indigofera suffruticosa* is a legume used in popular medicine but its properties still are unknown. Our aim is to evaluate the effect of the extract of *I. suffruticosa* in the bioavailability of the $Na^{99m}TcO_4$ in mice. The extract of *I. suffruticosa* was administered by intraperitoneal injection in a group of animals and the control group received saline, both for 7 days. In the 8th day the sodium pertechnetate was administered by intracaudal injection. After 30 minutes the animals were sacrificed their organs and tissues isolated, their masses obtained and the radioactivity counted in each. The percentage of radioactivity per gram of tissue (%ATI/g) was obtained dividing the radioactivity of each tissue by its mass. The values of %ATI/g of the animals treated with extract altered significantly ($p<0.05$) the uptake of $Na^{99m}TcO_4$ in thyroid, small intestine and lungs in comparison with the control group. In the thyroid the %ATI/g increased more than doubled in relation to the control. We suggested that the extract could act in the transport system of pertechnetate ions by the cell membranes of each tissue. Thus, *I. suffruticosa* extract was able of changes the bioavailability of a radiopharmaceutical $Na^{99m}TcO_4$ in mice, probably caused by presence of metabolites generated by plant extract.

Keywords: *Indigofera suffruticosa*; medicinal plant; bioavailability; sodium pertechnetate

Introduction

The image diagnostic is performed in the nuclear medicine using radioactive elements in the diseases diagnostic though of the detection of functional, biochemistry and metabolic process in the organs or tissues even before the appearance of anatomical events[1].

The images in the nuclear medicine are of high quality because are used scintigraphy techniques as computed tomography single photon emission (SPECT) and positron emission tomography (PET) helped in a rapid and efficient elucidation of diagnosis of various diseases or the rehabilitation of patients[2].

Technetium-99m (Tc-99m) radionuclide widely used in diagnosis by SPECT image due to the fact of having attractive physical characteristics, such as short half-life time and emission gama radiation of 140 keV which gives low ambient impact. Thus, images are produced with high efficiency using low doses of Tc-99m[1,3].

Tc-99m is administred in the body as sodium pertechnetate ($Na^{99m}TcO_4$) a radiopharmaceutical (radiobiocomplexes) which is used for obtain in nuclear medicine imaging of the organs and tissues of patients[4, 5]. Thus is possible study morphological or physiological changes in blood flow and to observe absorption drug delivery, metabolism and excretion in target organs[6, 7].

The $Na^{99m}TcO_4$ after injected intravenously is bound to plasma proteins (70-80%) and spreads slowly through the vascular fluid. Then, it suffer dissociation in the pertechnetate ion (TCO^{-4}) and leaves the capillary membranes to the interstitial fluid where are captured by different organs stomach, intestine, thyroid and salivary glands[8, 9, 10]. Thus, the pertechnetate sodium is used as a marker in the diagnosis of thyroid imaging, gastric mucosa, intestinal tract[11,12].

Several factors can interfere on the bioavailability of $Na^{99m}TcO_4$ in the body as treatment with synthetic and natural drugs, therapy with radiation, terms of diet, disease processes and surgical procedures[13, 14, 15, 16, 17, 18, 19, 20, 21, 22]. This fact leads to

possible error or inadequacy in the interpretation of the examination, leading thus the unnecessary repetition of the test with new exposure to radiation [1, 23, 24].

Some studies have demonstrated the effect of natural drugs in the biodistribution of complexes radioatives in animals[25, 7, 26]. Medicinal plants may contain natural components of potential toxic contaminants such as heavy metals[27], which can act in this procedure diagnostic.

Indigofera suffruticosa Mill (Leg. Papilionoideae) is a legume popularly known as “anil”, widely found in the range of semi-arid region of northeastern Brazil. This plant has some medicinal properties against joint pain, circulatory disorders and respiratory problems. As well as, its leaves are used as a compound for entnomedicina antispasmodic, sedative, diuretic and purgative[28, 29, 30].

In relation to the effects of medicinal plants on the body still exists little knowledge. This work aimed to evaluate the effect of the aqueous extract of *Indigofera suffruticosa* to the bioavailability of sodium pertechnetate in organs and tissues of mice.

Metodology

Plant Material

The leaves of *Indigofera suffruticosa* was collected in March 2008 in the city of Sao Caetano - PE, semi-arid region of northeastern Brazil. The plant was taxonomically identified by Dr. Marlene Carvalho de Alencar Barbosa from the Departament of Botany, Federal University of Pernambuco (UFPE), Brazil, institution where a Voucher specimen has been deposited in the Herbarium UFP Geraldo Mariz (number 45.217).

Extraction and Isolation

The powdered (50g) from the leaves of *I. suffruticosa* were extracted at room temperature with distilled water, for 14-16 hours in electric homogenizer. The liquid phase was removed by decantation and the residue was new extraction with water. At the end, the extracts were united, filtered and lyophilized, and obtained the aqueous extract of this plant.

