

# Synthesis, biological evaluation and biodistribution of the $^{99m}\text{Tc}$ –Garenoxacin complex in artificially infected rats

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Received: 20 October 2010 / Published online: 12 December 2010  
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**Abstract** The labeling of garenoxacin (GXN) with technetium-99m ( $^{99m}\text{Tc}$ ) using different concentrations of GXN, sodium pertechnetate ( $\text{Na}^{99m}\text{TcO}_4$ ), stannous chloride dihydrate ( $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ ) at different pH was investigated and evaluated in terms of in-vitro stability in saline, serum, binding with multi-resistant *Staphylococcus aureus* (MDRSA) and penicillin-resistant *Streptococci* (PRSC) and its biodistribution in artificially MDRSA and PRSC infected rats.  $^{99m}\text{Tc}$ –GXN complex with  $97.45 \pm 0.18\%$  radiochemical stability was prepared by mixing 3 mg of GXN with 3 mCi of  $\text{Na}^{99m}\text{TcO}_4$  in the presence of 150  $\mu\text{L}$  of  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  (1  $\mu\text{g}/\mu\text{L}$  in 0.01 N HCl) at a pH 5.6. The radiochemical stability of the complex was evaluated in normal saline up to 240 min of reconstitution. It was observed that the complex showed maximum RCP values after 30 min of the reconstitution and remained more than 90% up to 240 min. The complex showed radiochemical stability in normal saline at 37 °C up to 16 h with a 17.80% de-tagging. The complex showed saturated in-vitro binding with living MDRSA and PRSC as compared to the insignificant binding with heat killed MDRSA and PRSC. Biodistribution behavior of the complex was assessed in artificially infected with living and heat killed MDRSA and PRSC rats. It was observed that

the accumulation of the complex in the infected (live MDRSA and PRSC) tissue of the rats was almost five fold than in the inflamed and normal tissue. The high radiochemical stability in normal saline at room temperature, promising in-vitro stability in serum at 37 °C, saturated in-vitro binding with living MDRSA and PRSC, specific biodistribution behavior and high infected (target) to normal (non-target) tissue and low inflamed (non-target) to normal (non-target) tissue ratios we recommend  $^{99m}\text{Tc}$ –GXN complex for in-vivo localization of infection caused by MDRSA and PRSC effective stains.

**Keywords**  $^{99m}\text{Tc}$ –Garenoxacin complex · Biodistribution · MDRSA · PRSC · Infection

## Introduction

In suspected infectious and non-infectious inflammatory processes the role of Nuclear Medicine Scintigraphy (NMS) is promising and the diagnosis of infectious diseases in early phase remains prominent in clinical practices. The advanced diagnostic techniques like Ultrasonography (US), Computerized Tomography (CT) and Magnetic Resonance Imaging (MRI) provides the diagnostic information with high sensitivity but failed to differentiate infection from inflammation [1, 2].

Based on the promising results of the existing radiopharmaceuticals [3–15] and our recently reported  $\gamma$ -emitting  $^{99m}\text{Tc}$  labeled antibiotics [16–24] intended for infection localization and its discrimination from inflammation, buoyant us to seek more sensitive and specific infection radiotracers.

Garenoxacin (GXN) (Fig. 1a) (1-cyclopropyl-8-(difluoromethoxy)-7-[(1R)-1-methyl-2,3-dihydro-1H-isindol-5-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid) a newly developed quinolone having no fluorine atom at C-6 position

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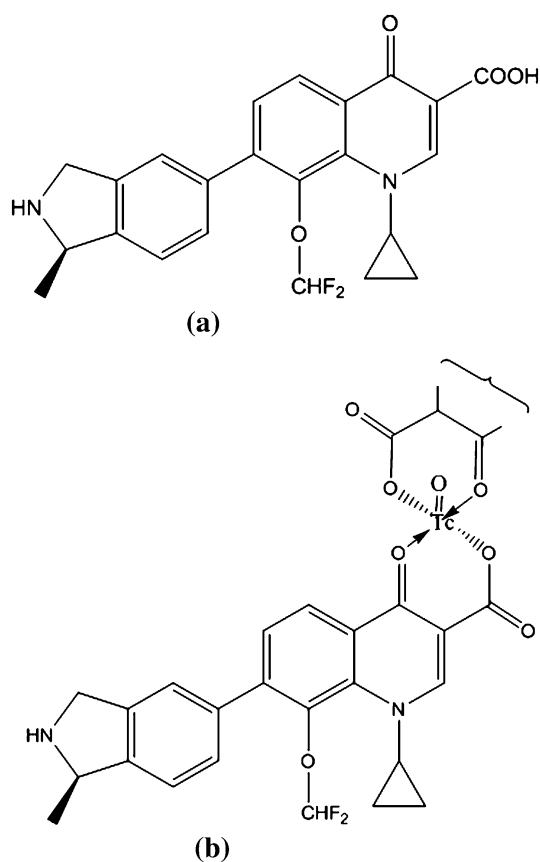
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**Fig. 1** **a** Chemical structure of the Garenoxacin (GXN). **b** Proposed structure of the  $^{99m}\text{Tc}$ -GXN complex

