Chapter 9

IN VIVO BIODISTRIBUTION STUDY

9.1 Introduction

In vivo biodistribution study is a method of tracking the compound as it travels through the body of animal or human subject. Improving drug efficacy and limiting potential side effects are cardinal steps in the development of new pharmaceutical compounds and/or formulations. In vivo biodistribution studies provide pivotal information about accumulation of compounds and/or formulations in various organs or tumors. This information helps to optimize drug formulations and improve biodistribution properties of compounds. [1]

Biodistribution studies involve the estimation of drug in various tissues and organs at different time points post administration of dosage form. It can be done by sacrificing the animal, isolating the different organs and determining the concentration of drug in each organ/tissue. This method is invasive and requires sacrificing large number of animals for obtaining statistically pertinent results. Moreover, sophisticated instruments like high-performance liquid chromatography (HPLC), Enzyme-linked immunosorbent assay (ELISA) etc, which can measure very small tissue concentrations are also required. [2] However, the most economic, rapid and non-invasive method of biodistribution study is Imaging Technology. Among the different imaging techniques like PET, MRI, CT-Scan etc., Gamma Scintigraphy is widely accepted method as it is inherently quantitative. [3] In addition, Gamma Scintigraphy is a computer-based system attached to the software to perform a variety of analysis options. The most common analysis tool is region-of-interest analysis, which determines the number of counts in a designated area. [2, 4] Scintigraphic imaging employs a gamma-emitting radionuclide label to track and quantitate the distribution of formulation in the body. It has ability to image the total organism in a single whole body scan that makes it superior to other techniques.

Among all the radioisotopes, "Technetium-99m" (^{99m}Tc) is most commonly used radionuclide for the biodistribution studies. Radioactive Technetium has proved to be appropriate for labeling both large and small biomolecules, proteins and peptides as well as formulations. Technetium-99m is a short-lived metastable nuclear isomer produced from molybdenum 99. ^{99m}Tc has been widely used as a radiotracer in nuclear medicine

and in biomedical research to label molecular and cellular structures as well as studying the biodistribution properties of the drug and/or delivery systems due to its many desirable characteristics: it emits a 140 -keV gamma ray with 89 % abundance, which is suitable for imaging with gamma cameras. Moreover, due to short physical as well as biological half-life, it causes less exposure to the animal body and the environment leading to its fast clearing from the body after an imaging process. [2, 5]

In ^{99m}Tc labeling of compounds, prior reduction of ^{99m}Tc from +7 state to a lower oxidation state is required. Among various reducing agents, stannous chloride is the most commonly used reducing agent. The use of stannous chloride has a disadvantage in that it also readily undergoes hydrolysis in aqueous solution at approximately pH 6 to 7 and forms insoluble colloids. These colloids bind to reduced ^{99m}Tc and thus compromise the labeling yield. To prevent this colloid formation, an acid is added to prevent the hydrolysis of Sn^{2+} before the reduction of technetium. [6]

9.2 Materials and Equipment

Materials

Diethylene triamine penta acetic acid (DTPA), stannous chloride dihydrate ($SnCl_2.2H_2O$) and Cholesterol were purchased from Sigma Aldrich, India. Sodium pertechnetate separated from Molybdenum-99 by solvent extraction method was procured from Regional center for Radiopharmaceutical division (Northern Region), Board of Radiation and Isotope Technology, Delhi, India. Raloxifene Hydrochloride (RLX) was kindly gifted by Aarti Drugs Ltd. (Mumbai, India). Leuprolide Acetate (LA) was a gift sample from Sun Pharma Advanced Research Centre, Vadodara, India. 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) was purchased from Avanti Polar Lipids Inc. (Alabama, USA). Silicon tubing (medical grade) was purchased from VWR International (Leicestershire, United Kingdom). Silica gel (SG)-coated fiberglass sheet was obtained from Gelman Sciences Inc., Ann Arbor, MI. All other chemicals and solvents were of analytical reagent grade and were used without further purification.

Equipment

- Rotary evaporator (IKA RV10, Karnataka, India)
- Probe Sonicator (Sartorius LABSONIC M, Germany)
- Digital Analytical balance (ATX 224, Shimadzu, Japan)
- pH meter (PICO pH meter, LABINDIA Analytical, Mumbai, India)
- Gamma Counter (Caprac T well counter, Capintec NJ, United States)
- Gamma Camera (Single Photon Emission Computerized Tomography (SPECT, LC 75–005, Diacam, Siemens AG, Earlangan, Germany)

9.3 Radiolabelling of Drugs

9.3.1 Radiolabelling of Raloxifene Hydrochloride

RLX was labeled with ^{99m}Tc by direct labeling method as described by Richardson et al, 1977. [12] Briefly, 1 mL of ^{99m}Tc-pertechnetate (37 MBq/mL) was mixed with 50 µL of stannous chloride solution (1 mg/mL solution in water containing 0.1N HCl). pH was adjusted to 4.5 using 0.1N HCl. This mixture was added to 5 mL of RLX solution (1 mg/mL, in hydro-alcoholic mixture (6:4)) and incubated for 10 to 15 minutes at room temperature. Percentage labeling efficiency and stability of the labeled complexes was then further assessed. [2, 8]

9.3.2 Radiolabelling of Leuprolide acetate

LPA was labeled with ^{99m}Tc-pertechnetate by direct labeling method as described by Richardson et al, 1977 [7] and Patel et al, 2016 [2] with slight modifications. Briefly, 1 mL of ^{99m}Tc-pertechnetate (37 MBq/mL) was reduced with 50 µL of stannous chloride solution (1 mg/mL in distilled water containing 0.1N HCl). pH was adjusted to 7 using sodium bicarbonate solution. This mixture was added to 5mL of LPA solution (1 mg/mL, in distilled water) and incubated for 10 to 15 minutes at room temperature. Percentage labeling efficiency and stability of the radiolabeled complex was then assessed.

9.4 Quality Control Tests

Various in vitro physicochemical tests are essential for the determination of the purity and integrity of a radiopharmaceutical. In order to investigate the in vivo stability of the of the radio-complex, various quality control tests are carried out to establish the optimum conditions required to maintain highest labeling efficiency and purity.