Animals

Adult male mice (*Mus musculus*, 3-4 months of age, body mass 35-50g) were kept in a controlled environment, with ideal conditions of temperature (22 C ± 1), light and free access to food and water. Project approved by the Ethics Committee on animal experimentation, UFPE, Case Number 014413/2007-78.

Bioavailability of radiobiocomplex

The aqueous extract of *I. suffruticosa* (50mg/ml, solubilizated NaCl 0,9%) lyophilisate was administered by intraperitoneal injection in a group of mice (n=6), other than the control group that received saline, both for 7 days. In the 8th day was administered by intracaudal injection 0.5 ml of sodium pertechnetate ($Na^{99m}TcO_4$, 3.7 MBq). After 30 minutes the animals were sacrificed and their isolated organs (blood, heart, lung, stomach, liver, spleen, pancreas, kidney, large intestine, small intestine, muscle, bone, brain and thyroid) and masses were obtained by balance of precision and radioactive activity known in a counter of gamma radiation (Cobra II Auto-Gama, Packard BioScience, CT, USA). The percentage of radioactivity per gram of tissue (%ATI/g) was obtained dividing the radioactivity of each organ by its mass. A statistical analysis

(ANOVA test, with significance level $p < 0.05$, $n = 6$) was utilized to compare the values found.

Results

The effects on the bioavailability of the sodium pertechnetate ($Na^{99m}TcO_4$) in different tissues from mice treated with the *I. suffruticosa* crude extract (50mg/mL) or not treated (control group) are presented in Table 1.

The values of the percentage of radioactivity per gram of tissue (%ATI/g) of animals treated with extract increase significantly ($p<0.05$) the uptake of $Na^{99m}TcO_4$ in thyroid more than doubled (2.24 ± 0.72 to 6.22 ± 0.62) in relation to the control group (Table 1).

However, there was decreasing of the %ATI/g in the small intestine (0.29 ± 0.10 to 0.06 ± 0.08) and lungs (0.52 ± 0.06 to 0.32 ± 0.09) of the treated group with *I. suffruticosa* in comparison with the control group (Table 1). The other tissues not demonstrated significant changes.

Discussion

The plants utilized in ethnomedicine can cause positive or negative effect in the organism depending of its properties its. These natural products need to be studied in laboratory in order to prevent complications on its implementation in diseases the treatment or diagnostic. Leaves of *Indigofera suffruticosa* are used in popular medicine and important biological activities had been reported[31, 32, 33].

Studies assess the ability of plants products to interfere in the radiolabeling of tissues or organs with the radiobiocomplexe sodium pertechnetate in image examination[34, 35]. However, other naturals products are not interfere in this process, such as *Pfaffia sp*.[36, 13].

Therefore, the knowledge of the natural drug interaction in the body is an important factor for the nuclear medicine clinic, because allows a correct interpretation of the scintigraphic images and avoids an undesired radiation dose distribution, besides obtain a poor quality image of the organ. Moreover, the uptake of the sodium pertechnetate in tissues is also of interest for experimental studies *in vivo* wich allows knowledge of biological properties of extracts and natural products. The uptake mechanism of this radiopharmaceutical in tissues occurred of different forms. 2 to 4% of the $^{99m}TcO^{-4}$ is captured in the thyroid by active transport, as a sodium-iodide symporter[1]. The 99mTc has many advantages as less emission radiation, exam in the even day and less relative cost in relation to iodine-131, radionuclide also used in nuclear medicine[1]. When the $Na^{99m}TcO_4$ is administred oral or intramuscular way the $^{99m}TcO^{-4}$ íon is absorved by the digestive tract by simple diffusion. In others tissues this transport is performed, mainly, by calcium canals[37].

The results have showed the increased in the uptake of $^{99m}TcO^{-4}$ by the thyroid of mice treated with *I. suffruticosa* extract in comparison with control group. The transport of pertechnetate ion by the membrane of tissues have been related with the effect of drugs in the transport system of stannous and pertechnetate ions in erythrocytes [8, 9]. And studies suggest that effects of the plant components increased the fixation of the pertechnetate ion in the pancreas, kidney, spleen, liver, and thyroid from Wistar due the changes in the in the transport system of ions of the tissues membrane[38].

In addition, the uptake of $^{99m}TcO^{-4}$ decreased significantly in the small intestine and lungs of the mice in the presence of the *I. suffruticosa* extract in comparison with control group. Similar works reported that treatment with *Ginkgo biloba* reduces the uptake in the duodenum of the Wistar rats [32]. Other authors have verified even effect in the lung of the rats treated with different natural products [16, 31]. These vegetal extracts can generate metabolites capable to promote morphological and/or physiological

modifications in tissues [7], which could explained the altering the bioavailability of $Na^{99m}TcO_4$ in treated animals.

