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Technetium-99m-Labeled Sulfadiazine: a Targeting Radiopharmaceutical for Scintigraphic Imaging of Infectious Foci Due To *Escherichia coli* in Mouse and Rabbit Models

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Abstract Bacterial infection is one of the vital reasons of morbidity and mortality, especially in developing countries. It appears silently without bothering the geological borders and imposes a grave threat to humanity. Nuclear medicine technique has an important role in helping early diagnosis of deep-seated infections. The aim of this study was to develop a new radiopharmaceutical ^{99m}Tc-labeling sulfadiazine as an infection imaging agent. Radiolabeling of sulfadiazine with technetium-99m (^{99m}Tc) was carried out using stannous tartrate as a reducing agent in the presence of gentistic acid at pH = 5. The quality control tests revealed ~98% labeling efficiency. Paper chromatographic (PC) and instant thin-layer chromatographic (ITLC) techniques were used to analyze radiochemical yield. Biodistribution and infection specificity of the radiotracer were performed with *Escherichia coli* (*E. coli*) infection-induced rats. Scintigraphy and glomerular filtration rate (GFR) study was performed in *E. coli*-infected rabbits. Scintigraphy indicated *E. coli* infection targeting potential of ^{99m}Tc-SDZ, while biodistribution study showed minimal uptake of ^{99m}Tc-SDZ in non-targeted tissues. The uptake in the kidneys was found 2.56 ± 0.06, 2.09 ± 0.10, and 1.68 ± 0.09% at 30 min, 1 h, and 4 h, respectively. The infected muscle (target) to non-infected muscle (non-target) ratio (T/NT) was found 4.49 ± 0.04, 6.78 ± 0.07, and 5.59 ± 0.08 at 30 min, 1 h, and 4 h, respectively.

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Introduction

Infection is explicitly a major cause of morbidity and mortality, and the symptoms of infection include fever, swelling, and pain. The diagnosis of infection is frequently based on clinical, pathological, and microbiological results. Despite the enhanced adroitness of human understanding of the mechanism of bacterial proliferation and action, medical science and world health organization still declare bacterial infection a bigger threat to humanity owing to the substantial diagnostic dilemma involved in bacterial infection [1, 2]. Molecular biology, immunology, and medical biotechnology offer various approaches for infection and inflammation imaging involving radiological standard imaging techniques such as computerized tomography (CT), magnetic resonance imaging (MRI), and radiolabeled leukocytes scintigraphy can pinpoint the site of infection and inflammation, but fail to discriminate between them. Compared with these two standard imaging techniques, ^{99m}Tc-labeled antibiotics (e.g., ^{99m}Tc-ciprofloxacin) scintigraphy is clinically more effective for bacterial infection and revealed discrimination between bacterial infection and inflammation [3]. In subsequent years, this approach has gained ample attention and other members of fluoroquinolone class and sister antibiotic classes have been labeled with ^{99m}Tc, and tested against different infections such as ^{99m}Tc-enorfloxacin [4], ^{99m}Tc-sitafloxacin [5], ^{99m}Tc-cefepime [6], and ^{99m}Tc-cefzolin [7] which showed promising diagnostic results as compared to ^{99m}Tc-leukocytes and ^{99m}Tc-ciprofloxacin [8–10]. Sulfonamide is the main functional group present in majority of drugs showing important biological properties such as antimicrobial, antifungal [11], antidiabetic, diuretics, anticonvulsants, dermatological, antiretroviral, hepatitis C antivirals, stimulant, and inhibitors of different enzymes could be an attractive choice of labeling with technetium-99m. Sulfadiazine (SDZ) is an active antibacterial agent of a sulfonamide family, which works by inhibiting the dihydropteroate synthetase enzyme. Dihydropteroate synthetase produces dihydropteroate in bacteria which is an important intermediate in folate synthesis. Folate further helps in DNA and RNA synthesis, required for cell division. Therefore, dihydropteroate synthetase enzyme could be a good target for ^{99m}Tc-SDZ to diagnose bacterial infection. In present study, sulfadiazine was labeled with technetium-99m to assess the imaging potential of ^{99m}Tc-SDZ in *Escherichia coli* bacteria-induced infected rat and rabbit models.

