10.1. Introduction

Biodistribution studies are in vivo test-article distribution or localization studies performed in selected nonclinical species to support early bio-therapeutic drug development. Once a chemical has entered the vascular system, it is distributed throughout the body fluids according to the physiochemical properties of the drug and its ability to penetrate the barriers [1].

Modern imaging technologies offer high-sensitivity and high-resolution visualization of imaging probes. In the last decade, in vivo imaging has been gradually growing in importance as an aid for the development of new drug delivery systems and approaches to controlled dosage release. These two fields are powerful in their own right, but together they can generate considerable new knowledge in a cost-effective manner with regard to the pharmacokinetics, biodistribution, bioavailability, local concentration, and clearance of drug substances for the treatment of a variety of diseases including, in particular, the treatment of cancer. In the pharmaceutical industry, controlled release formulations are widely used, and become increasingly popular for reasons of improving patient compliance, reducing side effects, and extending patent protection. Non-invasive imaging techniques allow rapid, repetitive (and thus potentially high throughput) assessment of the drug deposition in various tissues in the body, which can vastly facilitate the eventual translation of novel dosage forms into the clinic [2-4].

Molecular imaging may be defined as the imaging of molecules either delivered to the body or already present in the body. Generally, this refers to in vivo imaging that is, imaging within a living multicellular organism. The process requires an imaging instrument, a subject, and, commonly, an imaging agent. These tools enable longitudinal assessment of delivered materials, specific targets, mechanisms of action, and biological processes. Molecular imaging has already found utility in the clinic [4, 5].

Physical properties such as radioactive decay; absorbance or reflectance of light, sound, or X-rays; and behaviour in a magnetic field are used to generate images. Machines sensitive to such physical changes are used to create images. These require appropriate tissue and spatial resolution. If the imaging is performed from one angle, a two-dimensional (2D) image is generated. If the imaging is performed at multiple angles, with appropriate mathematical algorithms and the speed of modern computers, three-dimensional (3D) images can be created [4, 6].

Imaging Modalities

Light-based imaging

- Primarily CCD (charge coupled device) -based cameras
- White light
- With filters: fluorescence, near infrared, infrared
- Raman spectroscopy
- Penetration depth limited: external cameras, internal cameras such as endoscopes

Nuclear medicine

- Gamma camera-based imaging
- Positron Emission Tomography (PET)

Magnetization

- Magnetic Resonance (MR)

Sound

- Ultrasound
- Photo-acoustic imaging (light input, sound output)
- Thermo-acoustic imaging (radiofrequency input, light output)

X-rays

- Radiography
- Computed Tomography (CT)

Among percutaneous imaging methods that may also be applicable in humans, nuclear medicine offers the highest sensitivity, in the nano-molar range. For such imaging, a radiopharmaceutical is delivered to the patient. It includes a radionuclide whose decay in the body is imaged. Radionuclides are unstable atoms. Atoms are made up of neutrons, proton, and electrons. In their ground state, nucleons (protons and neutrons) are stable, but if the ratio of neutrons to protons is not optimal or the nucleons are not in their ground state, they may release energy/particles, including gamma rays with characteristic energy. This released energy signature is used to distinguish radioactive decay arising directly from the radionuclide from background/scatter reactions that result in different energies from the source of interest. The de-excitation may be immediate or delayed. The latter is referred to as a metastable state. The decay of ^{99m}Tc (m for metastable) is commonly imaged. The released characteristic energy is imaged using a gamma camera, which may be used to perform 2D planar imaging or single photon emission computed tomography (SPECT) 3D imaging. In the present study, gamma scintigraphy technique was used to explore in vivo behaviour of the developed formulation [4].

The efficacy of the treatment can be defined as the extent to which a drug has the ability to bring about its intended effect under ideal circumstances [7]. A critical aspect of drug efficacy is its behaviour in vivo. Many compounds that appear active in cell culture systems fail when tested in animals [8]. In the present study, the efficacy of the developed formulations was studied in vivo in rabbit model using ultrasound imaging techniques.

10.2. Materials and Methods

10.2.1. In vivo Biodistribution Study

10.2.1.1. Radiolabelling of Drugs

Sodium pertechnetate $Na^+[TcO_4]^-$ freshly eluted from ^{99}Mo by solvent extraction method, was procured from Regional centre for radiopharmaceuticals, Board of radiation and isotope technology (BRIT), Department of atomic energy, India (B. No. P7352). The direct labelling method using ^{99m}Tc described in literatures was employed

to label PTX with slight modifications [9]. Stannous chloride ($SnCl_2$) solution (concentration 1 mg/mL) was freshly prepared in 0.1N hydrochloric acid. 1 mL of ^{99m}Tc-pertechnetate (37 MBq/mL) was added into 50 µL of $SnCl_2$ solution and mixed properly. The pH of the solution was adjusted to 6.5 using 0.1 N sodium hydroxide solution to achieve maximum radio labelling efficiency [10]. The prepared solution was then added into 5 mL of PTX solution of concentration 1 mg/mL, in hydro-alcoholic mixture (6:4) and allowed to incubate at room temperature for 15-20 minutes. The drug labelling efficiency and the stability of the labelled complex in different media were evaluated. Similar radiolabelling procedure was used for CBP and the labelling efficiency and the stability of the labelled complex in different media were evaluated.