In conculsion, the components from aqueous *Indigofera suffruticosa* extract were able of change the bioavailability of a radiopharmaceutical the sodium pertechnetate ($Na^{99m}TcO_4$) in mice, probably caused by metabolites generated by plant extract. Thus, this study allows know about possibile medicament interaction between natural drug and radiopharmaceutical and the biological properties of a natural product used by the popular medicine.

Acknowledgments

This work was financially supported by CNPq

References

[1] Hladik III WB, Saha GB, Study KT. Essentials of Nuclear Medicine Science. William and Wilkins, London, 1987.

[2] Saha, G B. Characteristic of Specific Radiopharmaceuticals: *Fundamentals of nuclear pharmacy*. New York : Springer-Verlag.ed, 2004.

[3] Early P J, Sodee D B. Principles and Practice of Nuclear Medicine, New York: Mosby Year Book, 1996.

[4] Chandra, R. Nuclear Medicine Physics the Basics 5th edition. London: Williams & Wilkins, 1998.

[5] Saha, G B. *Fundamentals of nuclear pharmacy*. New York : Springer-Verlag.ed, 1998.

[6] Peremans, K, B. Cornelissens, B. Van Den Bossche, K. Audenaert, C. Van De Wiele, Vet. Radiol. Ultrasound 2005; 46:162.

[7] Gomes LM, Oliveira de N B M e Bernardo-Filho M B. Drug interaction with radiopharmaceuticals: Effect on the Labeling of Red Blood Cells with Technetium-99m and on the Bioavailability of Radiopharmaceuticals. Brazilian Archives of Biology and Techonology 2002; 45: 143-9.

[8] Zuckier LS, Dohan O, Li Y, Chang CJ, Carrasco N, Dadachova E . Kinetics of perrhenate uptake and comparative biodistribution of perrhenate, pertechnetate, and iodide by NaI symporterexpressing tissues in vivo. J Nucl Med 2004; 45:500–507.

[9] Owunwanne A, Patel M, Sadek S . Preparation of Radiopharmaceuticals. The Handbook of Radiopharmaceuticals. Chapman & Hall: London, 1995.

[10] Narra KR, Sastry KSR, Goddu SM, Howell RW, Strand SE e Rao DV, 1994. Relative biological effectiveness of 99mTc-radiopharmaceuticals. Med.Phys 1994; 21: 1921-1926.

[11] Moreno SRF , Carvalho JJ, Nascimento ALR, Freitas RS, Diré GF, Lima, EA , Lima-Filho GL, Rocha EK e Bernardo-Filho M.2 2 4 2 Biodistribution of Sodium Pertechnetate and Light Microscopy of Organs Isolated from the Rats: Study of the Effects of a *Ginkgo biloba* Extract Pakistan Journal of Nutrition 2004; 3 (1): 64-67.

[12] Harbert JC, Eckelman WC, Neumann RD Nuclear Medicine Diagnosis and therapy. Thieme Medical, New York , 1996.

[13] Bernardo-Filho M, Santos-Filho SD, Moura EG, Maiworm AI, Orlando MMC, Penas ME, Cardoso VN, Bernardo LC, Brito LC. Drug interaction with radiopharmaceuticals: a review. Braz Arch Biol Technol 2005; 48:13–28.

[14] Brito D M M, Gomes, M L, Rodrigues P C, Paula E F, Gutfilen B e Bernardo-Filho M. Effect of the chemotherapeutic drugs on the biodistribution of 99mTc-DTPA in Balb/c mice. J Exp Clin Cancer Res 1998; 17:313–316.

[15] Capriles PV, Dias AP, Costa TE, Oliveira MB, Faria MV, Moura EG, Abreu BA, Bernardo-Filho M 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 2000; 48:771–776.

[16] Diré G, Lima E, Gomes M, Bernardo-Filho M. The effect of a chayotte (*Sechium edule*) extracts (decoct and macerated) on the labeling of blood elements with Technetium-99m and on the biodistribution of the radiopharmaceutical sodium pertechnetate in mice: an in vitro and in vivo analysis. Pakistan J Nutr 2003; 2:221–227.

[17] Mattos DMM, Gomes ML, Freitas RS, Rodrigues PC, Nascimento VD, Boasquevisque EM, Paula EF, Bernardo-Filho M. Assessment of the vincristine on the biodistribution of 99mTc-labelled glucoheptonic acid in female Balb/c mice. Nucl Med Commun 2000; 21:117–121.

[18] Fonseca AS, Frydman JN, Santos R, Bernardo-Filho M. Influence of antipyretic drugs on the labeling of blood elements with technetium-99m. Acta Biol Hung 2005; 56:275–282.

[19] Holanda CMCX, Leite RCH, Catanho MTJ, Souza GM, Bernardo-Filho M . The effect of glucantime TM on the labeling of blood constituents with technetium-99m. Acta Cir Bras 2005; 20:81–85.