like other fluoroquinolones but having a difluoromethoxy group at C-8 position. Such structural changes enhanced the potency of the GXN against DNA gyrase and topoisomerases. GXN has shown improved potency against Gram positive and Gram negative pathogens including methicillin-resistant *Staphylococci* (MDRSA) and penicillin-resistant *Streptococci* (PRSC) [25, 26].

To utilize the enhanced potency of the GXN for localization of infection caused by MDRSA and PRSC in the current investigation, radiolabeling of the GXN with  $^{99m}\text{Tc}$  was assessed. The efficacy of the labeled GXN was evaluated in terms of radiochemical stability in saline at room temperature, in-vitro stability in serum at 37 °C, in-vitro binding with living and heat killed MDRSA and PRSC and in-vivo biodistribution in rats artificially infected with living and heat killed MDRSA and PRSC.

## Experimental

### Materials

Garenoxacin (GXN) (Bristol-Myers Squibb, Syracuse, NY, United Kingdom, TLC (Merck) and all the other chemicals

and solvents of analytical grade (Sigma). RP-HPLC (Shimadzu, Japan) well counter and scalar count rate meter (Ludlum, USA) Dose calibrator (Capintech, USA) and Gamma camera GKS-1000 (GEADE Nuclearmedicine system, Germany).

### Methods

#### Synthesis of the $^{99m}\text{Tc}$ -Garenoxacin complex

The radiocomplex of technetium-99m ( $^{99m}\text{Tc}$ ) and garenoxacin (GXN) was prepared by mixing stannous chloride 25–250  $\mu\text{L}$  (with 25  $\mu\text{L}$ :  $\mu\text{g}/\mu\text{L}$  0.01 N HCL) sodium pertechnetate 0.5–5.0 mCi (with 0.5 mCi increment) in 10 separate sterilized vials. The pH of the vials was set between 5.1 to 6.0 (with 0.1 unit ascend). Thereafter, the reaction mixtures were swirled for 5 min and 0.5–5 mg (with 0.5 mg augment) GXN was added to the vials followed by incubation at 25 °C for 30 min. The final reaction mixture was filtered through Millipore filter.

#### HPLC analysis of the $^{99m}\text{Tc}$ -Garenoxacin complex

Radiochemically the  $^{99m}\text{Tc}$ -GXN complex was characterized by using the HPLC. SCL-10 AVP, Shimadzu HPLC equipped with SDP-10 AVP, UV detector operating at 254 nm, Packard 500 TR series flow scintillation analyzer, binary pump, using online degasser. The flow rate 1 mL/min was sustained for 15 min using 25 mmol/L triethylammonophosphate (2.25 pH buffer) (TEAP) and a methyl alcohol (MetA) as the mobile phases. For 0–3 min (100% TEAP), 3–6 min (100–75% TEAP), 6–8 min (75–66% TEAP), 8–10 min (34–100% MetA), 10–12 min (100% MetA) and 12–15 min (100% MetA to 100% TEAP).

#### Stability of the $^{99m}\text{Tc}$ -Garenoxacin complex in saline

Thin layer chromatography (TLC) was used for determination of the radiochemical stability of the  $^{99m}\text{Tc}$ -GXN complex in saline using two mobile phases (acetone and ethanol:water:ammonia (2:5:1)). Aliquots, 1  $\mu\text{L}$  of the  $^{99m}\text{Tc}$ -GXN complex at 30, 60, 90, 120 and 240 min after reconstitution were spotted on the separate TLC strip (stationary phase) and developed in acetone (mobile phase). The same process was repeated using ethanol:water:ammonia (2:5:1) as the mobile phase. Thereafter, the developed strips were divided into two equal parts at  $R_f$  5 and counted for activity using single well gamma ( $\gamma$ ) rays detecting counter interface with scalar count rate meter. The RCP values of the  $^{99m}\text{Tc}$ -GXN was compared with the  $^{99m}\text{Tc}(\text{CO})_3$ -GXND and  $^{99m}\text{TcN}$ -GXND complex [22, 24].