10.2.1.2. Radiochemical Purity

Instant Thin Layer Chromatography (ITLC) technique was used to evaluate labelling efficiency of ^{99m}Tc-labelled PTX and CBP. Acetone was used as mobile phase for the ITLC. The silica gel (SG)-coated fiberglass sheet (Gelman Sciences Inc, Ann Arbor, MI, USA) was cut into strips of 1 x 12 cm area. The radiolabelled complex approx. 2-3 µL volume was applied at 1 cm above the end of ITLC strips. The strips were placed in the glass chamber containing mobile phase and allowed to run till solvent front reached 8 cm from the point of application of the test substance. Free $^{99m}[TcO_4]^-$ was found to have R_f value between 0.9 and 1.0 whereas the labelled formulation and radio-colloids (reduced/hydrolysed ^{99m}Tc), if developed, were retained at the point of application. Free $^{99m}[TcO_4]^-$ was estimated after separating the ITLC sheets into two equal parts transversely and counting radioactivity of each section using well type γ-scintillation counter (Caprac T well counter, Capintec NJ, United States). The radio colloid content was determined using pyridine: acetic acid: water (3: 5: 1.5 v/v/v) as mobile phase, wherein radio-colloids (R/H ^{99m}Tc) remained at the bottom, whilst both the free $^{99m}[TcO_4]^-$ and the labelled complex travelled to the top of the ITLC strip along with the solvent front. Free $^{99m}[TcO4]^-$ and colloids formation were estimated and radiochemical purity calculated [10].

10.2.1.3. In vitro stability study

The radiolabelled drug was analysed for its stability in physiological saline and serum by ascending instant thin layer chromatography. For in vitro stability in physiological saline and serum, 100 µl of the radiolabelled complex was mixed with 1.9 ml of normal saline and serum separately. The mixtures were incubated at 37°C and the changes in labelling efficiency were monitored at predetermined time intervals over a period of 48 h by ITLC [11].

10.2.1.4. DTPA Transchelation Study

Diethylene Triamine Penta Acetic acid (DTPA) challenge test was performed to evaluate stability of the radiolabelled drug complex since the reduced species of ^{99m}Tc are chemically reactive and form complexes with chelating agents. Three different concentrations i.e. 5, 10 and 15 mM of DTPA was prepared in distilled water. The radio labelled drug complex (500 µl) was incubated for 2 hours at 37°C with different concentrations of DTPA solution. The trans-chelation of the ^{99m}Tc with DTPA was measured using by thin layer chromatography using 100% acetone as mobile phase. The radio labelled complex remains at the point of application (R_f = 0.0), while free $^{99m}[TcO_4]^-$ (R_f = 0.9 to 1.0) and all known chemical forms of ^{99m}Tc-DTPA complex migrate (R_f = 0.5 to 0.8) along with the solvent front with different R_f values. The developed ITLC strip was then cut into 3 parts (Proportion = 5:3:2) and radioactivity counts were measured in well-type gamma counter (Caprac T well counter, Capintec NJ, USA).

10.2.1.5. In vivo biodistribution studies in Rabbits

All animal experiments carried out in accordance with the guidance by the Institutional Animals Ethics Committee of the Institute of Nuclear Medicine and Allied Sciences (INMAS), DRDO, New Delhi, India. Healthy sexually mature female New Zealand white rabbits weighing approximately 2.5 ± 0.5 kg were selected for the study. The rabbits were housed in animal house facility at INMAS, DRDO, New Delhi. The animals provided free access to food and water and maintained under controlled light-dark cycles of 12 h each and temperature (20-25°C) conditions. A dose of 1000 µCi of the

labelled drug formulation and labelled plain drug solution was measured using dose calibrator (CRC®-25R Dose Calibrator, CAPINTEC, INC. RAMSEY, NJ, USA) and administered intravaginally to each rabbit using medical grade silicone tubing and disposable syringe. The rabbits were fixed on board and the scintigraphic imaging was carried out at predetermined time intervals i.e. 0, 1, 2, 24 and 48 h using Single Photon Emission Computerized Tomography (SPECT, LC 75–005, Diacam, Siemens AG, Earlangan, Germany) gamma camera. The deposition of the formulation administered by intravaginal route into whole body and the target organ was measured by taking into account gamma camera attenuation, background and radioactive decay. The activity counts were measured for the region of interest by delineating outline covering the respective site in the scintigraphic images obtained by the gamma camera. Percentage deposition in the uterus was calculated in respect to the whole body deposition.

10.2.1.6. Quantitative systemic absorption study

The systemic absorption of the drug from the developed formulation and the plain drug solution was monitored during in vivo biodistribution studies. About 0.5 ml blood samples were collected from the dorsal vein of the ear of both the rabbits, at predetermined time intervals i.e. 1, 2, 24 and 48 h using 24 gauge needle and disposable syringe. The radioactivity in the collected blood samples was measured immediately in a well-type gamma counter (Caprac-t well counter, Capintec NJ, USA) and was calculated as the percentage of the injected dose.

10.2.2. In vivo efficacy study

In the present study, the efficacy of the developed formulations was evaluated on rabbit model to establish the effectiveness of novel formulations for the treatment of carcinomatous endometrial tissues. As the developed formulations are designed for the novel intravaginal route for the uterine targeting, it is important to evaluate the effectiveness of these delivery systems in vivo.

