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Effect of a plant origin drug on the biodistribution of ^{99m}Tc-DTPA in *Wistar* albino rats

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Article

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Abstract: In recent years all over the world, medicinal plants are used quite a lot but side effects of biological and chemical contents and radiopharmaceutical interactions for each consumer in question aren't entirely well-known. The studies of plant origin drug interaction with radiopharmaceuticals are highly relevant and desired. One of them is passiflora syrup (*Passiflora incarnata* L., Passifloraceae) which is widely used for depression, insomnia, anxiety and menopause period. The aim of current study is to evaluate possible effects of passiflora syrup on the biodistribution of ^{99m}Tc-DTPA and its blood cells uptake. DTPA was labeled with ^{99m}Tc radionuclide. Biodistribution studies were performed on male *Wistar* albino rats which were treated via oral feeding-gavage-method with either passiflora syrup or 0.9 % NaCl as control group for ten days. Blood samples were obtained by cardiac blood withdrawal from the rats and they were radiolabeled. The biodistribution results showed that the passiflora syrup decreased the uptake of ^{99m}Tc-DTPA in kidneys and in blood cells. ^{99m}Tc-DTPA being used widely as a kidney diagnostic agent in nuclear medicine seems to be interacting with orally taken passiflora. Passiflora syrup may modify the uptake of ^{99m}Tc-DTPA by kidney. The knowledge of this negative effect may contribute to reduce the risk of misdiagnosis and/or repetition of the examinations in nuclear medicine.

Introduction

The use of medicinal plants for treatment of various diseases has increased in the last years in all over the world (Everson & McQueen, 2004; Barbosa-Filho et al., 2008). Frequently, it is based on empirical knowledge, and their side effects, chemical composition and possible drug interaction are not fully known (Hu et al., 2005). Particularly in nuclear medicine, including medicinal plant origin drugs interaction with radiopharmaceuticals is not completely understood. It may arise as a result of a variety of factors including the pharmacological action of the drug, physiochemical interactions between drugs and radiotracers, and competition for binding sites. It has already reported that the biodistribution of radiopharmaceuticals used in diagnostic imaging in nuclear medicine is also altered by including plant origin drugs (Hesslewood & Leung, 1994; Britto et al., 1998; Gomes et al., 2001; Mattos et al., 1999). It has been described that extracts of medicinal plants could interfere with the biodistribution of ^{99m}Tc sodium pertechnetate particularly (Rebello et al., 2007; Rebello et al., 2008; Bernardo-Filho et al., 2005; Moreno et al., 2007; Jankovic & Djokic, 2005; Valenca et al., 2005). In these studies, alterations on the uptake of radiopharmaceutical were observed.

Some authors have also described that medicinal plants can have an effect on the radiolabeling of blood cells constituent (Rebello et al., 2007; Rebello et al., 2008; Moreno et al., 2004; Braga et al., 2000; Benarroz et al., 2008; Oliveira et al., 2002; Diniz et al., 2008; Sampson 1993; Sampson 1996).

As the studies of plant origin drug interaction with radiopharmaceuticals are highly relevant and desired. One of them is passiflora syrup (*Passiflora incarnata* L., Passifloraceae) which is a sedative drug containing passiflora incarnate extract. It is widely used for depression, insomnia, anxiety and menopause period in Turkey (Yaris et al., 2005; Dhawan et al., 2004). The current study aims to evaluate in vitro and in vivo effects of passiflora syrup on the biodistribution of technetium labeled diethylenetriamine pentaacetate (^{99m}Tc-DTPA), which used for renal imaging and function testing, also known as ^{99m}Tc pentatate.

Materials and Methods

Passiflora syrup was purchased from Sandoz Ilaç San. A.Ş., Istanbul, Turkey (validity of the product was December 2010). All other chemicals were supplied from Merck Chemical Co. and Aldrich Chemical Co. and used as supplied. Experimental protocols followed in current study were approved by the Ethical Committee of the Institutional Animal Review Committee of Ege University (Number: 2009-127) Izmir, Turkey. Male *Wistar* albino rats (2.5 months, 130-180 g) were maintained in a controlled environment. The animals had free access to water and food with ambient temperature at 25 °C.

Animal treatments

Male *Wistar* albino rats (n=12) were treated either with *Passiflora* syrup (50 mg/kg) (Rebello et al. 2008) or with saline solution (0.9% NaCl), as control group, for 10 days. For this purpose, 7.2 µL of the passiflora syrup, which is included 1 mg the passiflora liquid extract, was diluted to 1 mL in saline solution (0.9% NaCl) and shacked for 2 min. Then, the rats (n=6) were treated by gavage with this prepared concentration of passiflora syrup.