9.4.1 pH of the Radiolabeled Drugs

All radiopharmaceuticals should have an appropriate hydrogen ion concentration or pH for their stability and integrity. The ideal pH of a radiopharmaceutical should be 7.4 (pH of the blood), although it can vary between 2 and 9 because of the high buffer capacity of the blood. [9] The pH of the solution can be accurately measured by a pH meter. Any deviation from the desired pH must be treated with caution and should be remedied. Radiopharmaceuticals must also have proper ionic strength, isotonicity, and osmolality in order to be suitable for human administration.

In general, the pH values in the range of 4.5–7.5 are quite acceptable since blood has very good buffering capacity. Since the pH of the final drug formulation may likely vary from batch to batch, it is important that the actual pH of the final drug formulation is tested using a suitably calibrated pH meter.

9.4.2 Determination of Radiolabelling efficiency

The labeling efficiency of ^{99m}Tc- RLX and ^{99m}Tc- LA was estimated by instant thin layer chromatography (ITLC) using silica gel (SG)-coated fiberglass sheets (Gelman Sciences Inc, Ann Arbor, MI). The ITLC was performed using acetone as mobile phase. Approximately 2 to 3 μL of the radiolabeled complex was applied at a point 1 cm from one end of an ITLC-SG strip. The strip was developed in above-mentioned solvent and the solvent front was allowed to reach 8 cm from the point of application. The strip was cut horizontally into 2 halves, and the radioactivity in each segment was determined using well-type gamma ray counter (Caprac T well counter, Capintec NJ, USA). The free ^{99m}Tc-pertechnetate that moved with the solvent (R_f = 0.9) was determined. The reduced/hydrolyzed (R/H) technetium along with the labeled complex remained at the

point of application. Incorporation of excess of stannous chloride for reduction of ^{99m}Tc may lead to formation of radio colloids, which is undesirable. The colloid formation was determined in pyridine:acetic acid:water (3:5:1.5 vol/vol) as mobile phase. The radio colloids remains at bottom of the strip, while both the free pertechnetate and the labeled complex migrates with the solvent front. The activity moved with the solvent front using acetone was subtracted from that using pyridine:acetic acid:water as a mixture, the net % radiolabeled ^{99m}Tc-RLX and ^{99m}Tc-LA was calculated. [10]

9.4.3 Stability study of ^{99m}Tc-drug complex

The stability study of ^{99m}Tc-RLX and ^{99m}Tc-LA was determined in vitro using human serum and 0.9 % sodium chloride by instant thin layer chromatography. The complex (0.1 mL) was mixed with 1.9 mL of human serum and normal saline separately and incubated at 37 °C. ITLC was performed at different time intervals up to 24 hours to assess the stability of the radiolabeled complex. [2]

9.4.4 Transchelation Study (DTPA Challenge)

This study was performed in order to check the strength of binding of ^{99m}Tc with the RLX and LA. In brief, fresh solutions of DTPA (5, 10, and 15 mM) were prepared in distilled water. Five hundred microliters of the labeled drug was treated with different concentrations of DTPA and incubated for 2 hours at 37°C. The effect of DTPA on the labeling efficiency of complex was evaluated by ITLC using acetone as mobile phase. ^{99m}Tc-RLX or ^{99m}Tc-LA radio-complex remains at the origin (Rf = 0.0), while free pertechnetate (Rf = 0.9-1.0) and all known chemical forms of ^{99m}Tc-DTPA complexes migrate (Rf = 0.5-0.8). After developing, each paper was cut into 3 portions (0-5, 5-8, 8-10) and each portion was counted for radioactivity in gamma counter. [2]

9.5 In Vivo Biodistribution study

Female New Zealand white rabbits weighing approximately 2.5 ± 0.5 kg were housed in animal house facility at Institute of Nuclear Medicine and Allied Sciences (INMAS), DRDO, New Delhi under controlled light (12 h light: 12 h dark) and temperature (22-24°C) conditions. The animals were provided water and their respective chow. The

experimentation protocol was approved by CPCSEA. Animal handling and experimentation was carried out as per the guidelines of the Institutional Animal Ethics Committee of INMAS, DRDO, New Delhi.

9.5.1 Biodistribution study of Radiolabeled Raloxifene loaded Liposomes

Raloxifene was labeled with ^{99m}Tc as described previously in the Radiolabeling section 9.3.1 and entrapped within the liposomes during formulation preparation. Liposomal formulation containing radiolabeled drug was prepared by the method described in chapter 5, preparation of RLX- liposomes section 5.3. Dose of the drug to be administered in rabbits was calculated using the formula:

$$\text{Human Equivalent Dose (mg/kg) (HED)} = \frac{\text{Animal Dose (mg/kg)} \times \text{Animal Km}}{\text{Human Km}}$$

Where Km is body weight (kg) divided by body surface area (m^2); the factor that varies for all animals. [11]

A dose of 37 MBq (1.5 ml) of the ^{99m}Tc-RLX loaded liposomes (containing 6mg of RLX) was administered intravaginally to each rabbit with the help of medical grade silicone tubing. To compare the targeting efficiency of Liposomes with plain drug, ^{99m}Tc-RLX was also administered in same dose as used in formulation in rabbits through vaginal route. Two rabbits were used one each for formulation and plain drug.

The rabbits were fixed on a board and imaging was performed at predetermined time intervals (0, 1, 2, 4, 18 and 24 hours) using a Single Photon Emission Computerized Tomography (SPECT, LC 75–005, Diacam, Siemens AG, Earlangan, Germany) gamma camera. The whole-body deposition was calculated by taking into account gamma camera attenuation, background, and radioactive decay. First, the uterine activity was calculated. The region of interest was defined by drawing outline around uterus on the images obtained by ^{99m}Tc. Uterine deposition was expressed as a percentage of the whole-body deposition. [2, 8]

9.5.2 Biodistribution study of Radiolabeled Leuprolide loaded Liposomes

LA was labeled with ^{99m}Tc as described previously in the Radiolabeling of LA section 9.3.2 and entrapped within the liposomes during formulation preparation. Liposomal formulation containing radiolabeled drug was prepared by the method described in chapter 5, preparation of LA-liposomes section 5.4. Dose of the drug to be administered in rabbits was calculated using the formula:

$$\text{Human Equivalent Dose (mg/kg) (HED)} = \frac{\text{Animal Dose (mg/kg)} \times \text{Animal Km}}{\text{Human Km}}$$

Where Km is body weight (kg) divided by body surface area (m^2); the factor that varies for all animals. [11]

Similar method was performed as described by Patel et al, 2016. [2] A dose of 22.2 MBq of the ^{99m}Tc-LA loaded liposomes (0.5 ml containing 0.308 mg of LA) was administered through the vaginal tract of rabbit with the help of medical grade silicone tubing. To compare the targeting efficiency of Liposomes with plain drug, ^{99m}Tc-LA was also administered in same dose as used in formulation in rabbits through vaginal route. Two separate rabbits were used one each for formulation and plain drug.

