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Effect of rosemary (*Rosmarinus officinalis*) extract on the biodistribution of ^{99m}Tc sulphur colloid and on the radiolabeled blood constituents

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Abstract: With this study we evaluated the effects of the herb rosemary (*Rosmarinus officinalis* L. Lamiaceae) extract on the labeling of blood constituents with technetium-99m (^{99m}Tc) labeled sulphur colloid and on the biodistribution of ^{99m}Tc-Sulphur Colloid in Wistar albino rats. For this purpose, two groups of animals (male wistar rats, 130-140 g) were treated (1 mL) with a rosemary extract (750 mg/kg body wt.,n=9) and water (control, n=9) separately by gavage for five days. ^{99m}Tc-Sulphur Colloid was administrated by intravenous injection; organs/tissues were withdrawn and weighted. Blood was centrifuged, plasma and blood cells were isolated. The radioactivity was counted to calculate the percentage of activity per gram for each organ/tissue and percentage of activity in blood cells and plasma. A significant increase (p<0.05) in the uptake of ^{99m}Tc-Sulphur Colloid in the liver after the treatment with rosemary extract was observed. These results indicate that the substances or metabolites of the rosemary extract would change the biodistribution of ^{99m}Tc-Sulphur Colloid.

Introduction

The use of plant preparation as medicine is as old as mankind. One of the medicinal herbs rosemary (*Rosmarinus officinalis* L. Lamiaceae) owes wide range of biological activities due to the highest antioxidant properties it has in Lamiaceae family (Al-Sereitia et al, 1999). This plant grows in the dry warm regions of southern Europe, especially the Mediterranean area. It has been described as a medicinal plant and wonder-drug in various medieval drug monographs and literature (Sanchetti & Goyal, 2007). Rosemary which is used in traditional Turkish folk medicine for the treatment of hyperglycaemia, has a long history of medicinal use (Hanafy & Hassan, 2010).

In nuclear medicine, technetium-99m (^{99m}Tc) is the most widely available radionuclide for diagnostic radiopharmaceuticals, in the single photon emission computed tomography, due to its preferable properties such as short half-life (6.01 h), gamma energy (140 keV), low cost and general usefulness (Arano, 2002). Technetium exists in oxidation states varying from +7 to -1. ^{99m}Tc has an oxidation state +7 in the chemical form of pertechnetate ($^{99m}TcO_4^-$) when eluted from ^{99}Mo/^{99m}Tc generator. Chemical versatility of ^{99m}Tc suggests its use in various radiopharmaceutical designs for selective uptake by different body organs. Both ^{99m}Tc and chemical forms of it, makes many applications possible which provides important information (Arano, 2002).

Many radiopharmaceuticals which can be radiolabeled with Tc-99m are used in nuclear medicine for diagnostic purposes. Among them ^{99m}Tc-Sulphur Colloid (^{99m}Tc-SC) is utilizable for liver, spleen and possibly bone marrow scintigraphy. It is known that the biodistribution of intravenously injected colloids depends on phagocytic function of the reticuloendothelial system (Zolle, 2007).

On the other hand, there are many studies reporting the interaction between drugs and different radiopharmaceuticals (Moreno et al, 2007). Several authors have described that synthetic or natural products can have an effect on the labeling of red blood cells and on biodistribution of radiopharmaceuticals used for diagnostic imaging (Gomes et al, 2002). It is important to have knowledge about the interactions between drugs and radiopharmaceuticals to avoid from possibility of misdiagnosis, extra radiation dose to the patient due to repetition of examination (Bernardo-Filho et al, 2005).

Aim of this study was to evaluate the effect of aqueous extract of a widely consumed herb *Rosmarinus officinalis* on biodistribution of the sulphur colloid labeled with technetium-99m and on the labeling of blood constituents.

Materials and methods

Animals

All the experimental procedures with Wistar Albino rats were approved by the Department of Experimental Research and Surgery and the Animal Research Ethic Committees of the Ege University (Number: 2010-63). The animals were maintained under controlled conditions available water and food with ambient temperature at 25±2 °C.