10.2.2.1. Experimental setup and Induction of endometrial carcinoma (EC) in Rabbits

All the animal experiments were conducted as per the guidelines of Institutional Animals Ethics Committee (IAEC) of the Pharmacy Department, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India. Healthy sexually mature female New Zealand white rabbits (aged 8 to 10 weeks) weighing approximately 2.5 ± 0.5 kg were procured from the Animal Vaccine Institute, Gandhinagar, Gujarat, India. The rabbits were kept in animal house facility at Shri G. H. Patel Bldg., Maharaja Pratapsinhrao Gaekwad Parisar, Vadodara, Gujarat. The animals were provided free access to food and water and maintained under controlled light-dark cycles of 12 h each and temperature (Approx. 25°C) conditions. Rabbits were divided into four groups of one animal each as below:

Group I: Negative Control (Normal Rabbit- Receiving No treatment)

Group II: Positive Control (Induced Endometrial Cancer- Receiving only placebo)

Group III: PTX-UDNVs formulation treated rabbit

Group IV: CBP-UDNVs formulation treated rabbit

Endometrial carcinoma was induced in rabbits of group II, III and IV by injecting 100 µg/kg Estradiol Dipropionate (ED) four times once a week subcutaneously, followed by weekly dose of 20 mg/kg N-methyl-N-nitrosourea (MNU) four times intraperitoneally [12-15]. Briefly, olive oil was sterilized by passing through 0.22 micron syringe filter and collected in standing centrifuge tube. Accurately weighed quantities ED was taken (to produce 1 mg/ml solution) and added in sterile vehicle and mixed thoroughly. The mixture was bath sonicated and the centrifuge tubes vortexed to solubilize ED and obtain homogeneous solution and stored in refrigerator. The MNU solution was freshly prepared at the time of injecting to prevent degradation by hydrolysis. Briefly, sterile normal saline was used as a vehicle and the weighed quantity (to produce 12 mg/ml resultant solution) of MNU was added to the solution and vortexed to mix and solubilize MNU. pH of the final solution was controlled between

5.0 to 6.0 to avoid pH dependent degradation of MNU. The solutions of ED and MNU were then injected subcutaneously and intraperitoneally using disposable syringe and 22 gauge needle. The treatment was repeated weekly till the tumour developed which typically takes around 2 months. From the third week of the treatment, ultrasound sonography was used to detect tumour formation in rabbits every week. The confirmed induction of the EC was ensured by ultrasound scanning of the uterine horns. The rabbits were subjected to their specific treatment as per the respective groups. The intravaginal rods loaded with the formulations of PTX and CBP equivalent to 35 mg/m^2 and 80 mg/m^2 dose respectively were carefully inserted into the vaginal lumen of the rabbits with the help of vaginal insert applicator under the guidance and supervision of the veterinarian. Rabbits were scanned using ultrasound to monitor and detect tumour regression every week. The images were recorded for each group of the rabbit.

Ultrasound scanning

The rabbits were shaved carefully on the dorsal sides of the abdomen to remove fur to facilitate smooth scanning for the sonography probe. Ultrasonography of the rabbits were performed at Veterinary Polyclinic, Vadodara, India under the guidance and monitoring of the veterinarian doctors. The ultrasound gel was applied on the shaved surface of the rabbit to establish conductive medium that creates a bond between the skin and the ultrasound transducer to achieve better clarity of the ultrasound imaging using mobile ultrasound system (MyLab™30Gold VET, Esaote, Italy). The normal uterine structures and the internal anatomy of the rabbit uterine horns was thoroughly explored by ultrasound scanning.

10.3. Results and Discussion

10.3.1. In vivo Biodistribution Study

10.3.1.1. Radiochemical purity

Radiochemical purity for PTX radio labelling procedure was calculated after assessment of free $^{99m}[TcO4]^-$ and radio-colloids (R/H ^{99m}Tc). The results of mean radiochemical purity are tabulated in **Table 10-1** and represented in **Fig. 10-1**.

Radiochemical purity of labelled PTX was found to be 96.5 ± 1.2 even after 48 h of incubation. The results indicate that the PTX was successfully labelled using ^{99m}Tc.

Table 10-1: Mean radiochemical purity for radiolabelled PTX

Incubation Time (h)	Mean Radiochemical Purity (%) (n=3)
1	99.3 ± 0.3
2	99.0 ± 0.7
4	98.2 ± 0.4
24	97.6 ± 1.0
48	96.5 ± 1.2

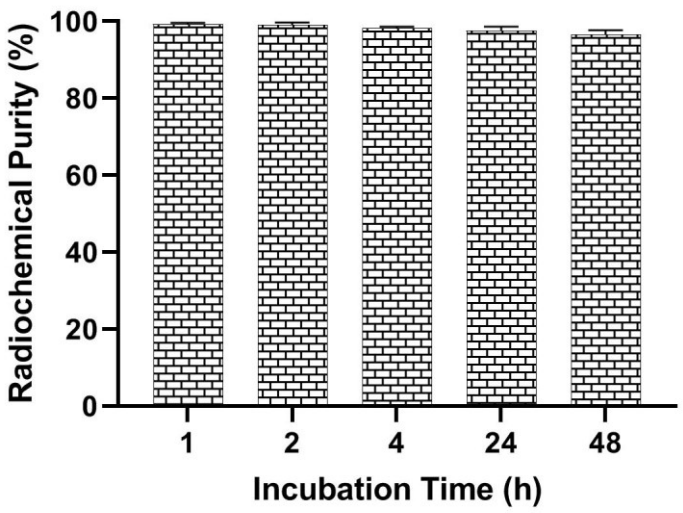

Fig. 10-1: Mean radiochemical purity for radiolabelled PTX (n=3)

The results of radiolabelling of CBP are represented in **Table 10-2**. The dissociation of the ^{99m}Tc and the CBP from the complex was only 23.62 % which was reduced to 2.55 % at the end of 3 h incubation time. The results indicate that the radiolabelling of the CBP was not optimum to be utilized for bio distribution studies. Hence, it was not studied further in animal models.