The rabbits were fixed on a board and imaging was performed at predetermined time intervals (0, 1, 2, 4, 18 and 24 hours) using a Single Photon Emission Computerized Tomography (SPECT, LC 75–005, Diacam, Siemens AG, Earlangan, Germany) gamma camera. Each image was scanned for 2 minutes. The whole-body deposition was calculated by taking into account gamma camera attenuation, background, and radioactive decay. First, the uterine activity was calculated. The region of interest was defined by drawing outline around uterus on the images obtained by ^{99m}Tc. Uterine deposition was expressed as a percentage of the whole-body deposition. [2]

9.6 Results and Discussion

In the present investigation, RLX and LA loaded liposomes were formulated for the treatment of Uterine Fibroids and Endometriosis. Since, the marketed formulations of both the drugs faces severe systemic side effects, intravaginal administration was done to target the drugs to the site of action i.e. Uterus. In order to examine the drug targeting efficiency, in vivo biodistribution studies was carried out using ^{99m}Tc labeling. Since drugs by and large influence body biochemistry and physiology, it follows that radiolabeling and imaging procedures provide a unique tool to study the biodistribution of drugs noninvasively in the body in real time.

9.6.1 pH of the Radiolabeled complex

All the radiolabeled formulations, intended for in vivo administration, should maintain relatively stable pH (a measure of hydrogen ion concentration) over an optimal range, preferably very close to the pH of blood. In order to get the highest labeling efficiency, pH was adjusted in the range of 4.5 to 7.5 using either 0.1N HCl or 0.5M $NaHCO_3$ solutions appropriately. RLX-^{99m}Tc was found to be stable with highest labeling efficiency at pH 4.5, adjusted using 0.1N HCl while LA-^{99m}Tc showed stability with high labeling efficiency at pH 7. pH value less or more than 4.5 and 7 for RLX and LA respectively, led to lowering of the labeling efficiency.

9.6.2 Determination of Radiolabeling efficiency

9.6.2.1 Radiolabeling efficiency of Raloxifene Hydrochloride

The mean labeling efficiency of RLX was >98 % at pH 4.5 (adjusted using 0.1 N HCl). Less than 1 % radioactivity was dissociated after 24 hours incubation, which indicates the suitability of the complex for its in vivo use. Results are shown in Table 9.1

Table 9.1: Effect of Incubation Time on the Labeling Efficiency of RLX with ^{99m}Tc

Incubation time (Hours)	%RLX Radiolabeled
0.5	99.71±0.87
1	99.30±1.1
2	99.51±0.56
3	99.57±1.2
4	99.68±1.4
24	99.56±0.84

Results are mean of three separate experiments (n=3)

9.6.2.2 Radiolabeling efficiency of Leuprolide Acetate

In the current study, LA was successfully labeled using ^{99m}Tc by direct labeling method with a mean labeling efficiency of >98 % at pH 7.0. On the basis of chromatographic analysis the radiolabeling efficiency was found to be more than 98% consistently. Less than 1 % radioactivity was dissociated after 24 hours incubation, which indicates the stability of the complex. Results are shown in Table 9.2.

Table 9.2: Effect of Incubation Time on the Labeling Efficiency of LA with ^{99m}Tc

Incubation time (Hours)	%LA Radiolabeled
0.5	99.96±1.1
1	98.41±1.0
2	99.03±0.8
3	98.55±0.87
4	98.76±0.98
24	98.88±1.2

Results are mean of three separate experiments (n=3)

9.6.3 Stability of ^{99m}Tc-drug complex

As a part of quality control, radiolabeled preparations were checked for their in vitro stability in the presence of serum and normal saline 0.9 % at 37^{o}C. These conditions were selected for stability study to mimic in vivo environment such as serum proteins and physiological pH.

9.6.3.1 Stability of RLX-^{99m}Tc complex

Incubation of ^{99m}Tc-RLX in human serum and 0.9 % saline at 37^{o}C revealed that the labeling of the RLX was extremely stable. As shown in Table 9.3, less than 1 % to 5 % radioactivity was dissociated after 24 hours incubation in serum and saline, which indicates the suitability of the complex for its in vivo use.

Table 9.3: Stability of RLX-^{99m}Tc complex in Human Serum and Physiological Saline at 37^{o}C

Incubation time (Hours)	**% RLX Radiolabelled**	
	In Human Serum	**In 0.9% Saline**
0.5	98.1±1.2	99.54±1.7
1	97.5±1.5	99.20±0.57
2	96.9±1.1	98.51±1.6
3	96.2±1.3	99.01±1.2
4	95.8±1.4	98.68±1.2
24	95.7±1.2	98.12±1.23

The samples were subjected to ITLC with 100% acetone as mobile phase. Results are the mean of 3 separate experiments (n=3)

9.6.3.2 Stability of LA-^{99m}Tc complex

The radiolabeled complex was stable in serum and normal saline for 24 h. In these media, at all-time points ^{99m}Tc-labeled preparation showed more than 95% radiolabeling as seen in Table 9.4, indicating the usefulness of the label as a marker for the biodistribution studies.