[20] Lima-Filho GL, Lima GM, Freitas RS, Aleixo LC, Moreno SR, Catanho MT, Bernardo-Filho M. Evaluation of the phytic acid effect on the labeling of blood elements with technetium-99m and on the survival of a strain of *Escherichia coli* treated with stannous fluoride. Mol Cell Biochem 2003; 247:121–126.

[21] Sampson CB. Complications and difficulties in radiolabelling blood cells: A review. Nucl Med Commun 1996; 17:648–658.

[22] Passos MCF, Ramos CF, Bernardo-Filho M, Mattos DM, Moura EG. The effect of protein or energy restriction on the biodistribution of Na 99mTcO4in Wistar rats. Nucl Med Comm 2000; 21:1059–1062.

[23] Hart D, Wall BF. UK nuclear medicine survey 2003–2004. Nucl Med Commun 2005; 26:937–946.

[24] Hesslewood, S., E. Leung, Eur. J. Nucl. Med. 1994; 21: 348.

[25] Moreno S R F, Arnobio A., Carvalho JJ de, Nascimento A L, Timoteo M O, Olej B, Rocha EK e Caldas L Q de A. The ingestion of a *Nectandra membranacea* extract changes the bioavailability of technetium-99m radiobiocomplex in rat organs. Biology research 2007; 40:131-135.

[26] Santos-Filho S D, Bernardo-Filho M. 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 Cirúrgica Brasileira 2005; 20(1).

[27] Kam, P. C. A. and Liew, S.. Traditional Chinese herbal medicine and anaesthesia. Anaesthesia, 2002, 57(11), 1083 – 1089.

[28] Dominguez X A, Martinez C, Calero A., Hinosa M, Zamudio A, Watson W H e Zabel V. Chemical components from Jiquelite *Indigofera suffruticosa Mill*. Planta Medica 1978; 34: 172-175.

[29] GARCEZ, WS. GAECEZ, FR. HONDA, NK. & DASILVA, A.JR. A Nitropropanoyl – glucopyranoside from *Indigofera suffruticosa* . Phytomistry 1989, 28: 1251-1252..

[30] Ribeiro L R, Batista A RPL, Silva A R, Sales L A, Salvadori D M F e Maia PC. Toxicological and toxicogenetic effects of plants used in popular medicine and in cattle food. Memórias do Instituto Oswaldo Cruz 1991; 86: 89-91.

[31] Leite SP, Silva LLS, Catanho MT J A, Lima EO, Lima VLM. Atividade antiinflamatória do extrato de *Indigofera suffruticosa*. Revista Brasileira de Ciências da Saúde 2003; 7: 47 – 52.

[32] Leite SP, Medeiros PL, Silva EC, Maia MBS, Lima VLM, Saul DE. Embryotoxicity in vitro with extract of *Indigofera suffruticosa* leaves. Reproductive Toxicology 2004; 18: 701-705.

[33] Leite SP, Vieira JRC, Medeiros PL, Leite RMP, Lima VLM, Xavier HS, Lima EO. Antimicrobial activity of *Indigofera suffruticosa*. Evidence- Based Complementary and Alternative Medicine 2006 3(2):261-265.

[34] Soares S F, Brito L C, Souza DE, Bernardo LC, Oliveira JF, Bernardo-Filho M. Potential pitfalls in the nuclear medicine imaging: Experimental models to evaluate the effect of natural products on the radiolabeling of blood constituents, bioavailability of radiopharmaceutical and on the survival of Escherichia coli strains submitted to the treatment with stannous ion. Nuclear Instruments and Methods in Physics Research A 2006; 569: 505–508.

[35] Moreno SR F, Carvalho JJ, Nascimento AL, Pereira M, Rocha EK, Diré G, Arnobio A., Caldas LQ de A e Bernardo-Filho M. Bioavailability of the Sodium Pertechnetate and Morphometry of Organs Isolated from Rats: Study of Possible Pharmacokinetic Interactions of a Ginkgo Biloba Extract. Brazilina archives of Biology and Technology2005; 48:73-78.

[36] Fernandes J FO, Brito LC, Frydman JNG, Santos-Filho SD, Bernardo-Filho M. An aqueous extract of *Pfaffi a* sp. does not alter the labeling of blood constituents with technetium-99m and the morphology of the red blood cells. Revista brasileira de farmacognosia, 2005; 15(2): 126-132.

[37] Narra KR, Sastry KSR, Goddu SM, Howell RW, Strand SE e Rao DV. Relative biological effectiveness of 99mTc-radiopharmaceuticals. Med.Phys. 1994; 21: 1921-1926.

[38] Santos-Filho S D, Maiworm A I, Presta G A, Paoli S P, Giani TS e Bernardo-Filho M. Aqueous extract of the medicinal plant *Mentha crispa* alters the biodistribution of the radiopharmaceutical sodium pertechnetate in Wistar rats. Med Chem Res 2007; 16:230–237.