Table 10-2: Radiochemical purity of ^{99m}Tc-CBP

Incubation Time (h)	Radioactivity (%)

	Free $^{99m}[TcO_4]^-$	Radio-colloids (R/H ^{99m}Tc)	^{99m}Tc-CBP
0.25	0.18	76.21	23.62
1	1.14	94.81	4.05
3	0.16	97.29	2.55

10.3.1.2. In vitro stability study

The stability of radio labelled complex in normal saline and in serum is indicative of the suitability of the use of radiolabelled drug complex in vivo. The results of in vitro stability are represented in **Table 10-3** and **Fig. 10-2**. The results of radioactivity after incubation of 48 h revealed that % radiolabelling of 94.6 ± 1.3 and 91.9 ± 1.5 exhibited by PTX-^{99m}Tc in normal saline and serum respectively. The outcomes of the in vitro stability studies are indicative of its suitability to use for in vivo studies.

Table 10-3: In vitro stability of radiolabelled PTX (^{99m}Tc-PTX)

Incubation Time (h)	% Radiolabeled complex (^{99m}Tc-PTX)	
	Normal Saline	Serum
1	98.1 ± 0.3	97.5 ± 1.8
2	97.5 ± 1.2	96.7 ± 1.1
4	97.2 ± 1.2	93.9 ± 1.6
24	95.4 ± 0.9	92.4 ± 1.6
48	94.6 ± 1.3	91.9 ± 1.5

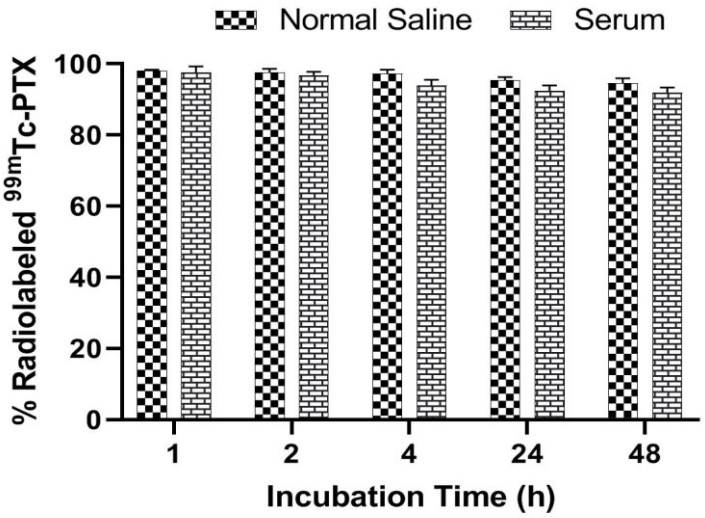

Fig. 10-2: In vitro stability of radiolabelled PTX (^{99m}Tc-PTX)

10.3.1.3. DTPA Transchelation Study

The reduced species of ^{99m}Tc are chemically reactive and form complexes with various chelating agents. DTPA is a chelating agent possess high affinity towards Tc for DTPA-Tc complex formation. As the dissociation of the Tc from the drug-Tc complex occurs, the DTPA-Tc complexes form readily. The formed complexes detected by ITLC technique and the stability of the Drug-Tc complex confirmed. The results of transchelation test at different molar concentrations of DTPA are represented in **Table 10-4** and **Fig. 10-3**. The results indicate that the there was no significant dissociation of activity from the labelled drug molecule at lower concentrations of the DTPA. Also there was only 2.9 ± 0.3 % transchelation was occurred at higher concentration (15 mM) of DTPA. This indicates that there was strong binding between drug molecule and the radioactive species ^{99m}Tc. The stable complex of ^{99m}Tc-PTX was found suitable for use for in vivo biodistribution studies.

Table 10-4: DTPA transchelation study for ^{99m}Tc-PTX complex

DTPA Concentration	Transchelation (%) (n=3)
5 mM	1.4 ± 0.2
10 mM	2.0 ± 0.3
15 mM	2.9 ± 0.3

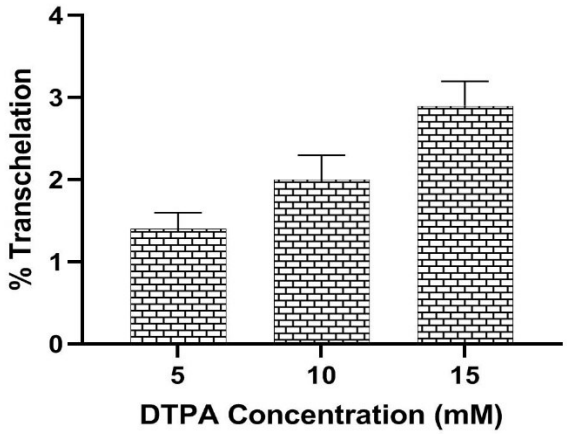

Fig. 10-3: DTPA transchelation study for ^{99m}Tc-PTX complex

10.3.1.4. In vivo biodistribution studies in Rabbits

Targeted drug delivery is emerging as a powerful tool for the treatment of cancer because of enhanced delivery of drugs to a tumour site with protection from the extracellular environment [16]. A given chemotherapeutic or drug can be very selective in blocking a specific cell process; however, this process may not be solely present in target cells, thus yielding toxicities that hamper successful therapeutic outcomes. The precise delivery of anticancer drugs to target cells represents one of the best approaches to improve treatment outcomes; however, developing a therapeutic with both enhanced targeting and pharmacological activities remains a difficult task [17]. Currently available chemotherapies for EC treatment are given as intravenous chemotherapy. Intravenous chemotherapy causes phlebitis at the site of administration that leads to poor patient compliance and more typical chemotherapeutic systemic side effects related with the drug and/or excipients. The vaginal route for drug administration have been well established through its long and well-studied history of safety and efficacy [18]. In recent years, the existence of direct transport mechanisms between the vagina and uterus has been demonstrated, resulting in preferential uterine delivery of hormones that are administered vaginally [19]. The phenomenon known as the first uterine pass effect. Hence, the uterine targeting efficiency of the developed formulation through intravaginal route was assessed to test this novel and unexplored hypothesis. Distribution of the PTX labelled with ^{99m}Tc loaded in UDNVs formulation along with plain PTX labelled with ^{99m}Tc was studied in female rabbits by intravaginal administration. The results of percentage dose distribution in various organs at different time intervals are represented in **Table 10-5** and **Table 10-6**.