Preparing procedure of ^{99m}Tc DTPA

One mg of DTPA was dissolved in 1 mL of distilled water. To this solution, 100 µL of $SnCl_2$ (1 mg $SnCl_2.2H_2O$ in 1 mL HCl) and 196.10 MBq (5.30 mCi)/275 µL ^{99m}Tc sodium pertechnetate were added under nitrogen atmosphere. The pH was adjusted to 5 with 1 M NaOH solution. The reaction mixture was shaken and allowed to stand for 30 min at room temperature. The quality control studies were done by using TLRC method.

Thin Layer Radio Chromatography (TLRC)

Radiochemical yield of ^{99m}Tc-DTPA was confirmed by using TLRC quality control method. ^{99m}Tc-DTPA was assessed by TLRC using flexible silica gel plates and TLRC solvent as saline solution. ^{99m}Tc-DTPA was set 1 cm from the lower end of the plates and submerged in different solvents. Relative front (Rf) values of the ^{99m}Tc-DTPA, reduced ^{99m}Tc and ^{99m}Tc sodium pertechnetate were calculated by using TLC Scanner (BioscanAR 2000).

Determination of the partition coefficient (logP) for ^{99m}Tc-DTPA

The partition coefficient was determined by mixing ^{99m}Tc-DTPA with equal volumes (0.2 mL) of 1-octanol and phosphate buffer (pH 7) in a centrifuge tube. The mixture was vortexed at room temperature for 1 min and then 0.1 mL of the radiolabeled compound was added to the mixture. The resulting solution was centrifuged [clinical centrifuge, 30 min, 850 g (2500 rpm/min force)]. From each phase, 0.1 mL of the aliquot was pipetted out and counted. Each measurement was repeated three times. Care was taken to avoid cross contamination between the phases. The partition coefficient was calculated using the equation; P = (cpm in octanol-cpm in background) / (cpm in buffer-cpm in background), as previously reported (Saji et al., 1993; Biber Muftuler et al., 2011). The final partition coefficient value was usually expressed as logP. Theoretical logP calculations were done with ACD/logP program [Advanced Chemistry Development, Inc., (ACD/Labs), Version 6.0 for Microsoft Windows] (Istanbul, Turkey).

In vivo *biodistribution studies on male Wistar Albino rats*

For the biodistribution assay, male *Wistar* albino rats [weighing approximately 130-180 g, (n=12)] were treated for 10 days orally administration with passiflora syrup (50 mg/kg) or with saline solution as control group. After sterilization by passing through a 0.22 µm membrane filter, ^{99m}Tc-DTPA was injected into the tail vein of the animals (2 µg/each rat). The activity was approximately 29.60 MBq (800 µCi)/rat. The rats were sacrificed post injection under ketamine anesthesia and tissues of interest (heart, lung, liver, kidney, small intestine, large intestine, stomach, spleen, pancreas, head, fat, thyroid, bladder, muscle, testis, prostate, bone) were removed. Blood samples were taken, organs were excised. All tissues were weighed and counted with Cd(Te) detector. The biodistribution in percentage of injected dose per gram of tissue weight (% ID/g) for some selected organs was given as the mean value of the measurements for three rats.

In vitro *radiolabeling of blood samples*

Blood samples (0.5 mL, n=12 for each treatment) were obtained under ketamine anesthesia by cardiac blood withdrawal from male *Wistar* albino rats treated with passiflora syrup (50 mg/kg) or with 0.9% NaCl, as control group. These samples were incubated with 0.1 mg DTPA, 0.1 mg stannous chloride solution and ^{99m}Tc sodium pertechnetate for 30 min. They were centrifuged [clinical centrifuge, 5 min, 850 g (2500 rpm/min force)], serum (S) and blood cells (BC) were separated. The radioactivity in the samples was counted by Cd(Te) detector and the percentage of radioactivity was calculated. The data are expressed as mean ± standard deviation of the percentage of radioactivity (Table 2).

Table 1. Biodistribution of ^{99m}Tc-DTPA on control group and treated group with *Passiflora* syrup at organ/muscle ratio of male *Wistar* albino rats.