Tabela 1. Percentage of radioactivity (%ATI/g) in mice tissues after of the treatment with aqueous *I. suffruticosa* extract**.**

Organs	Control	Treated
Blood	0.70 ± 0.13	0.58 ± 0.15
Heart	0.22 ± 0.02	0.18 ± 0.03
Lungs	0.52 ± 0.06	0.32 ± 0.09*
Stomach	2.03 ± 0.13	1.96 ± 0.84
Liver	0.26 ± 0.02	0.18 ± 0.06
Spleen	0.16 ± 0.02	0.15 ± 0.03
Pancreas	0.18 ± 0.03	0.20 ± 0.07
Kidneys	0.31 ± 0.02	0.24 ± 0.02
Large intestine	0.12 ± 0.01	0.10 ± 0.01
Small intestine	0.29 ± 0.10	0.06 ± 0.08*
Muscle	0.06 ± 0.01	0.06 ± 0.01
Bone	0.18 ± 0.07	0.12 ± 0.01
Brain	0.03 ± 0.01	0.02 ± 0.01
Thyroid	2.24 ± 0.72	6.22 ± 0.62*

(*) significant difference ($p<0.05$). Male mice were treated with *I. suffruticosa* (50 mg/mL) for 7 days and injected with 0.1ml of 99mTc. Animals organs were isolated, weighed and the radioactivity was counted in tissues and the %ATI/g calculated. Data are represented as mean ± standard deviation of the mean for six individual experiments.

CONCLUSÕES

- A resistência osmótica da membrana plasmática dos eritrócitos de ratos em solução hipotônica diminuiu devido à ação do extrato de *I. suffruticosa*;

- O extrato de *I. suffruticosa* interfere na marcação, *in vitro,* com tecnécio-99m de eritrócitos e de proteínas plasmáticas e celulares;

- A biodistribuição do Pertecnetato de sódio sobre os tecidos de camundongos foi modificada pelo extrato de *I. suffruticosa*, com grande influência sobre a tireóide;

- Os resultados mostram a interação de drogas naturais com radiofármacos como também o conhecimento de propriedades biológicas de uma planta medicinal.

ANEXOS

Trabalhos Enviados Para Congressos

XXXVII Reunião Annual da Sociedade Brasileira de Bioquímica e Biologia Molecular,

Sbbq, Águas de Lindóia-SP – 2008

PURIFICATION AND PARTIAL CHARACTERIZATION OF AN *Indigofera suffruticosa* LEAF LECTIN

Pereira, D.R.; Correia, M.T.S.; Coelho, L.C.B.B.; Paiva, P. M. G.; Lima, V.L.M.

Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, Pernambuco, Brazil

Indigofera suffruticosa Mill (Fabaceae) is a legume used in popular medicine by its claimed sedactive and diuretic actions. Lectins are proteins with hemagglutinating activity (HA) present in large numbers of plants that shows biological functions. The aims of this work were the isolation and partial characterization of an *I. suffruticosa* leaf lectin (IsuLL). An extract (10%, w/v) was obtained by mixture of dried leaves with 0.15 M NaCl and evaluated through HA assay using different erythrocytes. The extract was chromatographed on chitin column (5 ml) and adsorbed lectin was eluted with 1.0 M acetic acid, pH 4.0. The Lowry method was used for protein measurement of extract and isolated protein. HA of extract and purified lectin was evaluated with carbohydrates, glycoproteins and various pH values (3–10). The lectin was submitted to polyacrylamide gel electrophoresis under denaturing conditions (SDS-PAGE). The extract showed highest specific HA (215) with human erythrocytes (type A) and had its HA partially inhibited by xylose, galactose, sucrose, N-acetylglucosamine and glycoproteins (bovine serum albumin and azocasein). The best pH for extract activity ranged from 6.5 to 7.5. The lectin (0.78 mg) with specific HA of 63,015 was inhibited by monossacharides and glycoproteins. SDS-PAGE resolved IsuLL as a single band. In conclusion, one chitin-bind lectin can be purified from *I. suffruticosa* leaves. Further studies are in progress to evaluate its biological activity.

Supported by: CNPq.

Keywords: *Indigofera suffruticosa*, lectin, leaves.

Data de apresentação: 19/05/2008

Guide For Authors

Journal of Natural Products

Template for Submission of Manuscripts to American Chemical Society Journals

Word 2000, Version 2 (Revised November 2001)

This template is to be used to prepare manuscripts for submission to any American Chemical Society (ACS) primary research journal. As a result, it contains paragraph styles that may not normally be used in the journal you have selected for submission. Please consult the Instructions to Authors or a recent issue of the ACS journal where you plan to submit this paper for the appropriate paragraph styles. Use of this template is a benefit to the author in that the entire manuscript (text, tables, and graphics) may be submitted in one file. Inserting graphics and tables close to the point at which they are discussed in the text of the manuscript can also be a benefit for the reviewer. Use of the template is not a requirement for submission.
When you submit a manuscript using this template, you will not actually see the page formatting that appears in the printed journal. This will occur as part of the editorial production process using the paragraph tags you inserted from the template. Please read the general instructions given below on how to use the template. If you are unfamiliar with the use of templates, additional instructions can be found at the site where you downloaded this template.