Experimental

Materials

Sulfadiazine was obtained from Neo Quimica, Brazil. A locally produced fission-based PAKGEN ^{99}Mo/^{99m}Tc generator was used to elute pertechnetate ($^{99m}TcO_4^-$) in saline. Whatman No. 3 chromatographic paper (Maidstone, UK) and ITLC-SG (Gelman sciences Institute, USA) were used for radiochemical analysis. Ammonium hydroxide (NH_4OH), sodium hydroxide (NaOH), gentistic acid, and all other chemicals used were of analytical grade and purchased from Merck, Germany. *E. coli* bacterial strain (ATCC 25923) was obtained from the Department of Biological Sciences, Quaid-e-Azam University, Islamabad. The animal study was properly

approved by the animal ethics committee of the institute. Sprague-Dawley (SD) rats and New Zealand white rabbit were obtained from the National Institute of Health (NIH), Islamabad, for the purpose of biodistribution and scintigraphy, respectively. The animals were kept under standard conditions with free ingress to food and water.

Labeling of Sulfadiazine with Technetium-99m

Direct labeling of SDZ with technetitium-99m was carried out at room temperature. The influence of different parameters such as ligand concentration, reducing agent, radioactivity, and pH was assessed by serial changes with regular defined intervals. Typically, sulfadiazine (0.2 to 2.0 mg) and 4 μL (8.3 mg/mL) gentistic acid were taken into the sterilized glass vial followed by the addition of stannous tartrate (60–150 μg) as a reducing agent. The pH of solution was tested from 2 to 11 using 0.1 N NaOH or 0.1 N HCl. One milliliter of freshly eluted pertechnetate (60 to 200 MBq) solution was added to the above mixture in the vial. The volume of all experiments was adjusted to 2 mL using distilled water. The reaction mixture was vigorously shaken and quality control analysis was performed at different intervals from 10 to 110 min.

Radiochemical Purity of ^{99m}Tc-Sulfadiazine Analysis

Percent formation of ^{99m}Tc-SDZ, free $^{99m}TcO_4^-$, and reduced ^{99m}Tc (colloids) was determined by using PC (Whatman No. 3) and ITLC-SG analysis.

Paper Chromatography

In order to measure the percentage of the free $^{99m}TcO_4^{-1}$, a drop of radiochemical reaction sample (~2 μL) was spotted at the baseline of chromatogram (size 2 × 14 cm) and allowed to run with acetone as mobile phase. In this system, free $^{99m}TcO_4^-$ moves along with mobile phase and bound and hydrolyzed ^{99m}Tc remain at baseline. The strip for radioactivity counts was analyzed with NaI scintillation well-type γ-counter and radiometric 2π-scanner.

Instant Thin-Layer Chromatography

The percentage of hydrolyzed ^{99m}Tc was determined using ITLC-SG analysis with saline as mobile solvent system. In order to do this, a small drop of reaction mixture sample (~2 μL) was spotted at baseline of ITLC strip (size 2 × 14 cm) and allowed to run in saline as mobile phase. In this system, the ^{99m}Tc-SDZ and free $^{99m}TcO_4^-$ move along with solvent front while reduced ^{99m}Tc remains at baseline. The strip was then cut into 0.25-cm patches, and the counts were measured using NaI scintillation well-type γ-counter and radiometric 2π-scanner. The percentage of free $^{99m}TcO_4^{-1}$, colloids, and ^{99m}Tc-SDZ was determined by using the following formula;

$$\%\,\text{Free}\,TcO_4^-\,(\text{PC strip}) = \frac{\text{Radioactivity counts at}\,R_f\,0.75\text{–}1}{\text{Total radioactivity counts at}\,R_f\,0\text{–}1}$$

$$\%\text{Colloids}\,(\text{ITLC strip}) = \frac{\text{Radioactivity counts at}\,R_f\,0\text{–}0.25}{\text{Total radioactivity counts at}\,R_f\,0\text{–}1}$$

$$\%\ \text{Yield of}^{99m}\ \text{Tc - SDZ} = 100 - \left(\%\text{Free}\,TcO_4^- + \%\text{Colloids}\right)$$