%ID/g (Organ/Muscle)	Control Group	*Passiflora* Syrup Group	% ID/g (Organ/Muscle)	Control Group	*Passiflora* Syrup Group
Heart	3.03±1.49	1.20±0.49	Muscle	1.00±0.00	1.00±0.00
Lung	7.25±1.97	7.64±3.08	Head	0.75±0.36	0.14±0.06
Liver	3.59±1.40	4.23±1.80	Fat	1.71±0.68	0.30±0.32
Kidney	58.05±2.48	4.95±2.31	Thyroid	2.18±1.00	2.74±1.11
S. Intestine	2.71±1.19	1.92±0.80	Bladder	37.36±9.14	5.58±2.87
L. Intestine	1.02±0.52	0.63±0.09	Blood	11.96±5.48	3.56±1.43
Stomach	1.25±0.25	0.57±0.10	Testis	0.56±0.22	0.34±0.16
Spleen	2.27±1.12	0.95±0.36	Prostate	1.86±0.89	0.98±0.33
Pancreas	2.33±0.85	2.51±0.36	Bone	0.91±0.40	1.08±0.40

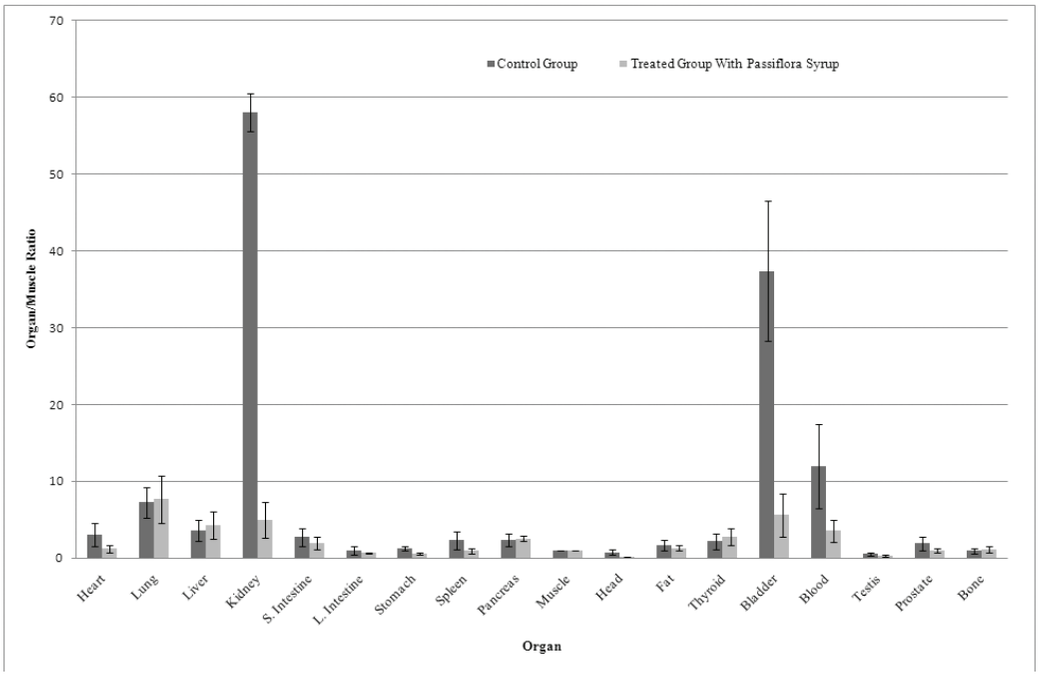

Figure 1. Effect of *Passiflora* syrup on the biodistribution of ^{99m}Tc-DTPA in organs isolated from male *Wistar* albino rats (organ/muscle).

Statistical analysis

The data are expressed as mean±standard deviation of % ID/g. The values were analyzed by SPSS 16 program (Univariate Variance Analyses and Pearson Correlation, SPSS, Inc., Chicago, IL) with a $p<0.05$ as significant level. Pearson correlation was carried out between different organs for ^{99m}Tc-DTPA.

Results

According to the TLRC chromatograms, Rf values of ^{99m}Tc-DTPA, reduced ^{99m}Tc and ^{99m}Tc sodium pertechnetate were 0.67, 0.02 and 0.90, respectively. Radiochemical yield of the ^{99m}Tc-DTPA was 98.35±1.75% (n=10).

It is known that partition coefficient has been calculated for the uncharged molecule theoretically. To verify it experimentally, we have properly calculated the corresponding logP values. Theoretical logP value of DTPA was -2.08±0.86. On the other hand, the experimental logP value of ^{99m}Tc-DTPA was -2.29±0.01. These values were similar each other.