Using the template

1. Abbreviated instructions for using the template follow. Additional instructions can be found in the readme file at the site where you downloaded this template.
2. If typing your manuscript directly into the template, select (highlight) the text of the template that you want to replace and begin typing your manuscript (i.e., the select the Title section for typing in your title).
3. If you have already prepared your document in a Word file, you will need to attach the template to your working document in order to apply the Word Style tags. Further instructions can be found in the readme file at the site where you downloaded this template.
    a. Go to the Word Style list on the formatting toolbar and you will see all the Word Styles from the template that have now been imported into the current document. A Styles toolbar has been generated that will display the different Styles for you to choose from. If this is not present, select **View**, **Toolbars**, and then select **Styles** and it should appear. You can close this at any time and then reopen it when needed.
    b. Click in the sentence or paragraph and then go to the Word Style menu on the toolbar and select the relevant Word Style. This will apply the Word Style to the entire text (sentence or paragraph). Do this for all sections of the manuscript.
4. In ACS publications there are many different components of a manuscript (i.e., title, abstract, main text, figure captions, etc.) that are represented in the template. See the Guide, Notes, Notice, or Instructions for Authors that appear in each publication's first issue of the year and the journal's homepage to determine which parts should be included for the manuscript that you are preparing.
5. To insert graphics within the text or as a figure, chart, scheme, or table, create a new line and insert the graphic where desired. If your graphic is not visible, ensure that the Word Style is "Normal" with an automatic height adjustment. If the size of the artwork needs to be adjusted, re-size the artwork in your graphics program and re-paste the artwork into the template (maximum width for single-column artwork, 3.3 in. (8.5 cm); maximum width for double-column artwork, 7 in. (17.8 cm)). **NOTE**: If you are submitting your paper to a journal that requires a Table of Contents graphic, please insert the graphic at the end of the file.
6. Delete all sections from the template that are not needed, including these instructions.

7. Save the file with the graphics in place: select **Save As** (**File** menu) and save it as a document file (.doc).
8. Proof a printout of the manuscript (from a 600 dpi or higher laser printer) to ensure that all parts of the manuscript are present and clearly legible.
9. Consult the Info for authors page from the home page of the ACS journal that you have selected for the latest instructions on how to proceed with the submission of your manuscript.
10. Ensure that page numbers are present on all pages before submitting your manuscript.

TITLE (Word Style "BA_Title"). The title should accurately, clearly, and concisely reflect the emphasis and content of the paper. The title must be brief and grammatically correct

AUTHOR NAMES (Word Style "BB_Author_Name"). Include in the byline all those who have made substantial contributions to the work, even if the paper was actually written by only one person. Use first names, initials, and surnames (e.g., John R. Smith) or first initials, second names, and surnames (e.g., J. Robert Smith). Do not use only initials with surnames (e.g., J. R. Smith) because this causes indexing and retrieval difficulties and interferes with unique identification of an author. Do not include professional or official titles or academic degrees. At least one author must be designated with an asterisk as the author to whom correspondence should be addressed.

AUTHOR ADDRESS (Word Style "BC_Author_Address"). The affiliation should be the institution where the work was conducted.

AUTHOR EMAIL ADDRESS (Word Style "BI_Email_Address")

RECEIVED DATE (to be automatically inserted after your manuscript is accepted if required according to the journal that you are submitting your paper to)

TITLE RUNNING HEAD (Word Style "AF_Title_Running_Head"). If you are submitting your paper to a journal that requires a title running head (condensed title appearing at the top of the journal page), please provide a 50-character or less summary of the title of the manuscript.

CORRESPONDING AUTHOR FOOTNOTE (Word Style "FA_Corresponding_Author_Footnote"). Clarify all corresponding authors' addresses by accompanying footnotes if they are not apparent from the affiliation line. Telephone numbers, fax numbers, and e-mail addresses may all be included in the corresponding author footnotes. If an author's address is different than the one given in the affiliation line, this information may also be included here.

ABSTRACT (Word Style "BD_Abstract"). All manuscripts must be accompanied by an abstract. The abstract should briefly state the problem or purpose of the research, indicate the theoretical or experimental plan used, summarize the principal findings, and point out major conclusions. The optimal length is one paragraph.

KEYWORDS (Word Style "BG_Keywords"). If you are submitting your paper to a journal that requires keywords, provide significant keywords to aid the reader in literature retrieval.

BRIEFS (WORD Style "BH_Briefs"). If you are submitting your paper to a journal that requires a brief, provide a one-sentence synopsis for inclusion in the Table of Contents.