### In-vitro stability of the $^{99m}\text{Tc}$ -Garenoxacin complex in serum

In serum at 37 °C the in-vitro stability of the  $^{99m}\text{Tc}$ -GXN complex was investigated using the reported method [22]. Briefly,  $^{99m}\text{Tc}$ -GXN, 0.2 mL was incubated with 1.8 mL of serum at 37 °C. Thereafter, 1  $\mu\text{L}$  aliquots of the mixture at 2, 4, 6, 8, 10, 12, 14 and 16 h of incubation were dappled on the TLC strips and after drying developed in  $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$  (9:1) (v/v) and counted for percent immovability using single well  $\gamma$ -rays detecting counter connected with scalar count rate meter. Further, the in-vitro permanence of the  $^{99m}\text{Tc}$ -GXN was ensured in a challenge assay by incubating equimolar  $^{99m}\text{Tc}$ -GXN with the increasing amount of cysteine ( $1 \times 10^7$  mol/L) followed by calculation of the stability intervals of incubation. The in-vitro stability of the  $^{99m}\text{Tc}$ -GXN in serum was compared with that of the  $^{99m}\text{Tc}(\text{CO})_3$ -GXND and  $^{99m}\text{TcN}$ -GXND complex [22, 24].

### In-vitro bacterial binding

In-vitro binding of the  $^{99m}\text{Tc}$ -GXN complex with the living and heat killed strains of multiresistant *Staphylococcus aureus* (MDRSA) and penicillin-resistant *Streptococci* (PRSC) was studied using the reported method [27]. Briefly, 0.1 mL of sodium phosphate buffer (Na-PB) was taken in a clean test tube followed by addition of 0.2 mL (10 MBq) of the  $^{99m}\text{Tc}$ -GXN complex. Subsequently, 0.8 mL (50%, v/v) 0.01 M acetic acid including  $1 \times 10^8$  colony forming units (CFU) of MDRSA was added trailed by incubation at 4 °C for 1 h with a closing pH 5. The mixture was centrifuged for 10 min (2,000 rpm) and discarded the supernatant. The pellets were resuspended in Na-PB (2 mL) and re-centrifuged for 10 min (2,000 rpm). The percent uptake was determined using single well  $\gamma$ -rays detecting counter connected with scalar count rate meter. Identical procedure was repeated for the determination of percent uptake by PRSC.

### Biodistribution in animal model rat

The percent in-vivo absorption of the  $^{99m}\text{Tc}$ -GXN complex in blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle of the rats artificially infected with living and heat killed MDRSA and PRSC. Healthy twenty male Sprague-Dawley rats (weight, 180–220 g) were selected and grouped into four groups of five rats in each group (A, B, C and D). Intramuscularly (I.M.) 0.2 mL sterile turpentine oil was injected to the left thigh of all the rats followed by 0.2 mL ( $1 \times 10^8$  CFU approx.) each of the living MDRSA to group A and heat killed MDRSA to group B. Likewise, 0.2 mL in normal saline containing approximately  $1 \times 10^8$  CFU of PRSC

each of living and heat killed were I.M. injected to the right thighs of the group C and D rats. Next, 18.5 MBq (0.5 mL) of the  $^{99m}\text{Tc}$ -GXN complex was intravenously (I.V.) administered to all the rats after 24 h. Thereafter the rats were killed in accordance with the approved rules and regulations of the Nuclear Medicine Research Laboratory (NMRL) University of Peshawar. Percent (%) absorption of  $^{99m}\text{Tc}$ -GXN complex in different organs of the group A, B, C and D rats model were was calculated using single well  $\gamma$ -rays detecting counter connected with scalar count rate meter.