Table 9.4: Stability of LA-^{99m}Tc complex in Human Serum and Physiological Saline at 37^{o}C

Incubation time (Hours)	**% LA Radiolabelled**	
	In Human Serum	**In 0.9% Saline**
0.5	98.3±1.1	99.26±1.3
1	97.5±1.2	98.22±1.1
2	96.5±1.0	99.03±1.0
3	95.9±1.2	98.0±0.87
4	95.3±1.3	97.76±0.18
24	95.4±1.1	97.26±1.2

The samples were subjected to ITLC with 100% acetone as mobile phase. Results are the mean of 3 separate experiments (n=3)

9.6.4 Transchelation study (DTPA Challenge)

The in vitro stability of radiolabeled complexes needs to be confirmed before using them for biodistribution studies. The in vitro stability of ^{99m}Tc-RLX and ^{99m}Tc-LA was assessed by DTPA challenge test. DTPA possess greater affinity to ^{99m}Tc. Upon incubation with the labeled complexes, the DTPA results in transchelation of labeled complex, and the degree of transchelation indicates the stability of the labeled complex. The low degree of transchelation indicates strong binding of technetium with the drug. Challenge study demonstrated that the labeling efficiency of the complex did not alter significantly in the presence of DTPA (Figure 9.1 A and B). Even at 15 mM concentration of DTPA, the transchelation was found to be less than 4.5 % for RLX and less than 6 % for LA (Table 9.5), indicating the stability of the radiolabeled complexes with higher binding affinity of technetium with RLX and LA.

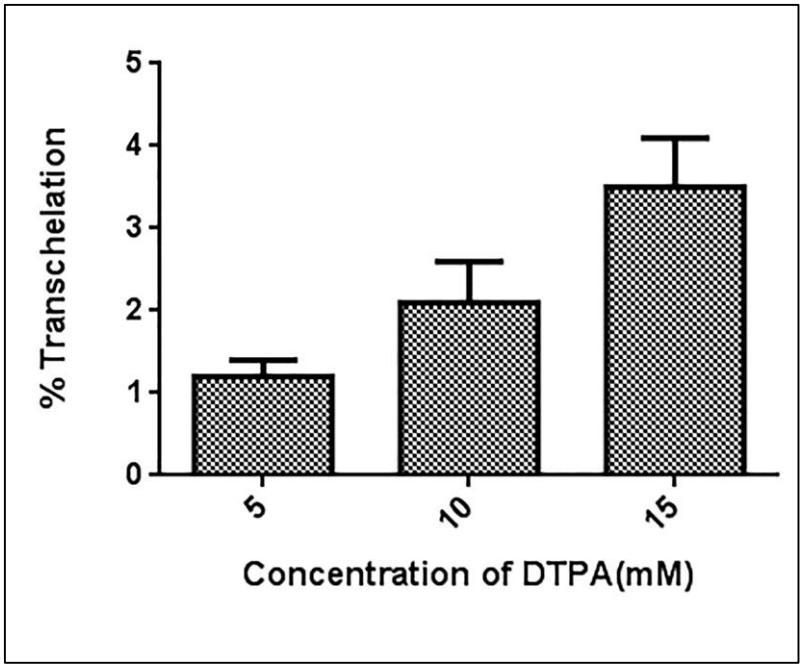

Figure 9.1 A)

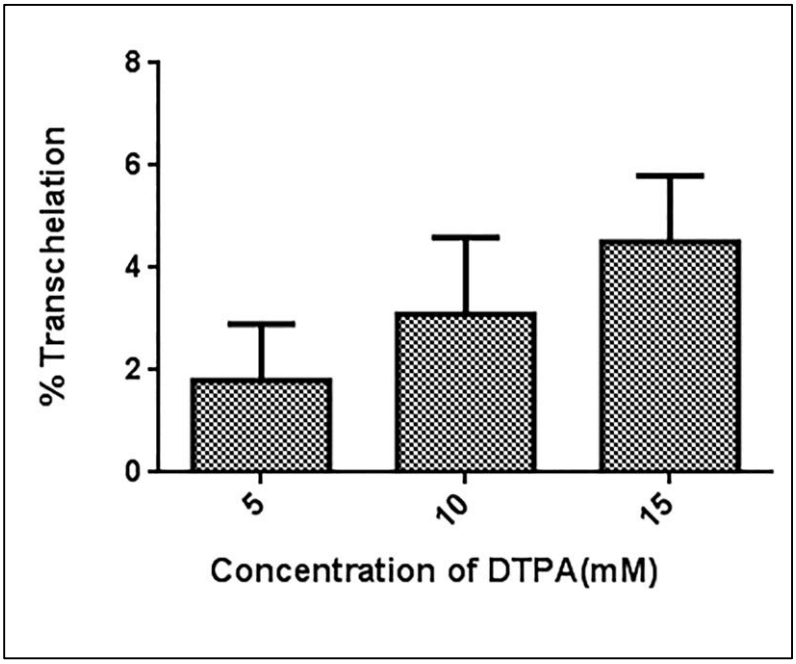

Figure 9.1 B)

Figure 9.1 DTPA challenge test of A) RLX-^{99m}Tc complex B) LA-^{99m}Tc complex

Table 9.5 DTPA challenge test of A) RLX-^{99m}Tc complex B) LA-^{99m}Tc complex

DTPA concentration	**% Transchelation**	
	RLX-^{99m}Tc	**LA-^{99m}Tc**
5mM	1.2±0.2	1.8±1.1
10mM	2.1±0.5	3.1±1.5
15mM	3.5±0.6	4.5±1.3

*experiment was done in triplicate (n=3)

9.6.5 Results of Biodistribution Study

In the past few years, targeted drug delivery has been one of the prime objectives of pharmaceutical developers, who wish to maximize desired effects of drugs on a target organ while minimizing any adverse consequences linked to systemic activity. Current marketed formulations of RLX and LA faces severe systemic side effects leading to poor patient compliance. Moreover, RLX has very low oral bioavailability (2%), requiring

administration of high dose to achieve the therapeutic effect. Researchers have reported that both Leuprolide as well as Raloxifene shows direct antiproliferative and cytotoxic effect on leiomyoma cells. [12, 13] With the aim of targeting both the drugs to the site of action, i.e. uterus, the present investigation was planned and worked out.

There has been a renewed interest in vaginal route of drug delivery, with growing evidence supporting the fact that drugs administered vaginally are preferentially uptaken in uterus where their tissue concentrations exceeds the systemic absorption. This may serve as a route of administration for substances used for the treatment of uterine fibroids and endometriosis. [14] Recently, numerous independent investigators [15, 16] have reported higher accumulations of drugs in the uterus when they were administered intravaginally. Higher concentrations were observed in the uterus even when levels in the blood were lower than those achieved by systemic administration (i.m., transdermal). These observations support the hypothesis that there is a high degree of direct vagina-to-uterus transport or a "first uterine pass effect." [15, 16]

9.6.5.1 Biodistribution of ^{99m}Tc-Raloxifene loaded liposomes

Biodistribution of RLX labeled with ^{99m}Tc entrapped in liposomes as well as ^{99m}Tc labeled RLX plain drug was examined in female rabbits, by administering them through vaginal route. The percentage of administered dose of drug in different organs at different time intervals is shown in Table 9.6 and 9.7.