Biodistribution

For biodistribution study, three healthy SD rats (weighing approximately 50–70 g) were used for each time point. The infection was introduced into right thigh muscles of all rats by injecting 100 μL saline suspension of 1×10^8 CFU (colony forming units) of *E. coli*. After 30 h, when visible swelling appeared in the infected thighs, ^{99m}Tc-SDZ was administrated after anesthetizing the animals with chloroform. The rats were sacrificed at 30 min, 60 min, and 4 h time points; different body organs such as the heart, liver, kidney, urinary bladder, and right and left thigh muscles (normal and infected) were separated and washed with saline and weighed. The distribution of ^{99m}Tc-SDZ activity in different organs was measured with NaI gamma scintillation counter in terms of percent injected dose per gram body organ.

Scintigraphic Study

The scintigraphic study was performed by using healthy male New Zealand white rabbits (~1.0–1.5 kg weight). To introduce infection, 300 μL saline solution of *E. coli* having 1×10^8 CFU was injected into the right thigh flank muscles, while 300 μL saline was injected in the left thigh flank muscles as control. After 30 h, visible swelling appeared in the infected thigh muscles, whereas minute swelling also appeared in negative control thigh muscles. The rabbits were then anesthetized by IV injection of diazepam followed by placing the rabbit under a dual-headed SPECT gamma camera (connected to an on-line dedicated computer system) by stretching the fore and rear legs out with polythene tape. Then, ^{99m}Tc-SDZ (185 MBq) was administrated through the rear ear vein of the rabbit and scintigraphic images were taken at 30 and 60 min.

Glomerular Filtration Rate Study

Glomerular filtration rate study was performed following the previously published protocol [12]. On the day of GFR study, the *E. coli*-infected rabbits were kept at fasting for about 6 h, with the exception of water intake to increase the urine flow. Then, 1 mL of ^{99m}Tc-SDZ was injected. Filtration rate through the kidney and collection of the urine activity in the urinary bladder was measured continuously in the form of GFR data using a dual-headed SPECT gamma camera connected to GFR software-installed computer system.

Results and Discussion

Reduced ^{99m}Tc can make complex with electron donor ligands. Fluoroquinolones and cephalosporin have certain electron donor groups which make complex with ^{99m}Tc. In all proposed structures of ^{99m}Tc-flouroquinolones, ^{99m}Tc uses two-electron donor oxygen atoms, i.e., carboxylic acid oxygen and carbonyl oxygen for complex formation [13]. However, other electron donor groups such as –S–, –NH–, $–NH_2$, and –COOH make coordinate covalent bond with ^{99m}Tc [6, 14, 15]. Sulfadiazine (Fig. 1) has =N–, –NH–, $–NH_2$, and $–SO_2–$ electron donor groups which facilitate stable complex chemistry of ^{99m}Tc-SDZ.

^{99m}Tc-SDZ purity, stability, formation of colloid, and free ^{99m}Tc were assessed by PC and ITLC methods. In paper chromatography using acetone as a mobile phase, the free $^{99m}TcO_4^{-1}$ migrated along with solvent front ($R_f = 1$), while ^{99m}Tc-SDZ and reduced ^{99m}Tc remained at the point of spotting ($R_f = 0$) (Fig. 2a). Using ITLC with saline solvent system, the ^{99m}Tc-SDZ

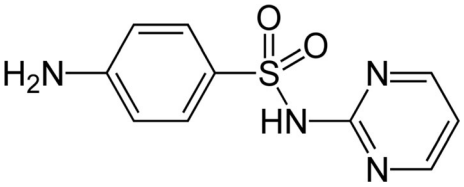

Fig. 1 Structure of sulfadiazine antibiotic

and free $^{99m}TcO_4^{-1}$ moved to the solvent front ($R_f = 1$), while the reduced ^{99m}Tc remained at spotting point ($R_f = 0$) (Fig. 2b). The both radiochromatograms were analyzed with radiometric 2π-scanner and well-type NaI scintillation gamma counter.