## Results and discussion

### Radiochemical purity (RCP) and HPLC characterization

Figure 1a garenoxacin (GXN) was labeled with  $^{99m}\text{Tc}$  using different concentration of the GXN (ligand), sodium pertechnetate (source of radioactive metal) and stannous chloride (as reducing agent) for the reduction of  $^{99m}\text{Tc}$  at different pH. A stable  $^{99m}\text{Tc}$ -GXN radiocomplex (Fig. 1b) with  $97.45 \pm 0.18\%$  RCP yield (maximum) was observed at 30 min after reconstitution by mixing 3 mg of GXN with 3 mCi of  $\text{Na}^{99m}\text{TcO}_4$  in the presence of 150  $\mu\text{L}$  of  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  (1  $\mu\text{g}/\mu\text{L}$  in 0.01 N HCl) at a pH 5.6. The values of the RCP yield (Fig. 2) decreased up to  $90.25 \pm 0.18\%$  within 4 h after reconstitution. The  $^{99m}\text{Tc}$ -GXN  $^{99m}\text{TcN}$ -GXND and  $^{99m}\text{Tc}(\text{CO})_3$ -GXND radiocomplexes showed almost similar RCP values as shown in Fig. 2.

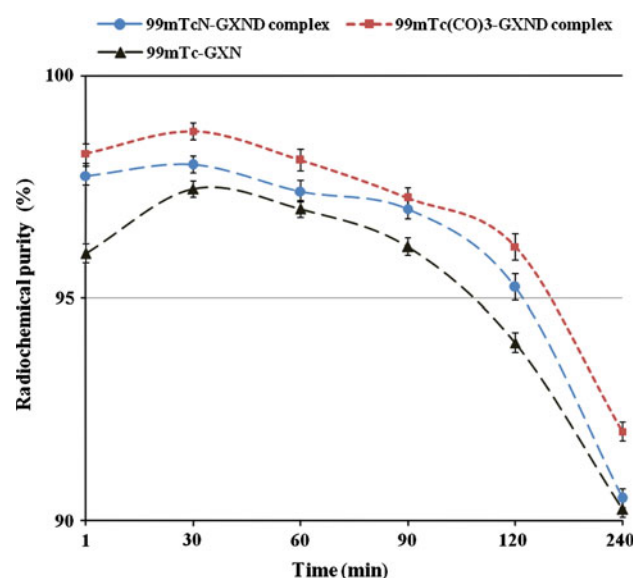


Fig. 2 Radiochemical stability of the  $^{99m}\text{Tc}$ -GXN complex in saline

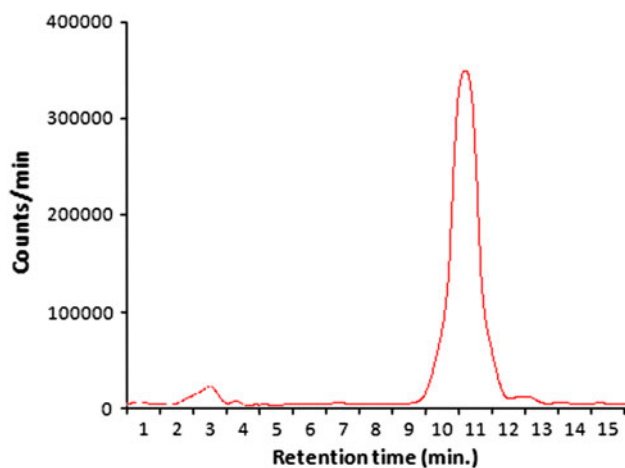


Fig. 3 HPLC radiochromatogram of the  $^{99m}\text{Tc}$ -GXN complex

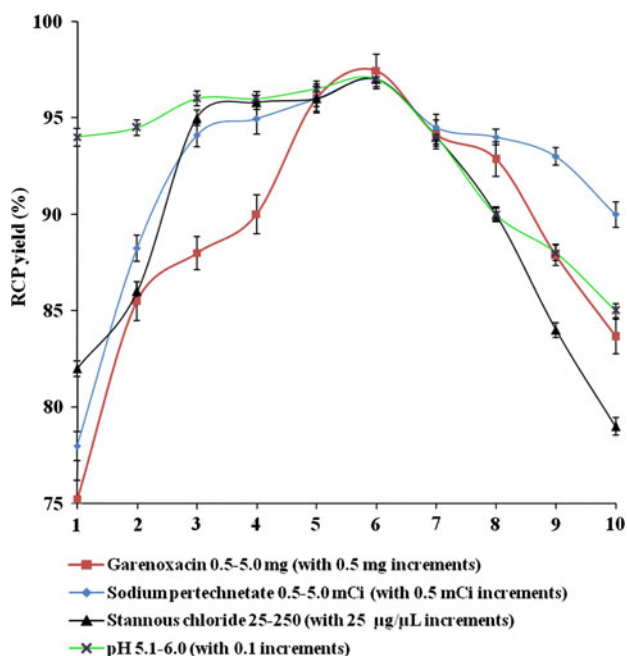


Fig. 4 The effect of GXN, sodium pertechnetate, stannous chloride amount and pH on the RCP values of the  $^{99m}\text{Tc}$ -GXN complex