Table 9.6: Biodistribution data of ^{99m}Tc-RLX loaded liposomes

Organ/Tissue	Percentage of administered dose in different organs					
	15 min	1 hr.	2 hr.	4 hr.	18 hr.	24 hr.
Uterus	81.48%	78.44%	76.72%	76.13%	75.20%	74.94%
Kidney	-	-	-	-	-	-
Lungs	-	-	-	-	-	-
Liver	-	-	-	-	-	-
Heart	-	-	-	-	-	-
Stomach	-	-	-	-	-	-
Intestine	-	-	-	-	-	-
Spleen	-	-	-	-	-	-

Radioactivity counts in different organs; results expressed as percentage of whole body deposition

Table 9.7: Biodistribution data of ^{99m}Tc-RLX plain drug

Organ/Tissue	Percentage of administered dose in different organs					
	15 min	1 hr.	2 hr.	4 hr.	18 hr.	24hr.
Uterus	75.86%	62.28%	58.48%	28.24%	-	-
Kidney	-	-	-	-	-	-
Lungs	-	-	-	-	-	-
Liver	-	-	-	-	-	-
Heart	-	-	-	-	-	-
Stomach	-	-	-	-	-	-
Intestine	-	-	-	-	-	-
Spleen	-	-	-	-	-	-

Radioactivity counts in different organs; results expressed as percentage of whole body deposition

As seen from the results in Table 9.6, when ^{99m}Tc-RLX loaded liposomes were administered via vaginal route, maximum amount of drug was found in uterus even after 24 hours (74.94%) with no distribution in any other organs of the body. These results are suggestive of the non-toxicity of the drug in other vital organs and highest targeting efficiency to uterus when the formulation is administered by vaginal route. While in case of ^{99m}Tc-RLX plain drug administration as seen in Table 9.7, 2.7 fold lesser concentration was able to retain in uterus (28.24 %) after 4 hours and further imaging, showed no radioactive counts in uterus. Figure 9.2 demonstrates graphically the comparison between uterine targeting efficiency of drug-loaded liposomes vs. plain drug.

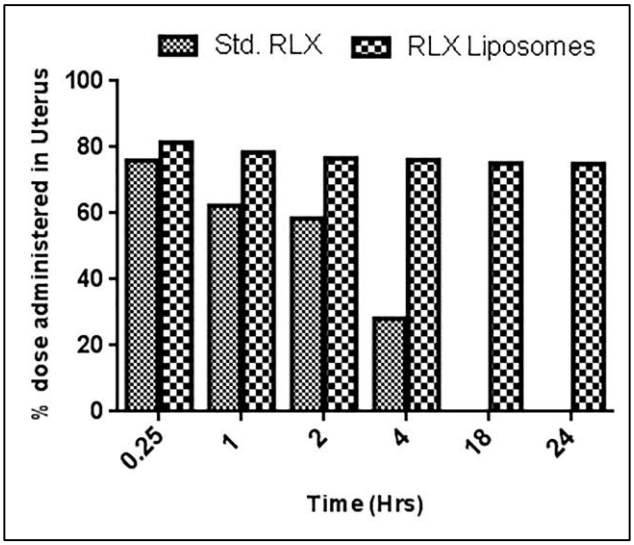

Figure 9.2 Uterine targeting efficiency of RLX loaded liposomes vs. plain drug

Figure 9.3 and 9.4 shows the Gamma Scintigraphic images of rabbits administered with formulation and plain drug respectively.