The maximum labeling yield (~98%) was achieved by reducing185 MBq $NaTcO_4$ with 120 μg stannous tartrate in the presence of 1.5 mg sulfadiazine and 4 μL (8.3 mg/mL) of gentistic acid at pH 5. The labeling reaction was carried out for 45 min at room temperature.

Effect of Labeling Parameters on Labeling Yield

The labeling yield of the ^{99m}Tc-SDZ was assessed by varying the labeling conditions; initially through hit-and-trail ways then after obtaining some pattern, the labeling was assessed through a regular variation in parameters.

Effect of Sulfadiazine Amount

It is noticed that 1.5 mg SDZ is stoichiometrically equal to 185 MBq $^{99m}TcO_4^-$ because at these concentrations, obtained maximum radiochemical and remained almost constant by subsequent increase in SDZ concentration (Fig. 3). By using 0.5 or 1 mg SDZ, the labeling yield remained below the maximum value. However, free and colloid impurities appeared in

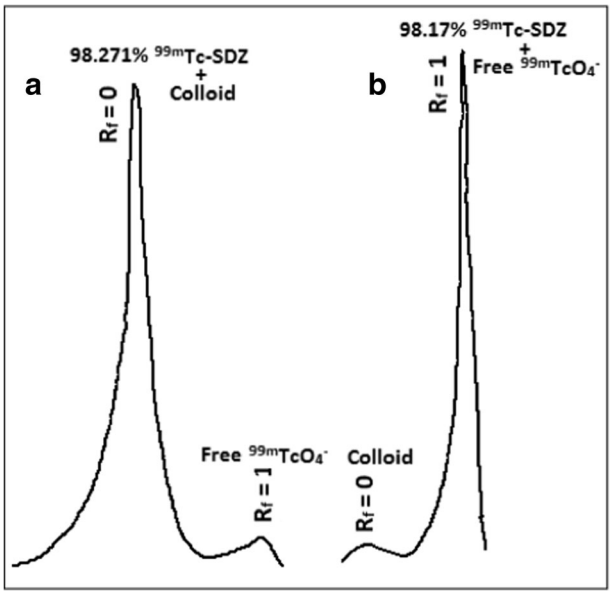

Fig. 2 Radiochromatogram profile of ^{99m}Tc-SDZ reaction mixture with paper chromatography *a*) The ^{99m}Tc-SDZ and colloid remained at radiochemical spotting point ($R_f = 0$) while free $^{99m}TcO_4^-$ migrated along with solvent front ($R_f = 1$), and ITLC *b)* The colloid at radiochemical remained at spotting point ($R_f = 0$) while free $^{99m}TcO_4^-$ and ^{99m}Tc-SDZ migrated along solvent front ($R_f = 1$)

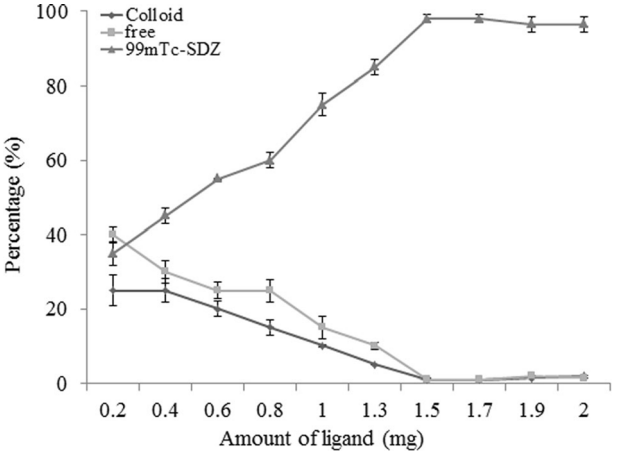

Fig. 3 The effect of sulfadiazine quantity on ^{99m}Tc-SDZ yield; 1.5 mg SDZ along with other optimized labeling parameters resulted in >98% radiochemical yield and <2% colloid and free $^{99m}TcO_4^-$