MANUSCRIPT TEXT (Word Style "TA_Main_Text"). For full guidelines, please see the Guide, Notes, Notice, or Instructions for Authors that appear in each publication's first issue of the year and on the World Wide Web at http://pubs.acs.org. This may include an introduction, experimental details (sections titled Experimental Methods, Experimental Section, or Materials and Methods), theoretical basis (sections titled Theoretical Basis or Theoretical Calculations), results, discussion, and conclusions.

ACKNOWLEDGMENT (Word Style "TD_Acknowledgments"). Generally the last paragraph of the paper is the place to acknowledge people, organizations, and financing (you may state grant numbers and

sponsors here). Avoid use of phrases like "we (I or the authors) would like to thank" and "we (I or the authors) wish to thank", instead use "we (I or the authors) thank". Follow the journal's guidelines on what to include in the Acknowledgments section.

SUPPORTING INFORMATION PARAGRAPH (Word Style "TE_Supporting_Information"). A brief statement in nonsentence format listing the contents of material placed here should be included at the end of the manuscript as a separate section titled **Supporting Information Available**. The contents of Supporting Information may include the following: (1) large tables, (2) extensive figures, (3) lengthy experimental procedures, (4) mathematical derivations, (5) analytical and spectral characterization data, (6) molecular modeling coordinates, (7) modeling programs, (8) crystallographic information files (CIF), (9) instrument and circuit diagrams, (10) and expanded discussions of peripheral findings. For complete instructions on how to prepare this material for publication, check the Guide, Notes, Notice, or Instructions for Authors that appear in each publication's first issue of the year and on the World Wide Web at http://pubs.acs.org.

FIGURE CAPTIONS (Word Style "VA_Figure_Caption"). Each figure must have a caption that includes the figure number and a brief description, preferably one or two sentences. The caption should immediately follow the figure with the format "**Figure X.** Figure caption.". All figures must be mentioned in the text consecutively and numbered with Arabic numerals. The caption should be understandable without reference to the text. It is preferable to place the keys to symbols used in the figure in the caption, not in the artwork. Ensure that the symbols and abbreviations in the caption agree with those in the figure itself and in the text and that the figure is already sized appropriately.

SCHEME TITLES (Word Style "VC_Scheme_Title"). Chemical reactions and flow diagrams may be called schemes. Schemes may have brief titles describing their contents. The artwork for each scheme should immediately follow the scheme title. The title should follow the format "**Scheme X.** Scheme Title". All schemes must be mentioned in the text consecutively and numbered with Arabic numerals. Schemes may also have footnotes (use Word Style "FD_Scheme_Footnote"), inserted after the artwork.

CHART TITLES (Word Style "VB_Chart_Title"). Lists of structures may be called charts. Charts may have brief titles describing their contents. The title should follow the format "**Chart X.** Chart Title". Charts may also have footnotes (use Word Style "FC_Chart_Footnote"). To insert the chart into the template, be sure it is already sized appropriately and paste it immediately after the chart title.

TABLES. Each table must have a brief (one phrase or sentence) title that describes its contents. The title should follow the format "**Table X.** Table Title" (Word Style "VD_Table_Title"). The title should be understandable without reference to the text. Put details in footnotes, not in the title (use Word Style "FE_Table_Footnote"). Define nonstandard abbreviations in footnotes.

Use tables (Word Style "TC_Table_Body") when the data cannot be presented clearly as narrative, when many precise numbers must be presented, or when more meaningful interrelationships can be conveyed by the tabular format. **Do not use Word Style "TC_Table_Body" for tables containing artwork.** Tables should supplement, not duplicate, text and figures. Tables should be simple and concise. It is preferable to use the Table Tool in your word-processing package, placing one entry per cell, to generate tables.

REFERENCES (Word Style "TF_References_Section"). In many journals, references are placed at the end of the article, while in others they are treated as footnotes. In any case, place your list of references at the end of the manuscript. The appropriate placement will be made as part of the editorial process. In ACS publications, references are cited in three ways: superscript numbers, italic numbers on the line and in parentheses, and by author name and year of publication in parentheses inside the punctuation. Please use the appropriate style for the journal for which you are submitting your manuscript. Authors are responsible for the accuracy and completeness of all references. Authors should check all parts of each reference listing against the original document. Detailed information on reference style can be found in *The ACS Style Guide,* 2nd ed., available from Oxford Press.

SYNOPSIS TOC (Word Style "SN_Synopsis_TOC"). If you are submitting your paper to a journal that requires a synopsis graphic and/or synopsis paragraph, see the Guide, Notes, Notice, or Instructions for Authors that appear in each publication's first issue of the year and the journal's homepage for a description of what needs to be provided and for the size requirements of the artwork.