Plant material

Dried commercial leaves of *Rosmarinus officinalis* L. Lamiaceae, were purchased from an herbalist (Izmir/ Turkey, Bagdat Baharat, No. 43011), identified by one of authors (ST) and a sample was kept in the laboratory for further reference. The leaves were powdered in a porcelain mortar and extracted with double distilled water (200 mL) by refluxing for 36 h at 55±5 °C (Sanchetti & Goyal, 2007). After filtration through filter paper, plant extract was stored at -20 °C temperature in a fridge. Then the frozen sample was lyophilized. A modular Shimadzu LC-10 system comprised of a LC-10Atvp pump, Cd(Te) solid-state detector, RAD 501 single channel analyzer and (SPD-10AV)UV-VIS detector was utilized for quality control of major components in rosemary extract. Analyses were performed on Nucleodur 100-5 C18 RP-C18 column (250 x 4,6 mm I.D.) (Macharey-Nagel) at 30 °C with a mobile phase solvent A; methanol and solvent B; 850 mL 10 mM acetic acid+150 mL acetonitrile at a flow rate of 1.5 $mLmin^{-1}$; with an injection volume of 20 µL; UV detection was at 285 nm.

Radiolabeling assay of Sulphur Colloid with ^{99m}Tc

To prepare ^{99m}Tc-SC, pH control was done (pH 1) after adding 300 µL 1M H_2SO_4 on 296 MBq/500 µL $^{99m}TcO_4^-$. After adding this solution on 4 mg sodium thiosulphate pentahydrate ($Na_2O_3S_25H_2O$), it was fulfilled to 1mL with saline solution. It was adjusted pH 6 and the mixture was cooled with ice bath right after being kept in boiling water for 10 min. Quality control was made by using Thin Layer Radiochromatography (TLRC) method. Methanol was used as solvent and silicagel as stationary phase.

Stability study of ^{99m}Tc-SC in serum

In vitro stability of ^{99m}Tc-SC in serum was determined by incubating 100 µL of the labeled compound with 300 µL of serum at 37 °C. The aliquots were then analyzed at time intervals of 0, 30, 60,120, 180, 240, 300 min and 24 h after incubation.

Labeling of blood constituents with ^{99m}Tc

A dose selection of *R. officinalis* extract was carried out on the basis of drug tolerance study previously described by Sancheti & Goyal (2007). In our study, total eighteen Wistar albino rats (male, 2-3 months, 130-140 g) were used. Half of them were treated with *R. officinalis* extract (750 mg/kg b. wt.) and the rest rats were treated with water as control group, by gavage for five days at 24 h intervals.

At the sixth day, 0.5 mL blood samples were withdrawn by cardiac puncture and incubated with 37 MBq ^{99m}Tc-SC for 30 min. Samples were centrifuged for 5 min, and then plasma and blood cells were isolated. The radioactivity in plasma (P) and blood cells (BC) were counted and percentage of radioactivity (%ATI) was calculated.

Biodistribution of ^{99m}Tc-SC

For the biodistribution assay, 10 µg sulphur colloid labeled with 37 MBq ^{99m}Tc was injected into the tail vein of all rats. Each rat was sacrificed after 15 min of the administration of ^{99m}Tc-SC, selected organs/ tissues (heart, lung, liver, kidney, intestine, stomach, spleen, pancreas, muscle, brain, thyroid, testis, blood) were withdrawn and weighed. Then the radioactivity was counted with Cd(Te) detector and the percentage of radioactivity per gram (%ATI/g) was calculated.

Statistical analysis

Statistical analysis was performed by the SPSS 16 program (Univariate Variance Analyses and Pearson Correlation). $p<0.05$ were considered significant. All data are expressed as mean±SD.

Results

An approximate yield of 16% extract (w/w) was obtained. According to High Performance Liquid Chromatography (HPLC) chromatogram Rt values of rosmarinic acid, carnosol and carnosonic acid were found as 8.1, 22.3, 27.7 min, respectively as seen in Figure 1. When compared with literature these results agree with Çeliktaş' s study (Çeliktas et al., 2007).

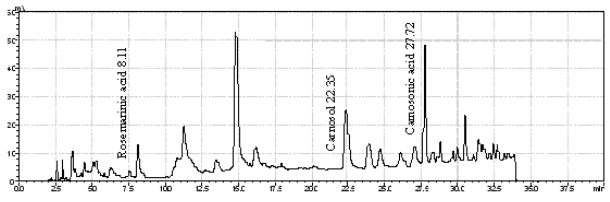

Figure 1. HPLC chromatogram of rosemary extract.