high percentage because below the stoichiometric amount of SDZ, most of the reduced $^{99m}TcO_4^-$ gets hydrolyzed. A similar interaction between ligand concentration and labeling yield was also reported by other authors [6, 16].

Effect of Reducing Agent Quantity

Optimal quantity of the reducing agent in ^{99m}Tc complex chemistry offers high radiochemical yield. Technetium-99m in its +7 oxidation state does not make a complex. Reduction of $^{99m}TcO_4^-$ offers maximum opportunity of making complex which is carried out with some appropriate reducing agent. The 120 μg of stannous tartrate was found to be optimal quantity to reduce maximum number of $^{99m}TcO_4^-$ ions to lower oxidation state. At 120 μg quantity of reducing agent, 185 MBq $^{99m}TcO_4^-$ provided maximum labeling yield; however, at low quantities of stannous tartrate such as 60, 90, and 110 μg, 45, 25, and 9% free $^{99m}TcO_4^-$ were obtained, respectively (Fig. 4). Similarly, a subsequent increase in stannous tartrate than the optimal quantity resulted in an increased concentration of colloids. The results revealed that to

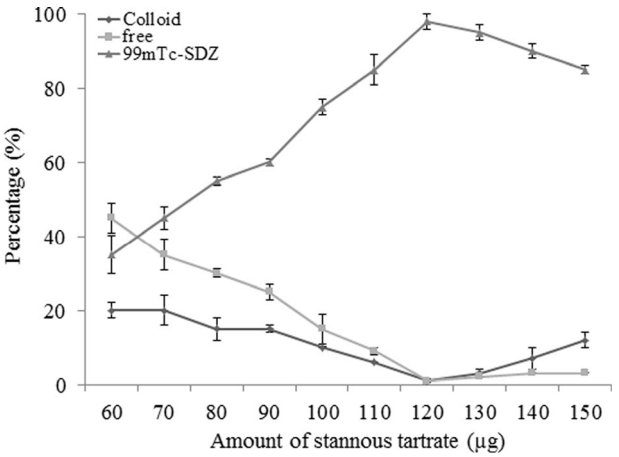

Fig. 4 The effect of stannous tartrate (reducing agent) on ^{99m}Tc-SDZ yield; the graph shows 120 μg of stannous tartrate is optimum to reduce $^{99m}TcO_4^-$ for maximum radiochemical synthesis

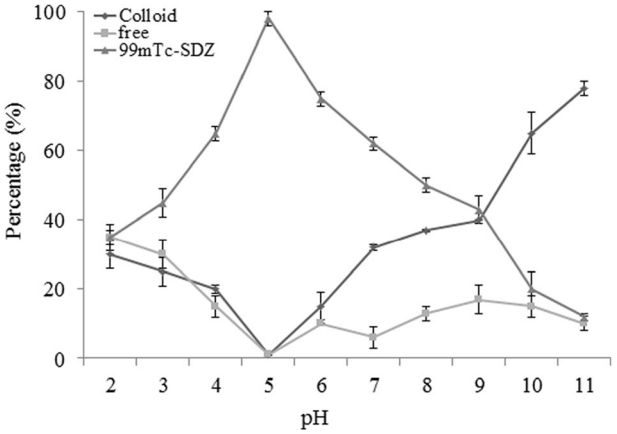

Fig. 5 The effect of pH on ^{99m}Tc-SDZ yield; at pH 5 maximum (>98%) radiochemical yield was obtained

perform maximum suppression of free $^{99m}TcO_4^{-1}$ and colloid formation, the optimal amount of reducing agent should be opted.