Nuclear Medicine and Biology

Official Journal of the Society of Radiopharmaceutical Sciences

Nuclear Medicine and Biology uses a Web-based online manuscript submission and review system. Please visit http://ees.elsevier.com/nucmedbio/ to submit your manuscript electronically. The website guides authors stepwise through the creation and uploading of the various files. Note that original source files, not PDF files, are required. A cover letter is generally required; please save this as a separate file for upload. Authors may send queries concerning the submission process, manuscript status or journal procedures to the Editorial Office. Once the submission files are uploaded the system automatically generates electronic (PDF) proof, which is then used for reviewing. All correspondence, including the Editor's decision and request for revisions, will be by e-mail.

Style

Manuscripts must be typewritten and double-spaced.

The first page should include:

(a) the title, which should be concise but informative.
(b) an abbreviated title of not more than 45 letters and spaces for abstracting and indexing purposes.
(c) the authors' names and institutions including postal address in full for the corresponding author.
(d) the author to whom correspondence should be sent, including e-mail address, telephone and fax numbers.
(e) for indexing purposes, please provide up to 6 key words.
An abstract of 80 to 250 words with subdivisions of Introduction, Methods, Results, and Conclusions must be supplied for all articles.

Animal experiments

In manuscripts describing experiments with living animals, authors should state clearly in the text that the experiments were carried out in compliance with the relevant national laws relating to the conduct of animal experimentation, or, where such laws do not exist, that the studies were performed in accordance with some internationally recognized code of practice such as the United Kingdom Biological Council's*Guidelines on the Use of Living Animals in Scientific Investigations*, 2nd edn.

Illustrations and Tables (see below) should not be placed in the body of the text, but their approximate location should be indicated in the margin. All figure captions must be typed in a list on a separate sheet at the end of the script.

Nomenclature

Preferably Systeme International (SI) units should be used throughout with equivalent quantities in older usage indicated in parentheses. NBS Special Publication 330 *The International System of Units* (SI) or the *Systeme International d'Unites* may be helpful in this respect. Chemical and biological nomenclature should conform to International Union of Pure and Applied Chemistry (IUPAC) recommendations. The IUPAC Rules can be found in the *Handbook of Chemistry and Physics*, CRC Press Inc., Boca Raton, Fla. Radioactive labels should be indicated by the radionuclide's symbol in square brackets before the labeled compound, e.g. [^{3}H]H_2O, [^{14}C]carbon dioxide, 2-amino- 4-([^{11}C]methylthio) butyric acid (not [^{11}C]methyl-methionine), N-[^{11}C]methyl-normophine, etc. Carrier-free compounds only should be designated as ^{3}H2O, $^{13}NH_3$, etc. No-carrier-added compounds should be written as [^{13}N]NH_3, [^{11}C]CO_2 unless proof of specific activity is given. Metastable (isomeric) and ground state of nuclei are designated by an "m" or "g" respectively, placed after the atomic-mass number, e.g.^{99m}Tc and ^{99g}Tc although the "g" may be omitted if no ambiguity results.

References

Text: Indicate references by number(s), in square brackets in line with the text. The actual authors can be

referred to, but the reference number(s) must always be given. List: Number the references (numbers in square brackets) in the order in which they appear in the text. List the first six authors for each reference.

Reference to a journal publication:
[1] Van der Geer J, Hanraas JAJ, Lupton RA. The art of writing a scientific article. J Sci Commun 2000;163:51-9.

Reference to a book:
[2] Strunk Jr W, White EB. The elements of style. 3rd ed. New York: Macmillan, 1979.

Reference to a chapter in an edited book:
[3] Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith TX, editors. Introduction to the electronic age.New York: E-Publishing Inc.; 1999, p. 281-304.

Abbreviated journal titles should be in accordance with *Chemical Abstracts Service Source Index (CASSI).*

Illustrations

Complete instructions for submission can be found at http://www.sciencedirect.com/science/journal/09698051 (click on Information for Authors). Color figures are welcome, and will appear in color on the web a no extra charge; however, there is a charge for the reproduction of color illustrations in print ($650 for the color figure and $100 for each additional color figure in the same article). Alternatively, the illustrations can be reproduced in black and white in the print edition at no extra charge.

All figures that contain images of biodistribution in vivo must contain the amount of radioactivity injected, the anesthesia used, and the length of the scan used to obtain that image in the figure caption. The same information should also appear in the Materials and Method section.

Tables

Each table should be typed, with caption, on a separate sheet at the end of the manuscript.

Extensive tables should be suitable for direct photographic reproduction, and thus are to be treated as illustrations (see above).

Proofs

Proofs will be despatched to the first-named author, unless otherwise requested. They should be returned with the least possible delay. Corrections to the proofs must be restricted to printer's errors only. Any substantial changes other than these will be charged to the author. Please note that authors are urged to check their proofs carefully before return, since late corrections cannot be guaranteed for inclusion in the printed journal.

Offprints

Offprints and copies of the issue (at a specially reduced rate) can be ordered on the offprint order form that will accompany the proofs. This should be returned to: Elsevier Inc., 360 Park Avenue South, New York, NY 10010-1710 USA.