Conspicuously two different HPLC peaks were observed at 2.8 and 10.2 min of retention as shown in Fig. 3. The radiopeak at 2.8 min corresponds to the amount of free technetium- $^{99m}\text{TcO}_4^-$  and that at 10.2 min the amount of  $^{99m}\text{Tc}$ -GXN complex.

The proposed structure of the  $^{99m}\text{Tc}$ -GXN radiocomplex (Fig. 1b) will have a square planar pyramidal geometry, 1:2 stoichiometry of Tc:Lig with the bidentate GXN.

The effect of GXN, sodium pertechnetate, stannous chloride amount and pH on the radiochemical stability of the  $^{99m}\text{Tc}$ -GXN complex is shown in Fig. 4. It was

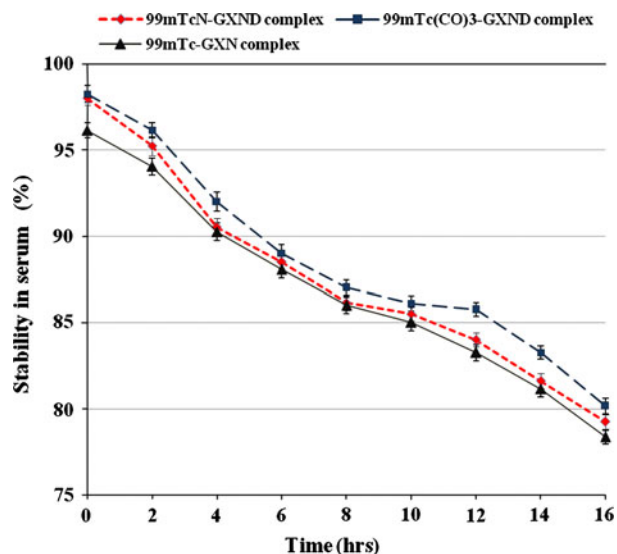


Fig. 5 In-vitro radiochemical stability of the  $^{99m}\text{Tc}$ -GXN in serum

observed that the complex showed highest RCP values using 3 mg GXN, 3 mCi sodium pertechnetate, 150  $\mu\text{L}$  of  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  (1  $\mu\text{g}/\mu\text{L}$  in 0.01 N HCl) at a pH 5.6. The amount of GXN, sodium pertechnetate, stannous chloride and pH lower or higher than the optimum amount substantially decreased the values of RCP.

#### In-vitro stability in serum

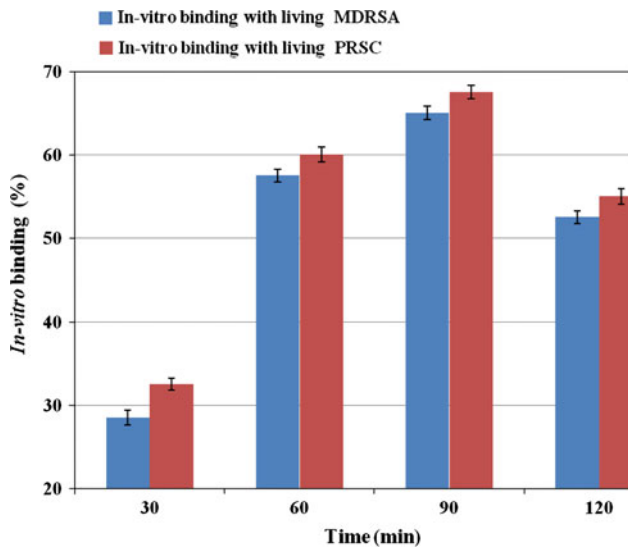
Figure 5 gives the radiochemical stability profile of the  $^{99m}\text{Tc}$ -GXN complex. At 37  $^\circ\text{C}$  the  $^{99m}\text{Tc}$ -GXN complex showed in-vitro stability in serum up to 16 h. The free radiolabeled  $^{99m}\text{Tc}$  part observed during the incubation was 17.80%. The  $^{99m}\text{Tc}$ -GXN  $^{99m}\text{TcN}$ -GXND and  $^{99m}\text{Tc}(\text{CO})_3$ -GXND radiocomplexes showed almost similar in-vitro stability results.