Effect of pH

The sensitivity of radiochemical formation at different pH is obvious from Fig. 5. The pH 5 was found to be the optimal value to obtain maximum labeling efficiency (>98%) at 120 μg stannous tartrate, 1.5 mg SDZ, and 185 MBq $^{99m}TcO_4^{-}$. At these conditions, other impurities (free $^{99m}TcO_4^{-}$ and colloids) decreased to less than 2%. Figure 5 shows a sharp increase and decrease in complex formation of ^{99m}Tc-SDZ which indicated the pronounced effect of pH on radiochemical complex formation and also showed the agreement with previously reported data which revealed the dominant effect of pH on ^{99m}Tc-labeling chemistry [6, 7, 16].

Effect of Radiochemical Reaction Time

After addition of all the radiochemical reaction components, the progress of reaction was monitored with an interval of 10 min. At 10 min of reaction, 35% reaction was completed leaving 72% free $^{99m}TcO_4^{-1}$ which increased to 45 and 85% at 20 and 30 min, respectively. At 40 min reaction period, the maximum radiochemical yield (~98%) was obtained (Fig. 6). Further, incubation of radiochemical up to 4 h at room temperature revealed that the complex is sufficiently stable (~97%) and could be safely administrated up to 4 h after its development.

Biodistribution and Scintigraphic Study

Table 1 shows the biodistribution results of ^{99m}Tc-SDZ in infected SD rats. The infected thigh (targeted organ) showed 4.27 ± 0.05%ID/g organ uptake at 1 h post injection, which washed out up to 2.09% at 4 h time point. In contrast to the infected thigh, the normal thigh (non-targeted organ) which was injected with saline as control showed 0.37 ± 0.06% at 30 min and 0.63 ± 0.02% at 1 h post injection. The T/NT ratio at 30 min, 1 h, and 4 h post injection was found 4.49 ± 0.04, 6.78 ± 0.07, and 5.59 ± 0.08, respectively; which is higher than the commercially marketed ^{99m}Tc-ciprofloxacin (infecton®; T/NT = 3.6 ± 0.4) [4]. Previous report on the assessment of ^{99m}Tc-SDZ (development using $SnCl_2{\cdot}2H_2O$ as reducing agent) in

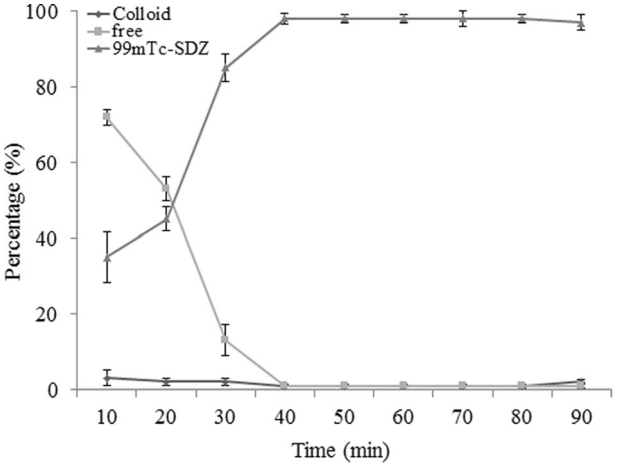

Fig. 6 The effect of reaction time on radiochemical yield; the maximum yield of ^{99m}Tc-SDZ was obtained in 40 min reaction period which remained constant at given conditions up to 90 min