Table 10-5: Results of biodistribution of ^{99m}Tc-PTX loaded UDNVs

Organ/Tissue	% dose distribution in different organs				
	0.25 h	1 h	2 h	24 h	48 h
Uterus	90.28	89.23	89.17	82.57	70.45
Spleen	-	-	-	-	-
Liver	-	-	-	-	-
Kidneys	-	-	-	-	-
Stomach	-	-	-	-	-
Intestine	-	-	-	-	-
Heart	-	-	-	-	-
Lung	-	-	-	-	-

Table 10-6: Results of biodistribution of ^{99m}Tc-PTX plain drug solution

Organ/Tissue	% dose distribution in different organs				
	0.25 h	1 h	2 h	24 h	48 h
Uterus	76.39	54.20	14.79	4.36	3.42
Spleen	-	-	-	-	-
Liver	-	-	-	-	-
Kidneys	-	-	-	-	-
Stomach	-	-	-	-	-
Intestine	-	-	-	-	-
Heart	-	-	-	-	-
Lung	-	-	-	-	-

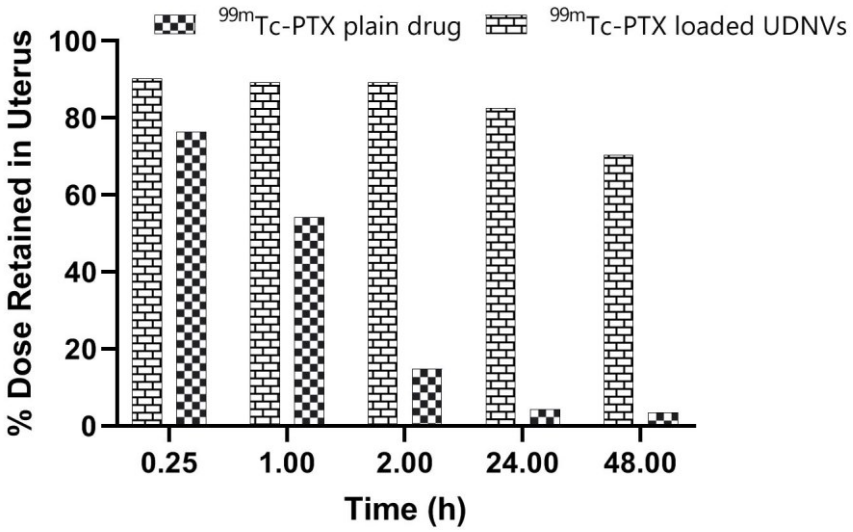

Fig. 10-4: Uterine targeting efficiency of PTX-UDNVs and Plain drug

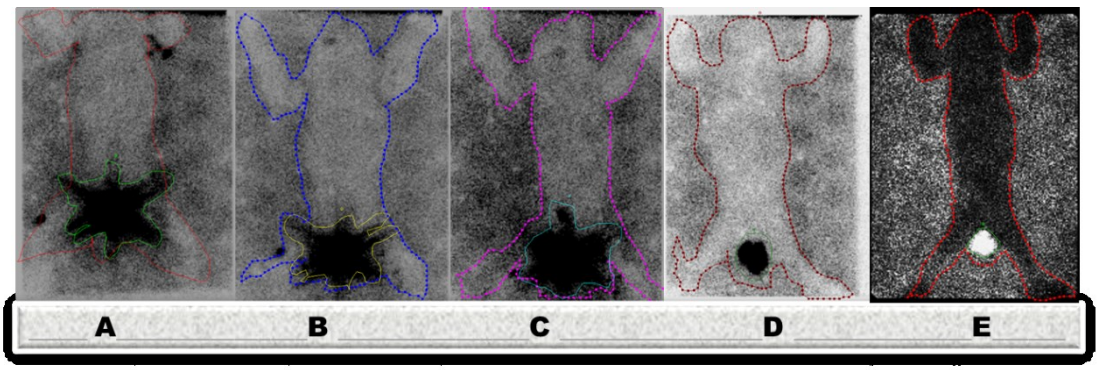

Fig. 10-5: Gamma scintigraphic images obtained at A) 0.25 h B) 1 h C) 2 h D) 24 h E) 48 h after ^{99m}Tc-PTX-UDNVs administration via vaginal route

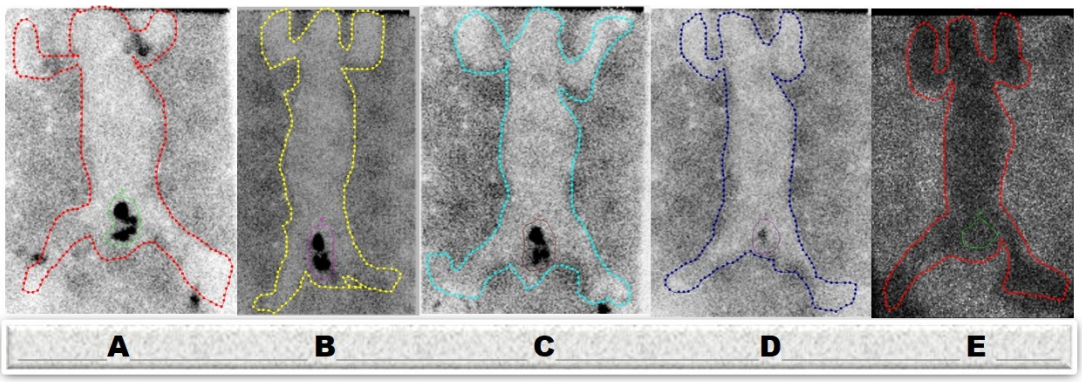

Fig. 10-6: Gamma scintigraphic images obtained at A) 0.25 h B) 1 h C) 2 h D) 24 h E) 48 h after ^{99m}Tc-PTX plain drug administration via vaginal route