#### In-vitro binding with MDRSA and PRSC

The  $^{99m}\text{Tc}$ -GXN complex showed comparable saturated in-vitro binding with living strains of MDRSA and PRSC as shown in Fig. 6. In comparison, the MDRSA showed higher binding affinity than PRSC.

#### Biodistribution of $^{99m}\text{Tc}$ -GXN complex in infected rats

The percent in-vivo absorption of the  $^{99m}\text{Tc}$ -GXN complex in blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle of the rats artificially infected with living and heat killed MDRSA and PRSC are given in Tables 1 and 2. In group A rats (infected with living MDRSA) the activity in blood after 30 min of I.V.



**Fig. 6** In-vitro binding of the <sup>99m</sup>Tc–GXN complex with MDRSA and PRSC

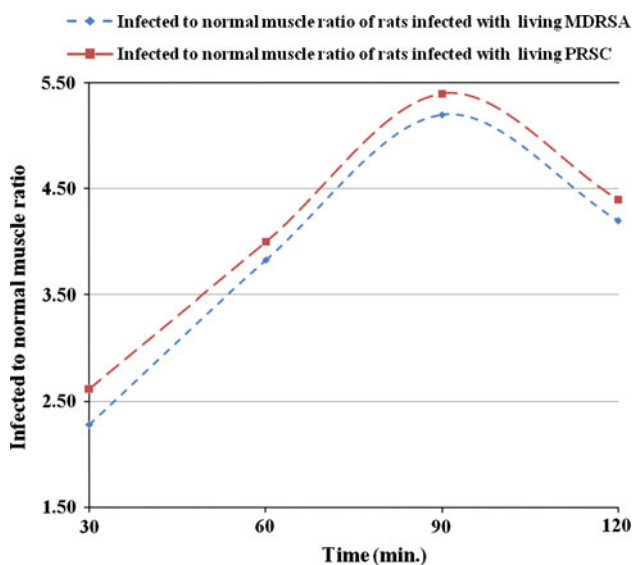
administration was high which reduced to  $5.15 \pm 0.19\%$  within 120 min from  $24.00 \pm 0.16\%$ . Similar trailing patron of activity in blood was noted in group **B** (infected with heat killed MDRSA), **C** (infected with living PRSC) and **D** (infected with heat killed MDRSA) rats. The similar uptake profile of the <sup>99m</sup>Tc–GXN complex was observed in liver, spleen, stomach and intestine, where the uptake of the tracer went down with time. Whereas, a reciprocal behavior was noted in kidneys, wherein the uptake gradually went up. The desertion of activity from the blood circulatory system and appearance in the urinary system established the usual course of excretion. Further, almost five times higher absorption of the <sup>99m</sup>Tc–GXN was observed in the infected muscle of the rats infected with living MDRSA and PRSC strain as compared to the inflamed and normal muscles as shown in Fig. 7. The rats infected with heat killed MDRSA and PRSC strains showed almost similar insignificant absorption.

**Table 1** Percent in-vivo absorption of the <sup>99m</sup>Tc–GXN radiocomplex in blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle of the rats artificially infected with living and heat killed multiresistant *S. aureus* (MDRSA)