Staphylococcus aureus infection showed T/NT ratio 3.6 [17] which is almost 50% lesser than we noticed in this study by using stannous tartrate as a reducing agent in *E. coli* infection-induced animals. Further, the radiochemical was found more bacterial infection-specific agent in terms of T/NT ratio, as compared to many reported ^{99m}Tc-labeled antibiotics for infection imaging such as ^{99m}Tc-gemifloxacin (T/NT = 3.5 and 1.89) [13, 18], ^{99m}Tc-sulfadimidine (T/NT = 2.25) [19], ^{99m}Tc-cefuroxime (T/NT = 1.8) [20], ^{99m}Tc-ceftriaxone (T/NT = 1.5) [21], ^{99m}Tc-cefotaxime (T/NT = 2.89) [16], ^{99m}Tc-cefprozil (T/NT = 5.5) [22], ^{99m}Tc-clindamycine (T/NT = 3.1) [23], and ^{99m}Tc-azithromycin (T/NT = 6.20) [24]. The nature of radiochemical may pose the accumulation effect; least accumulation in the normal muscles might be due to its low lipophilicity. Other non-targeted organs (the liver, heart, lungs, spleen, and kidneys) showed uptake of activity at 30 min post injection time point however declined to minimal quantity at 4 h post injection time point. The kidney showed the appreciable activity (2.64 ± 0.04%) at 30 min post injection and almost maintained up to 1 h post injection, which later on decline to 1.85 ± 0.11% at 4 h post injection. The continuous renal filtration of

Table 1 Organ biodistribution of ^{99m}Tc-sulfadiazine in *E. coli*-infected rat model representing percent injected dose per gram body organ (%ID/g organ)

Organs	%ID/g organ		
	30 min	1 h	4 h
Left kidney	2.64 ± 0.04	2.61 ± 0.12	1.85 ± 0.11
Right kidney	2.48 ± 0.07	1.58 ± 0.08	1.51 ± 0.08
Heart	3.32 ± 0.12	2.11 ± 0.17	2.46 ± 0.14
Spleen	0.74 ± 0.09	0.29 ± 0.09	0.25 ± 0.04
Liver	8.47 ± 0.89	3.02 ± 0.33	1.32 ± 0.09
Lung	0.73 ± 0.07	0.75 ± 0.08	0.44 ± 0.05
Urinary bladder	11.46 ± 2.56	6.35 ± 1.87	4.82 ± 1.54
Normal thigh muscles	0.37 ± 0.06	0.63 ± 0.02	0.39 ± 0.04
Infected thigh muscles	1.66 ± 0.03	4.27 ± 0.05	2.18 ± 0.02
T/NT	4.49 ± 0.04	6.78 ± 0.07	5.59 ± 0.08

T/NT= target to non-target ratio: the values are expressed as an average of triplicate measurements ($n = 3$) ± S.D.

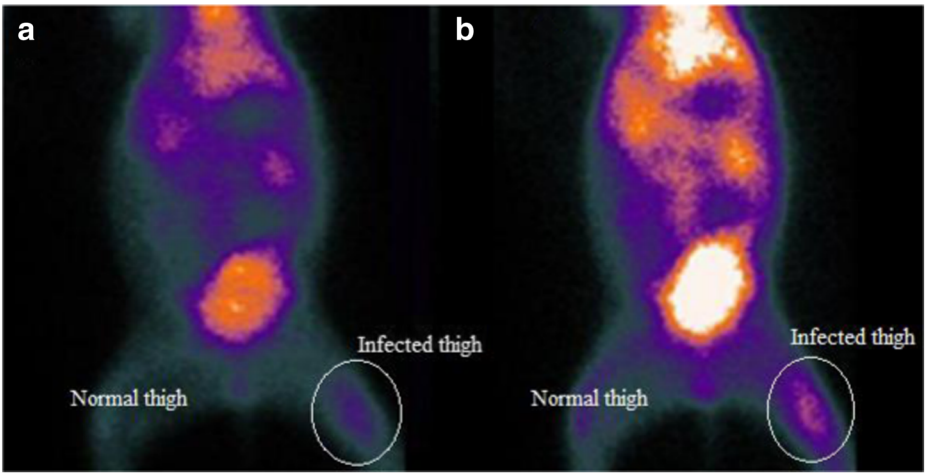

Fig. 7 Scintigraphy of *E. coli* infection-induced rabbit models at 30 min (*a*) and 1 h (*b*) post injection. *a* Visible uptake of ^{99m}Tc-SDZ but saline-injected thigh muscles (normal muscles) showing no uptake of activity. *b* Clear uptake in promising quantity and normal muscles showing minute uptake (T/NT ratio 6.78)