The qualitative evaluation of the biodistribution studies in rabbits after intravaginal administration of ^{99m}Tc-PTX-UDNVs formulation and ^{99m}Tc-PTX plain drug is represented in **Fig. 10-5** and **Fig. 10-6** at predetermined time intervals i.e. 0.25 h, 1 h, 2 h, 24 h and 48 h. The results shows that the PTX-UDNVs formulation was able to reach uterus and remain confined in the uterus for longer period of time as compared to PTX plain drug. The results also reveal that when drug was administered through intravaginal route, its systemic absorption becomes restricted which was further confirmed by quantitative systemic absorption study. In a nutshell, results of the investigation confirms uterine targeting of the formulation designed to reach uterus via intravaginal route and remain there for extended period of time avoiding systemic distribution.

10.3.1.5. Quantitative systemic absorption study

Systemic absorption of the ^{99m}Tc-PTX loaded UDNVs and plain drug was quantitatively estimated and compared. The results of quantitative systemic absorption study of ^{99m}Tc-PTX loaded UDNVs and plain drug were represented in **Table 10-7** and **Fig. 10-7**. Results indicate that the systemic absorption was considerably lower in the case of ^{99m}Tc-PTX loaded UDNVs formulation compared to plain drug when administered though intravaginal route.

Table 10-7: Quantitative systemic absorption study of ^{99m}Tc-PTX loaded UDNVs and plain drug

Time (h)	% Radioactivity remaining in Blood	
	^{99m}Tc-PTX loaded UDNVs	^{99m}Tc-PTX plain drug
1	0.01	0.10
2	0.01	0.45
24	0.25	2.53
48	0.89	3.83

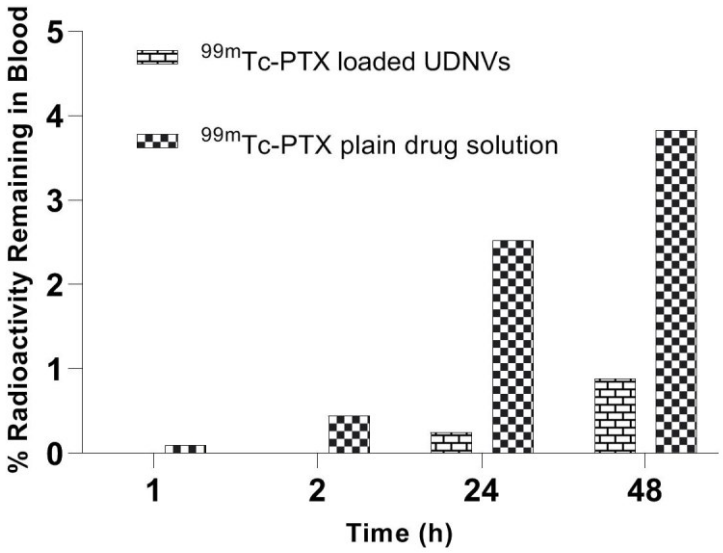

Fig. 10-7: Quantitative systemic absorption study of ^{99m}Tc-PTX loaded UDNVs and plain drug

The drug remains predominantly at the site of action i.e. uterus when administered after loading in UDNVs formulation whereas its leakage from the uterus was higher when given as a plain drug.

The results obtained in the present study with no radioactivity noted in other vital organs of the body of rabbit suggests the targeting efficiency of the route and also insignificant distribution of the drug to other organs which otherwise is a major concern caused by drug administered by other alternate routes exposing the drug to systemic circulation. Overcoming the limitations of currently available marketed

formulation of PTX the prepared formulation administered via vaginal route to treat endometrial carcinoma can increase the patient compliance and provide a cost-effective therapy.

10.3.2. In vivo efficacy study

Even though in vitro studies have decreased the numbers of animal studies still in vivo studies add significantly to understand the efficacy of the drug and formulations in development. Assurance of in vivo efficacy in animal models greatly increases confidence in clinical trials and also important to avoid failures. In vitro data with in vivo data offers the most complete portrayal about behaviour of the formulation. The in vivo efficacy of the PTX-UDNVs and CBP-UDNVs loaded formulations was evaluated in female rabbit model using ultrasound technique.