Organs/tissues (g)	In-vivo absorption of the <sup>99m</sup> Tc–GXN at different intervals (min)							
	Group A (living MDRSA)				Group B (heat killed MDRSA)			
	30	60	90	120	30	60	90	120
Infected muscle	5.70 ± 0.18	11.50 ± 0.15	13.00 ± 0.16	10.50 ± 0.15	3.00 ± 0.16	3.50 ± 0.18	3.00 ± 0.15	3.00 ± 0.15
Inflamed muscle	3.50 ± 0.15	4.00 ± 0.16	3.50 ± 0.18	3.00 ± 0.18	3.50 ± 0.18	4.00 ± 0.15	3.50 ± 0.14	3.00 ± 0.19
Normal muscle	2.50 ± 0.18	3.00 ± 0.18	2.50 ± 0.15	2.50 ± 0.17	2.50 ± 0.19	3.00 ± 0.19	2.50 ± 0.18	2.50 ± 0.16
Blood	24.00 ± 0.16	10.50 ± 0.15	8.25 ± 0.18	5.15 ± 0.19	19.00 ± 0.15	10.55 ± 0.19	8.25 ± 0.18	4.15 ± 0.16
Liver	18.00 ± 0.18	13.25 ± 0.19	10.00 ± 0.15	6.15 ± 0.17	18.50 ± 0.18	13.25 ± 0.15	8.50 ± 0.17	4.00 ± 0.16
Spleen	9.55 ± 0.18	8.15 ± 0.15	7.00 ± 0.17	4.85 ± 0.15	9.00 ± 0.15	7.15 ± 0.16	5.45 ± 0.18	3.85 ± 0.18
Kidney	8.25 ± 0.19	21.00 ± 0.17	22.55 ± 0.17	25.10 ± 0.16	9.50 ± 0.17	18.25 ± 0.18	20.50 ± 0.15	24.00 ± 0.17
Stomach and intestines	9.00 ± 0.15	8.10 ± 0.18	6.85 ± 0.19	4.15 ± 0.16	9.00 ± 0.19	8.15 ± 0.18	6.00 ± 0.15	3.85 ± 0.17

**Table 2** Percent in-vivo absorption of the <sup>99m</sup>Tc–GXN radiocomplex in blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle of the rats artificially infected with living and heat killed with penicillin-resistant *Streptococci* (PRSC)

Organs/tissues (g)	In-vivo absorption of the <sup>99m</sup> Tc–GXN at different intervals (min)							
	Group C (living PRSC)				Group D (heat killed PRSC)			
	30	60	90	120	30	60	90	120
Infected muscle	6.55 ± 0.14	12.00 ± 0.18	13.50 ± 0.16	11.00 ± 0.19	3.00 ± 0.19	3.50 ± 0.17	3.00 ± 0.17	3.00 ± 0.18
Inflamed muscle	3.00 ± 0.18	3.50 ± 0.16	3.50 ± 0.19	3.00 ± 0.15	3.00 ± 0.18	3.50 ± 0.15	3.50 ± 0.18	3.00 ± 0.16
Normal muscle	2.50 ± 0.16	3.00 ± 0.15	2.50 ± 0.18	2.50 ± 0.15	2.50 ± 0.18	3.00 ± 0.14	2.50 ± 0.15	2.50 ± 0.16
Blood	20.00 ± 0.15	11.15 ± 0.17	9.25 ± 0.18	5.50 ± 0.15	19.50 ± 0.15	12.00 ± 0.16	9.55 ± 0.18	5.25 ± 0.19
Liver	17.25 ± 0.15	12.45 ± 0.16	10.35 ± 0.18	6.00 ± 0.14	17.55 ± 0.15	13.00 ± 0.18	9.50 ± 0.16	5.85 ± 0.14
Spleen	9.00 ± 0.18	7.90 ± 0.15	6.75 ± 0.14	5.10 ± 0.16	9.10 ± 0.16	7.55 ± 0.18	6.00 ± 0.14	4.85 ± 0.17
Kidney	9.10 ± 0.16	20.55 ± 0.15	23.00 ± 0.18	24.25 ± 0.19	9.25 ± 0.14	18.50 ± 0.18	20.80 ± 0.15	22.95 ± 0.16
Stomach and intestines	8.90 ± 0.14	8.00 ± 0.15	6.75 ± 0.19	4.00 ± 0.18	9.10 ± 0.16	8.25 ± 0.15	5.95 ± 0.14	3.90 ± 0.18



**Fig. 7** Infected to normal muscle of rats infected with living MDRSA and PRSC

## Conclusion

Radiochemically a stable complex was achieved by mixing 3 mg of GXN with 3 mCi of  $\text{Na}^{99\text{m}}\text{TcO}_4$  in the presence of 150  $\mu\text{L}$  of  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  (1  $\mu\text{g}/\mu\text{L}$  in 0.01 N HCl) at a pH 5.6. The complex showed in-vitro stability in serum, saturated in-vitro binding with MDRSA and PRSC and normal biodistribution with five fold uptake in the infected muscle as compared to inflamed and normal muscle. The high stability in saline and serum, saturated in-vitro binding with MDRSA and PRSC stains and the biodistribution posed the  $^{99\text{m}}\text{Tc}$ -GXN complex as new promise in infection localization.

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