radiochemical indicated the absence of glomerular reabsorption which consequently omitted the chance of nephrotoxicity. The higher uptake of activity at 30 min post injection in the liver is due to the metabolic pathway of drug; while blood pool circulation through the heart was the cause of accumulation in the heart. The typical scintigrams of the rabbit with *E. coli*-infected thigh muscle at 30 min and 1 h post injection are shown in Fig. 7. The scintigram demonstrates mild uptake of activity in infected muscles at 30 min post injection which increased to significant quantity at 1 h time point. However, the non-infected thigh muscle remained cold. The trend of the activity uptake in the infected muscle and other organs were found to be almost similar to that of biodistribution data.

Glomerular Filtration Rate Study

Glomerular filtration rate study of ^{99m}Tc-SDZ revealed the maximum blood pool concentration was obtained within 1 min and 50% of the injected ^{99m}Tc-SDZ radiopharmaceutical excreted within 7 min as shown in Fig. 8. Both kidneys showed (blue and green lines) rapid decay toward horizontal axis as a consequence of renal filtration of ^{99m}Tc-SDZ which resulted in

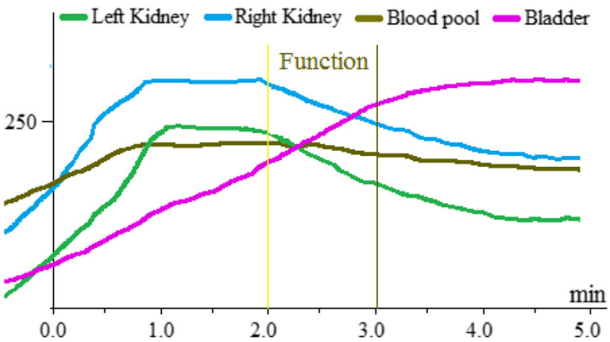

Fig. 8 The peak blood concentration within 1 min and renal radiochemical peak concentration at 2 min which become constant after gradual decrease up to 5 min. The bladder line shows the gradual increase of radiochemical in bladder

continuous increase in the bladder activity as indicated by bladder activity line in Fig. 8. Comparing with the gold standard GFR agent (^{99m}Tc-DTPA) which takes 11.4 min for 50% renal excretion and does not show nephrotoxicity, we argue the non-radionephrotoxicity of ^{99m}Tc-SDZ [25].

Conclusion

In this work, direct labeling of sulfadiazine with technetium-99m was carried out in the presence of stannous tartrate as reducing agent. The maximum radiochemical yield was obtained in a 40-min reaction at room temperature by subsequent addition of 185 MBq $^{99m}TcO_4^-$ freshly eluted from ^{99}Mo/^{99m}Tc generator, 120 μg stannous tartrate, 1.5 mg SDZ, 4 μL (8.3 mg/mL) of gentistic acid, and 0.1 N NaOH/HCl to achieve reaction pH 5. The T/NT ratio at 1 h post injection in *E. coli* infection-induced animal model was found 6.78 ± 0.07, which is higher than the commercially marketed infection imaging agent ^{99m}Tc-ciprofloxacin (infecton®, T/NT = 3.6 ± 0.4) [4] and also 50% higher than ^{99m}Tc-SDZ investigated for *S. aureus* infection imaging (T/NT = 3.6) [17]. On the basis of all these facts and the promising GFR value, the newly developed ^{99m}Tc-SDZ could be a suitable candidate for selective *E. coli* infection imaging in nuclear medicine technique.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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