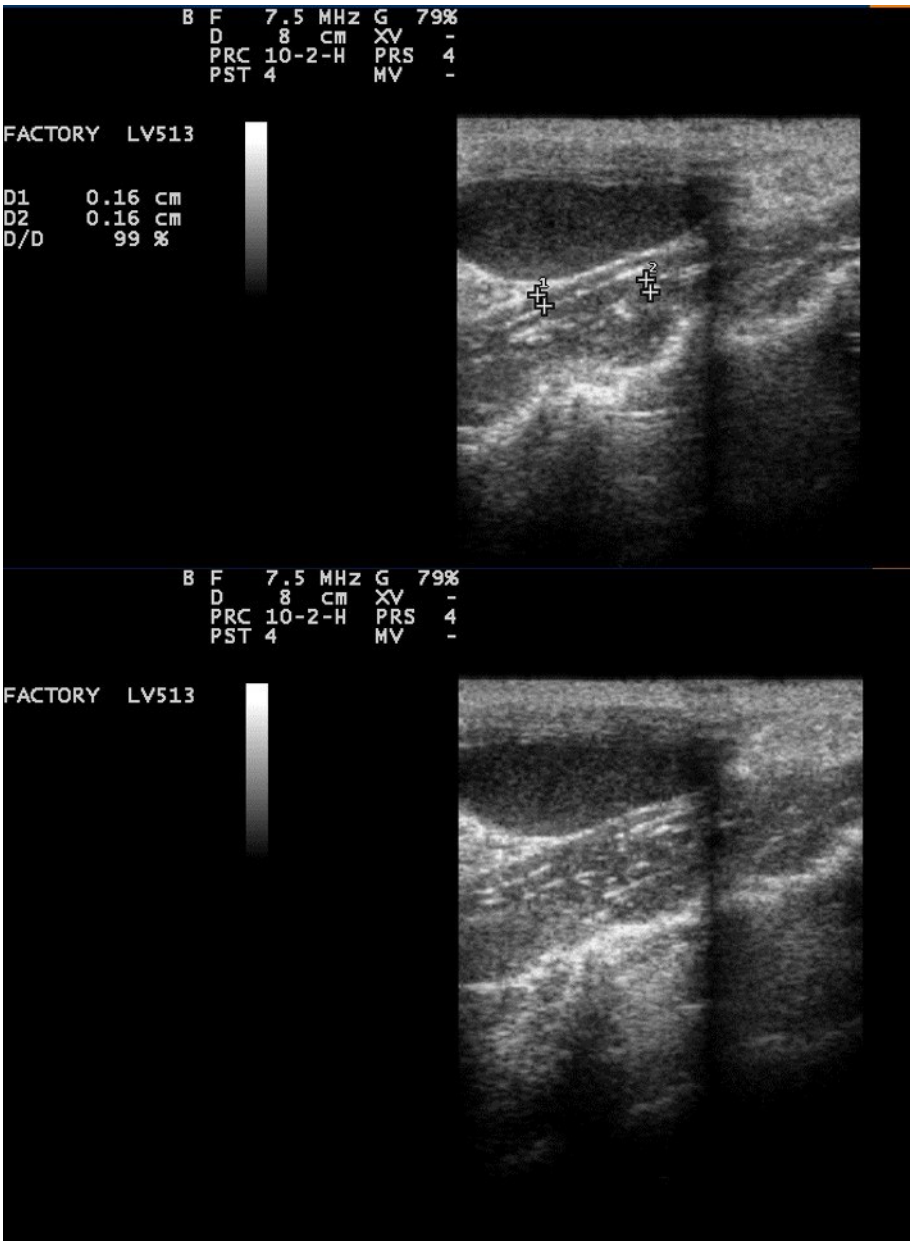

Fig. 10-8: Ultrasonography images of rabbit uterine horns showing clear lumen - Negative control

Tracing of the uterine horns was done successfully to observe lumen of both the uterine horns. Rabbits were kept with free access to water to inflate bladder which was observed as dark portion in the image representing cavity of the bladder filled with urine. The uterine horns were located with reference to urinary bladder and observed carefully and images were recorded as represented in **Fig. 10-8**. The uterine horns lumens could be clearly seen empty with no cellular mass in the cavity.