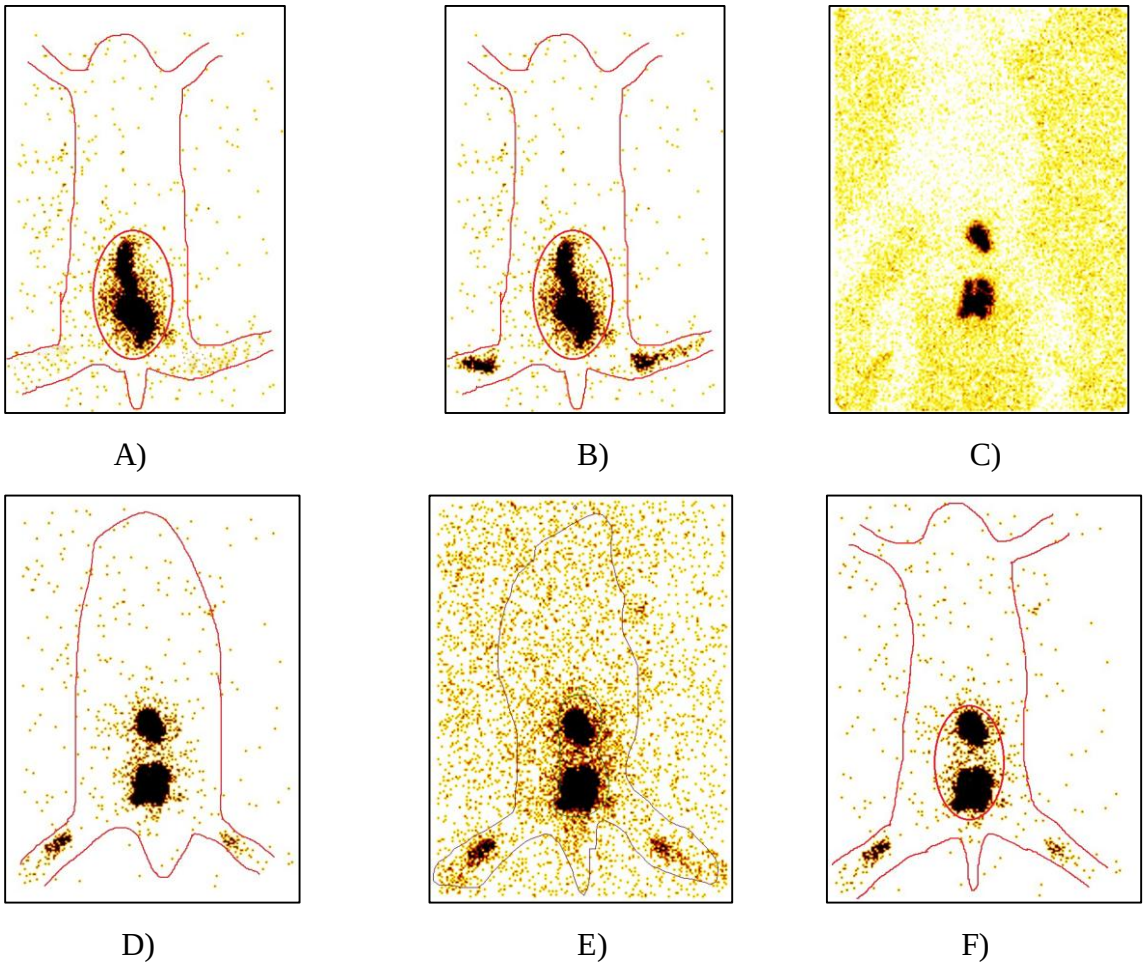

Figure 9.3: Gamma Scintigraphy of Rabbit, which was administered ^{99m}Tc-Labeled Raloxifene Hydrochloride loaded liposomes via Vaginal route, imaged after A) 0.25 hour; B) 1 hour C) 2 hours D) 4 hours E) 18 hours F) 24 hours

These findings are supported by the research carried out by Ettore Cicinelli and co-workers 2001, [16] who studied the absorption and preferential vagina to uterus distribution after vaginal administration of ^{99m}Tc-pertechnetate in post-menopausal women. They also evaluated the uterine uptake of the same radionuclide administered intravenously. They observed that pertechnetate administered vaginally concentrated in the uterus to a greater extent as compared to other organs. In contrast, when the

radionuclide was administered intravenously, this order of distribution was reversed. [16] These findings strongly suggest the existence of a preferential distribution of radionuclide from the vagina to the uterus.

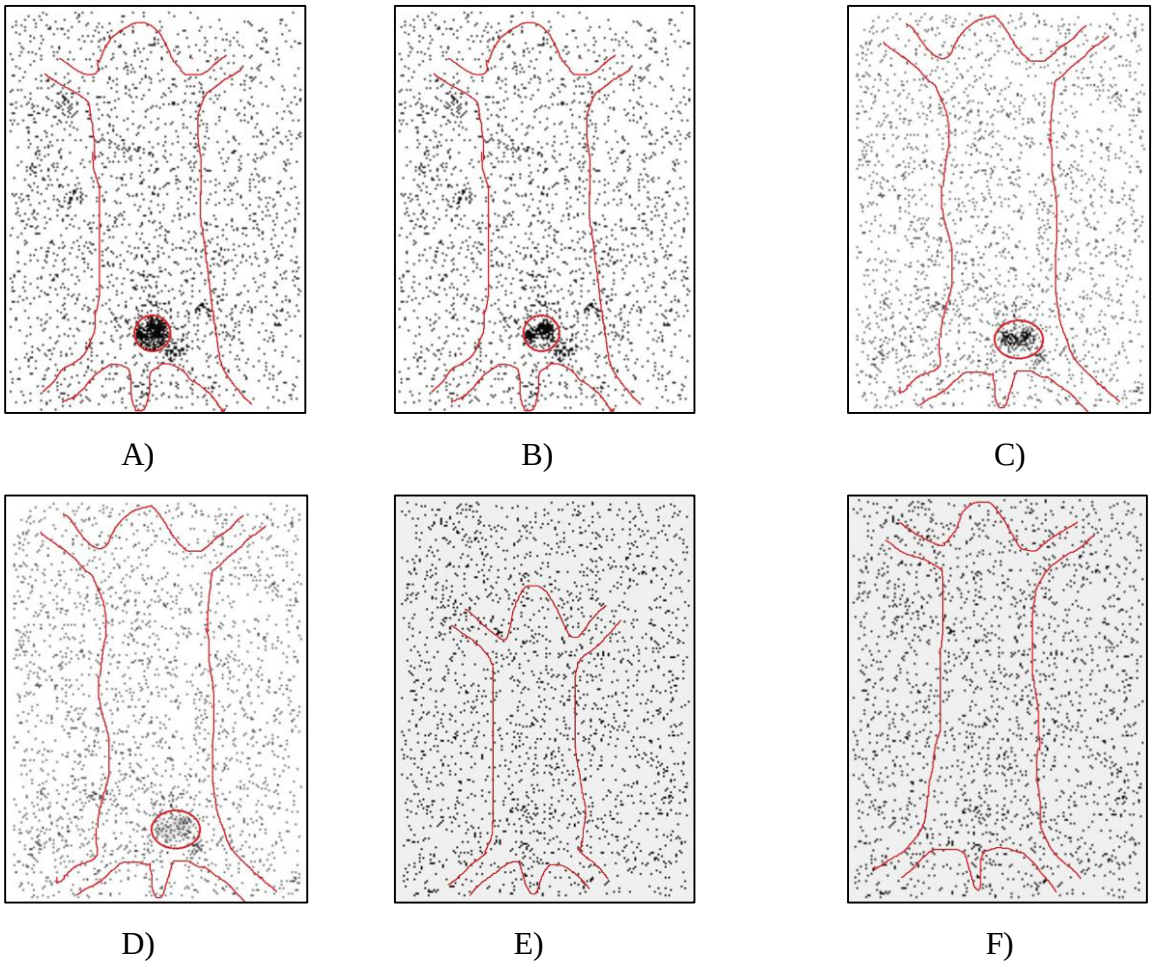

Figure 9.4: Gamma Scintigraphy of Rabbit, which was administered ^{99m}Tc-Labeled Raloxifene Hydrochloride plain drug via vaginal route, imaged after A) 0.25 hour; B) 1 hour C) 2 hours D) 4 hours E) 18 hours F) 24 hours

As seen in the figures 9.3 and 9.4, plain drug reached the uterus in very less amount as compared to liposomes loaded with drug. The formulation was able to remain at site for longer period of time than plain drug. Liposomes made up of lipids readily diffuse through the vaginal membrane and reach uterus more efficiently as compared to plain drug. Moreover the highly perfused uterus is able to retain lipidic liposomes for a longer period of time making them the most suitable formulation for uterine targeting. Results were in accordance to the study carried out by Refeurzo and Co-workers 2015 [17] who

prepared multilamellar vesicles of size 150-200 nm, loaded with Indomethacin and administered in pregnant CD1 mice. They observed that liposomes were localized within the uterus and did not cross the placenta to the fetus. Uterus was able to confine the liposomes within it and avoid its distribution elsewhere. [17] Similar results are obtained in the present study with no radioactivity noted in other vital organs of the body of rabbit suggestive of the targeting efficiency of the route and also the non-toxicity of the drug to other organs which other wise is a major problem caused by drug administered by other alternate routes exposing the drug to systemic circulation.