Table 1 and Figure 1 represent the values obtained for the % ID/g in male *Wistar* albino rats treated with passiflora syrup and control group. Data obtained from biodistrubition indicate that treatment with passiflora syrup has generally resulted in reduced uptake of ^{99m}Tc-DTPA in the kidney than control group. The treatment with passiflora syrup significantly ($p<0.05$) modify the % ID/g of in particular kidney for ^{99m}Tc-DTPA was decreased from 58.05±2.48 to 4.95±2.31. No significant

alteration on the % ID/g of tissues from heart, lung, liver, small and large intestine, stomach, spleen, brain, thyroid, testis, prostate and bone.

Table 2 represents the values obtained for the percentage of radioactivity of serum and blood cells from male *Wistar* albino rats treated with passiflora syrup and control group. According to *in vitro* radiolabeling of blood samples in the current study, the percentage of radioactivity on S and BC decreased (Table 2). Uptake of BC went down from 58.56±4.82 to 35.19±11.97.

Table 2. The percentage of radioactivity in serum (S) and blood cells (BC) for control group treated with SF and treated group with *Passiflora* syrup.

(n=6)	Serum (S)	Blood Cells (BC)
Control group treated with SF	41.44± 4.82	58.56 ± 4.82
Treated group with *Passiflora* syrup	64.81±11.97	35.19 ± 11.97

Discussion

Freitas et al. studied on ^{99m}Tc-DMSA and *Paullinia cupana* (guarana) extract, at low (0.1%) and high (1%) concentrations of extract showed decrease at blood cells from 19.56±4.75 to 15.13±2.19 (Freitas et al., 2007). In another study, *Ginkgo biloba* extract shifted uptake of ^{99m}Tc sodium pertechnetate on blood cells from 97.7±0.7 to 48.1±15.5 (Moreno et al., 2004). On the other hand Rebello et al. studied with ^{99m}Tc sodium pertechnetate and *Passiflora flavicarpa* fruit extract, results showed a decrease of uptake at intestine, spleen, stomach and blood and at others organs demonstrated no significant alterations (Rebello et al., 2008).

In other study, *Centella asiatica* extract which is an herbal drug was used and the biodistribution results showed an uptake decrease at spleen, heart, intestine, stomach, liver, muscle, kidney, testis and blood (Diniz et al., 2008). Moreno et al. studied with *Uncaria tomentosa.* They suggested that this extract can act on the biodistribution of ^{99m}Tc sodium pertechnetate in specific organs such as heart, pancreas and muscle (Moreno et al., 2007).

The radiolabeling of blood constituents has a great importance in nuclear medicine (Saha, 2004). Some studies have suggested that radiolabeling of blood constituents with just ^{99m}Tc could be performed to evaluate the biological effects of medicinal plant extracts (Moreno et al., 2004; Benarroz et al., 2008; Gomes et al., 2001; Abreu et al., 2006; Silva et al., 2006). Particularly Goncalves Filho et al. (2006) studied ^{99m}Tc sodium pertechnetate and unpeeled *Passiflora flavicarpa* extract, labeling of blood components. Results showed no alteration on distribution of radioactivity on blood cells and at plasma seen low decrease (Goncalves-Filho et al., 2006).

In our study, the changes at the biodistribution of the ^{99m}Tc-DTPA in the organs of the interest such as kidney and bladder could be explained by the presence of specific chemical compounds in the passiflora liquid extract or by the generation of active metabolites capable to interfere with the biodistribution of the ^{99m}Tc-DTPA. Also the reason of the passiflora liquid extract increased fixation of ^{99m}Tc-DTPA in kidney and bladder could be explained by the effect of the components of the passiflora liquid extract which would act in the transport of the pertechnetate (^{99m}Tc) ion through the cellular membrane of determined the organs.

When the drug interaction with a radiopharmaceutical is well known, the natural consequence is a right diagnosis. In current study the treatment with passiflora syrup decreased the uptake of ^{99m}Tc-DTPA by kidney and bladder. As a result of current findings relevant to the effect of the passiflora syrup which is widely used in Turkey have revealed important changes in the kidney after *in vivo* treatment with this syrup. Also *in vitro* radiolabeling of blood constituents (serum and blood cells) studies addressed here are compatible with each other. These findings can be considered as an example of plant origin drug interaction with radiopharmaceuticals.

The knowledge about this interaction represents vital clinical information for the best therapeutic decision and exact diagnosis. Although these experiments were carried out in controlled conditions and with rats, these findings should be worthwhile to avoid possible pitfalls in nuclear medicine imaging. Moreover, it is emerged that it is required to get evidence that possible unexpected alterations in the nuclear medicine examination may occur in patients that utilize passiflora syrup.

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