The radiochemical purity of ^{99m}Tc-SC was found as 99±1%. The obtained high radiochemical yield, verified by TLRC using methanol as developing media. R_f value of ^{99m}Tc-SC was found to be 0.015 while R_f value of $Na^{99m}TcO_4$ was 0.95. The stability of the complex in serum was investigated by the TLRC method. The results demonstrated that approximately 98% of ^{99m}Tc-SC existed as an intact complex in serum up to 24 h.

Distribution of radioactivity in BC and P are shown in the Table 1. The analysis of the results indicates that there is a significant alteration (p<0.05) in the radioactivity fixation in BC (from 67.19±7.66 to 98.02±0.85) and P (from 32.80±7.66 to 1.97±0.85).

Table 1. The percentage of radioactivity in plasma and blood cells for control group treated with water and treated group with rosemary extract.

(n=9)	% ATI	
	Plasma (P)	Blood Cells (BC)
Control group	32.80±7.66	67.19±7.66
Treated group with rosemary extract	1.97±0.85	98.02±0.85

The effect of rosemary extract treatment on the biodistribution of ^{99m}Tc-SC (%ATI/g) in the male rats is shown in Table 2. When we compare the control group and rosemary group the uptake of ^{99m}Tc-SC increased in some organs such as liver, lung, kidney, testis and blader.

Table 2. Effect of rosemary on the biodistribution of ^{99m}Tc-SC in organs isolated from male albino Wistar rats (organ/muscle).

%ID/g (Organ/Muscle)	Control Group	Rosemary Group
Heart	0.011±0.002	0.020±0.017
Lung	0.035±0.007	0.067±0.014
Liver	0.014±0.001	0.034±0.007
Kidney	0.017±0.003	0.038±0.012
Small intestine	0.026±0.005	0.042±0.007
Large intestine	0.004±0.002	0.004±0.003
Stomach	0.008±0.002	0.006±0.001
Spleen	0.029±0.009	0.024±0.014
Pancreas	0.053±0.006	0.049±0.002
Muscle	0.025±0.002	0.012±0.002
Brain	0.001±0.000	0.000±0.000
Fat	0.029±0.001	0.032±0.001
Bladder	0.023±0.004	0.067±0.030
Testis	0.010±0.002	0.032±0.004
Blood	0.006±0.001	0.016±0.004

Discussion

In recent years, consumption of medicinal plants is becoming widespread for curing various health disorders around the world. Accordingly, it is important from the point of interactions between these herbs and radiopharmaceuticals used while therapy and diagnosis of cancer (Bernardo-Filho et al, 2005).

Unexpected patterns of the biodistribution of radiopharmaceuticals cause possibility of misdiagnosis and/or repetition of the examination with an increase of radiation dose to the patient and staff (Santos-Filho et al., 2007).

Many studies about drug-radiopharmaceutical interactions report that medicinal plants are capable of uptake alterations in some organs/tissues (Abreu et al., 2006; Diniz et al., 2008; Moreno et al., 2007; Benarroz et al., 2007). In addition, alterations in radiolabeling of blood constituents related to extracts of some medicinal plants like *Thuya occidentalis* (Oliveira et al., 1996), chayotte (Diré et al., 2003), *Maytenus ilicifolia* (Oliveira et al., 2000), *Paullinia cupana* (Oliveira et al., 2002), *Mentha crispa* (Santos-Filho et al., 2004) have also been determined by research groups.

^{99m}Tc-SC is a liver specific radiopharmaceutical. Sometimes lung and kidney uptake has been observed (Klingensmith et al., 1976). In our study rosemary extract (Table 2) increased fixation of ^{99m}Tc-SC in the liver, lung, kidney, testis and bladder. Also, this extract was capable to alter radiolabeling of blood constituents (Table 1). It is possible that antioxidants in the extract would interact with ^{99m}Tc-SC or with structures in organ/tissues where these substances had high uptake. Santos-Filho and collaborators (2007) studied with *Mentha crispa* extract and reported increase fixation of sodium pertechnetate in the pancreas, kidney, spleen, liver and thryoid. These are similar to results obtained in our biodistribution assays. Rosemary is from same family (Lamiaceae) with *Mentha crispa* and it is possible to say that, increase of uptake in determined organs are because of same speculations.

In conclusion, these results can be explained by the presence of antioxidants in the rosemary extract capable to interfere with the biodistribution of ^{99m}Tc-SC. Also the effect of components of rosemary extract which would act in the transport of the pertechnetate ion through the cell membrane might increase involvement of ^{99m}Tc-SC by determined organs. In addition it is required to study about natural/synthetic drug and radiopharmaceutical interactions.

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