9.6.5.2 Biodistribution of ^{99m}Tc-Leuprolide loaded liposomes

Results of biodistribution study of ^{99m}Tc labeled-LA encapsulated in liposomes administered by vaginal route as well as ^{99m}Tc labeled-LA plain drug in New Zealand white female rabbits are shown in Table 9.8 and 9.9 as percentage of the administered dose of drug in different organs at different time intervals.

Table 9.8: Biodistribution data of ^{99m}Tc-LA loaded liposomes

Organ/Tissue	Percentage of administered dose in different organs					
	15 min	1 hr.	2 hr.	4 hr.	18 hr.	24hr.
Uterus	99.87%	94.81%	92.97%	91.63%	91%	90.21%
Kidney	-	-	-	-	0.55-left 0.45-right	0.48-left 0.46-right
Lungs	-	-	-	-	-	-
Liver	-	-	-	-	-	-
Heart	-	-	-	-	-	-
Stomach	-	-	-	-	-	-
Intestine	-	-	-	-	-	-
Spleen	-	-	-	-	-	-

Radioactivity counts in different organs; results expressed as percentage of whole body deposition

Table 9.9: Biodistribution data of ^{99m}Tc-LA plain drug

Organ/Tissue	Percentage of administered dose in different organs					
	15 min	1 hr.	2 hr.	4 hr.	18 hr.	24hr.
Uterus	85.86%	82.28%	78.48%	20.78%	-	-
Kidney	-	-	-	-	-	-
Lungs	-	-	-	-	-	-
Liver	-	-	-	-	-	-
Heart	-	-	-	-	-	-
Stomach	-	-	-	-	-	-
Intestine	-	-	-	-	-	-
Spleen	-	-	-	-	-	-

Radioactivity counts in different organs; results expressed as percentage of whole body deposition

As seen from the results in Table 9.8 and 9.9, when ^{99m}Tc-LA loaded liposomes were administered via vaginal route, maximum amount of drug was found in uterus even after 24 hours with insignificant amount found in kidneys 0.46-0.48%. There were no radioactive counts noted in any other organs of the body. These results are suggestive of the non-toxicity of the drug in other vital organs and highest targeting efficiency to uterus when the formulation is administered by intravaginally. While in case of ^{99m}Tc-LA plain drug administration as seen in Table 9.9, 4.5 fold lesser concentration of drug was able to remain in uterus (20.78 %) after 4 hours and further imaging of the rabbit, showed no radioactive counts in uterus. Figure 9.5 demonstrates graphically the comparison between uterine targeting efficiency of drug-loaded liposomes vs. plain drug.

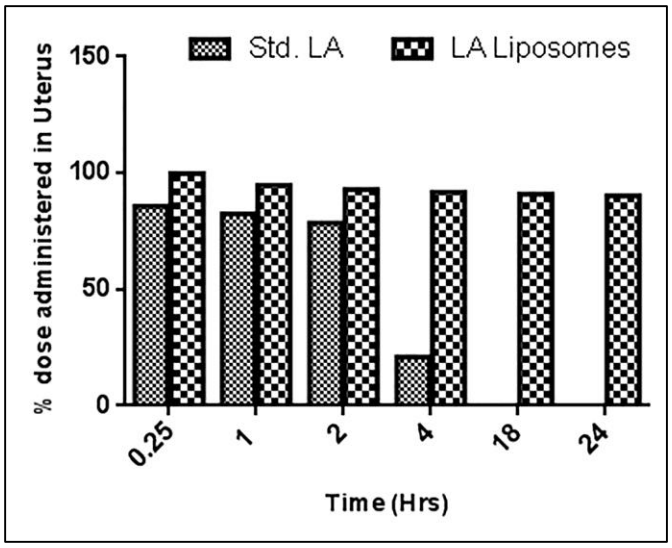

Figure 9.5 Uterine targeting efficiency of LA loaded liposomes vs. plain drug

Figure 9.6 and 9.7 shows the Gamma Scintigraphic images of rabbits administered with formulation and plain drug respectively. Similar results were observed like biodistribution of ^{99m}Tc-RLX loaded liposomes. LA reached the uterus in very less amount as compared to LA loaded liposomes. Uterus was able to confine the liposomes within it and avoid its distribution elsewhere.

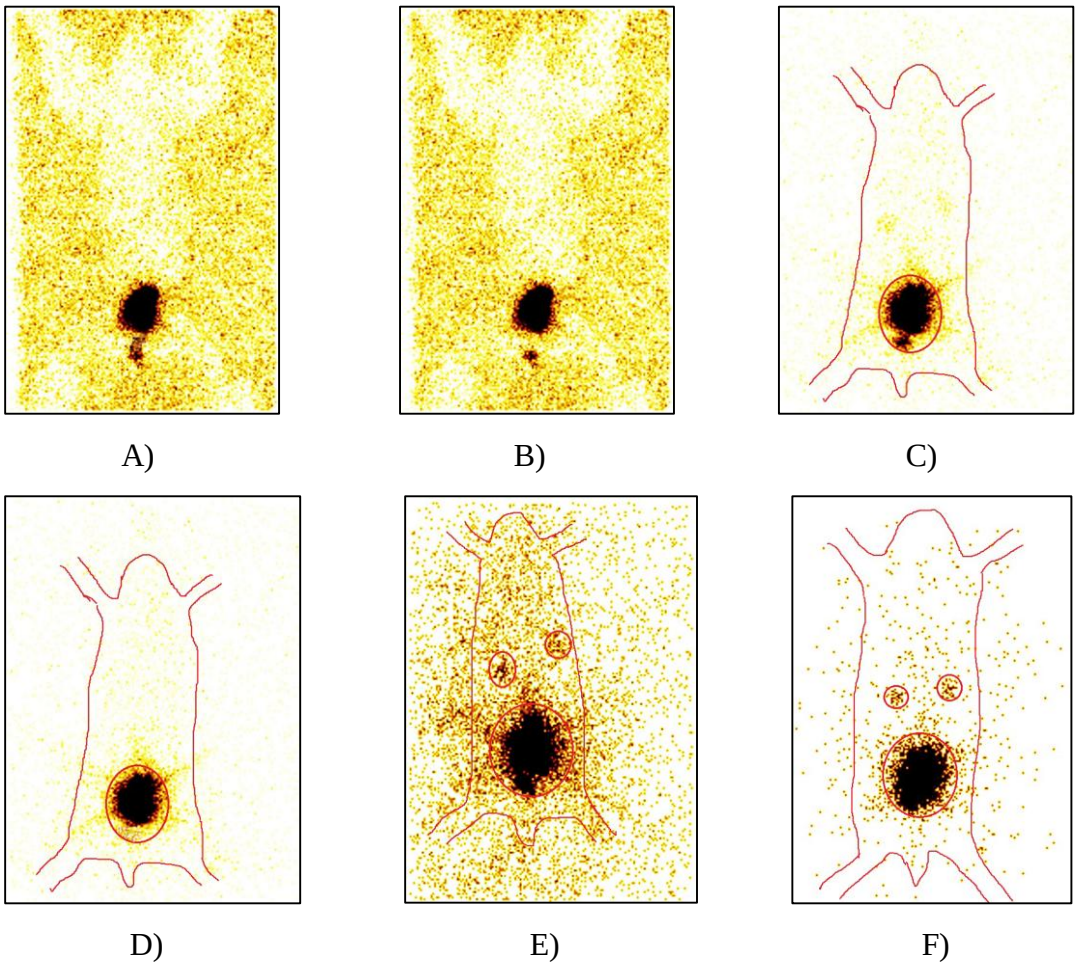

Figure 9.6: Gamma Scintigraphy of Rabbit, which was administered ^{99m}Tc-Labeled Leuprolide Acetate loaded liposomes via vaginal route, imaged after A) 0.25 hour; B) 1 hour C) 2 hours D) 4 hours E) 18 hours F) 24 hours

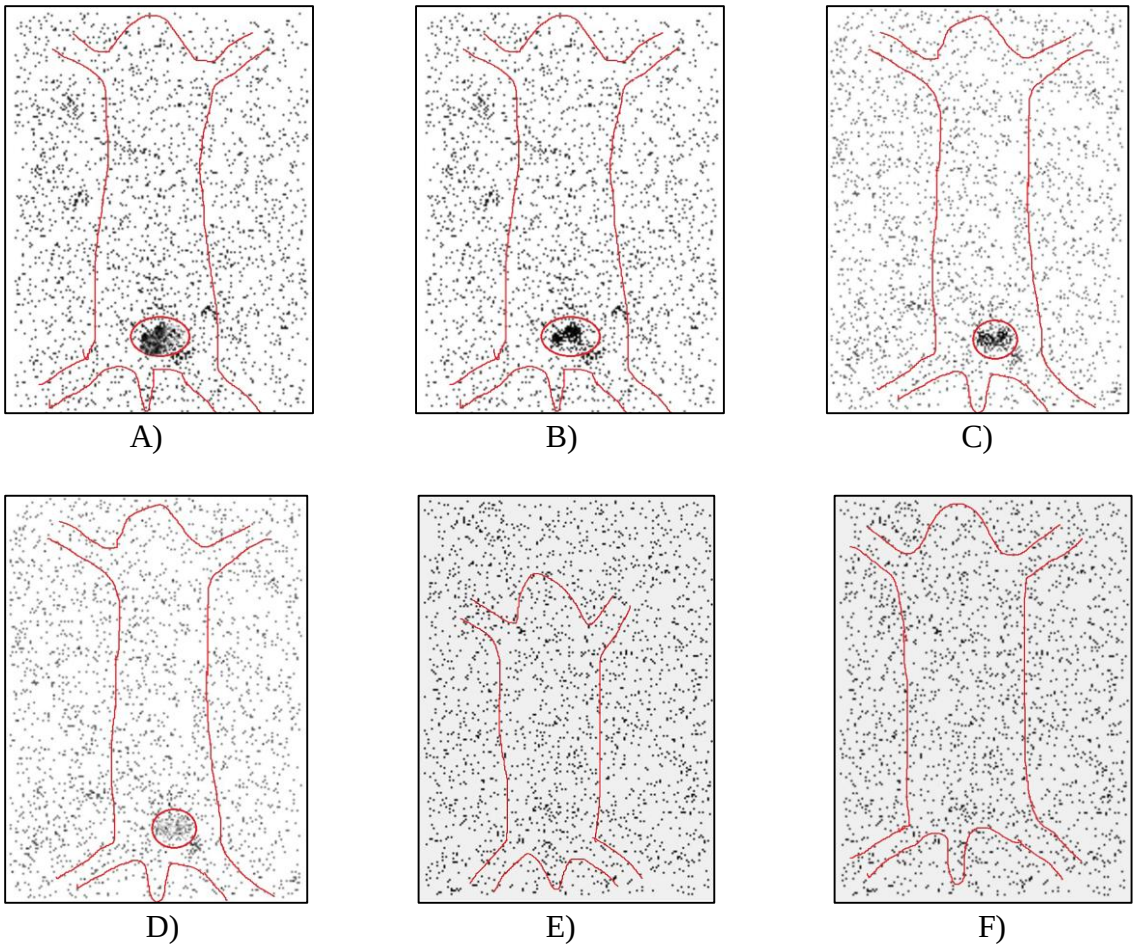

Figure 9.7: Gamma Scintigraphy of Rabbit, which was administered ^{99m}Tc-Labeled Leuprolide Acetate plain drug via vaginal route, imaged after A) 0.25 hour; B) 1 hour C) 2 hours D) 4 hours E) 18 hours F) 24 hours

This investigation shows that targeted drug delivery to the uterus is particularly appealing for the administration of substances designed to exert their primary action on the uterus, avoiding the systemic side effects, making the therapy more patient compliant. Overcoming the limitations of currently available marketed formulation of LA the prepared liposomes administered via vaginal route to treat fibroids and endometriosis can anticipate dose reduction and provide a safer and cost effective therapy.

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