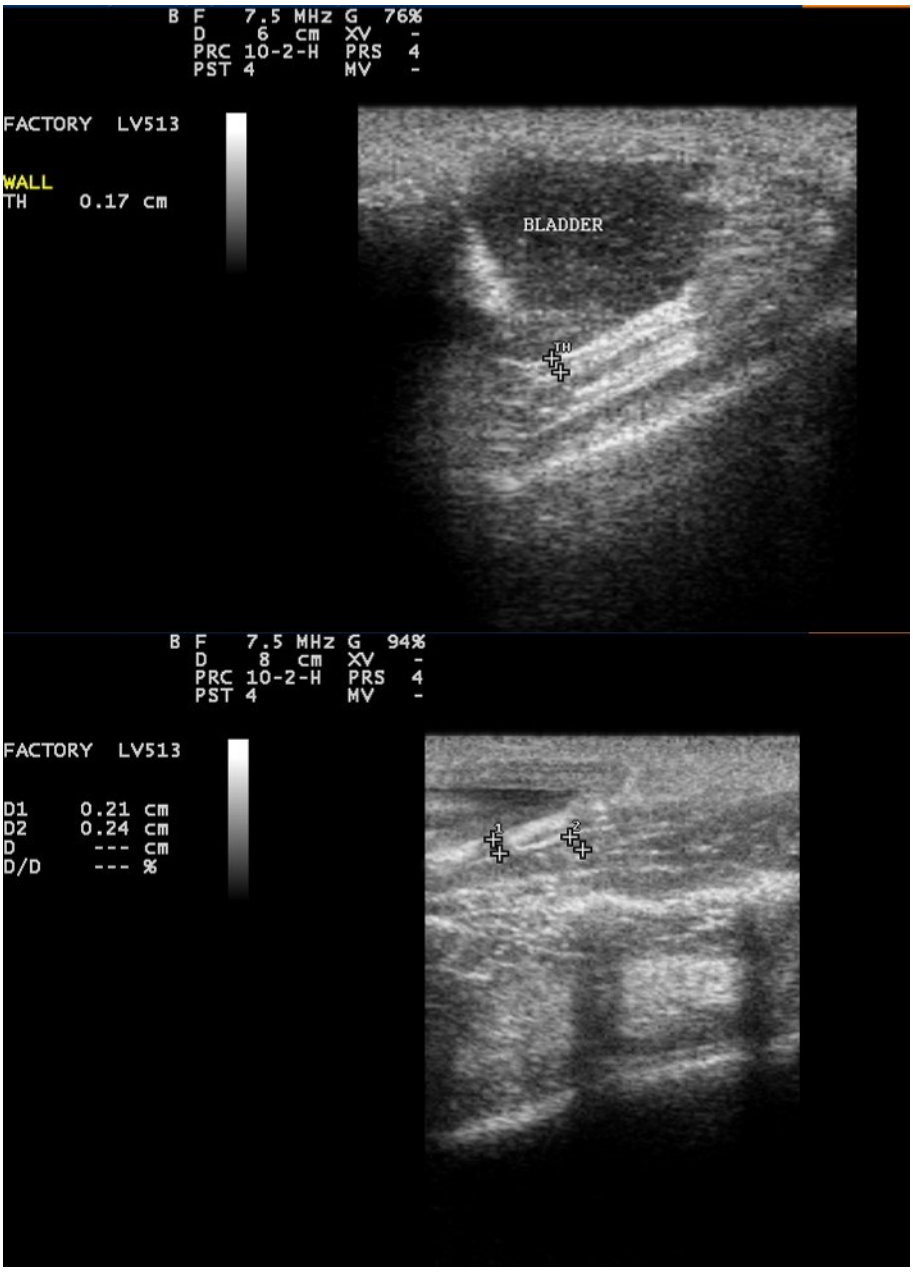

Fig. 10-9: Ultrasonography images of rabbit uterine horns showing lumen with Endometrial Carcinoma - Positive control

After confirmation of the induction of endometrial carcinoma, ultrasound images were recorded as represented in **Fig. 10-9**. The ultrasound graphics reveal that the lumen of both the uterine horns seen filled with cellular mass which could be the reason of proliferated lining of the endometrium inside uterus. The rabbit was considered as positive control for comparison purpose.

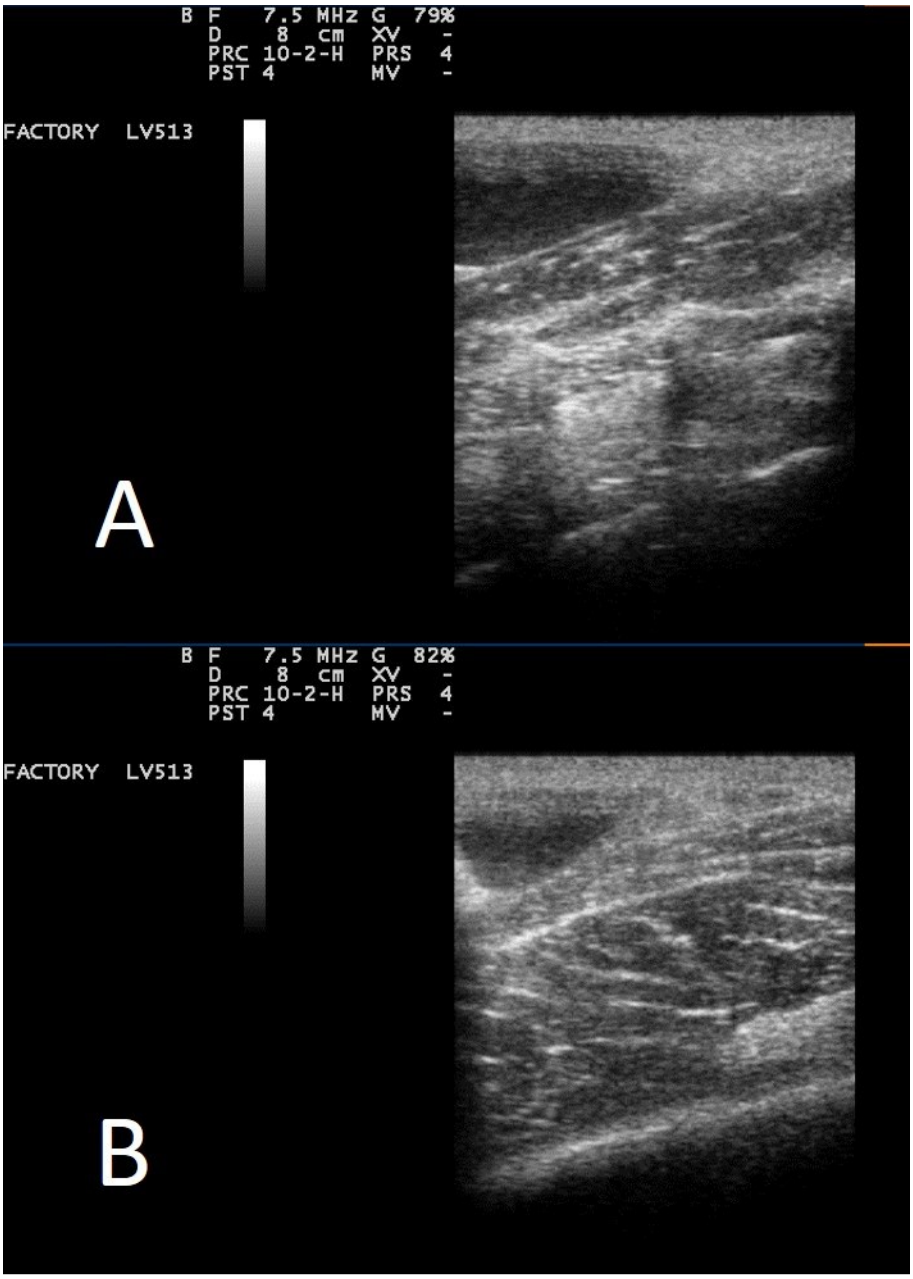

Fig. 10-10: Ultrasonography images of rabbits treated with PTX-UDNVs formulation A) after 2 weeks B) after 4 weeks

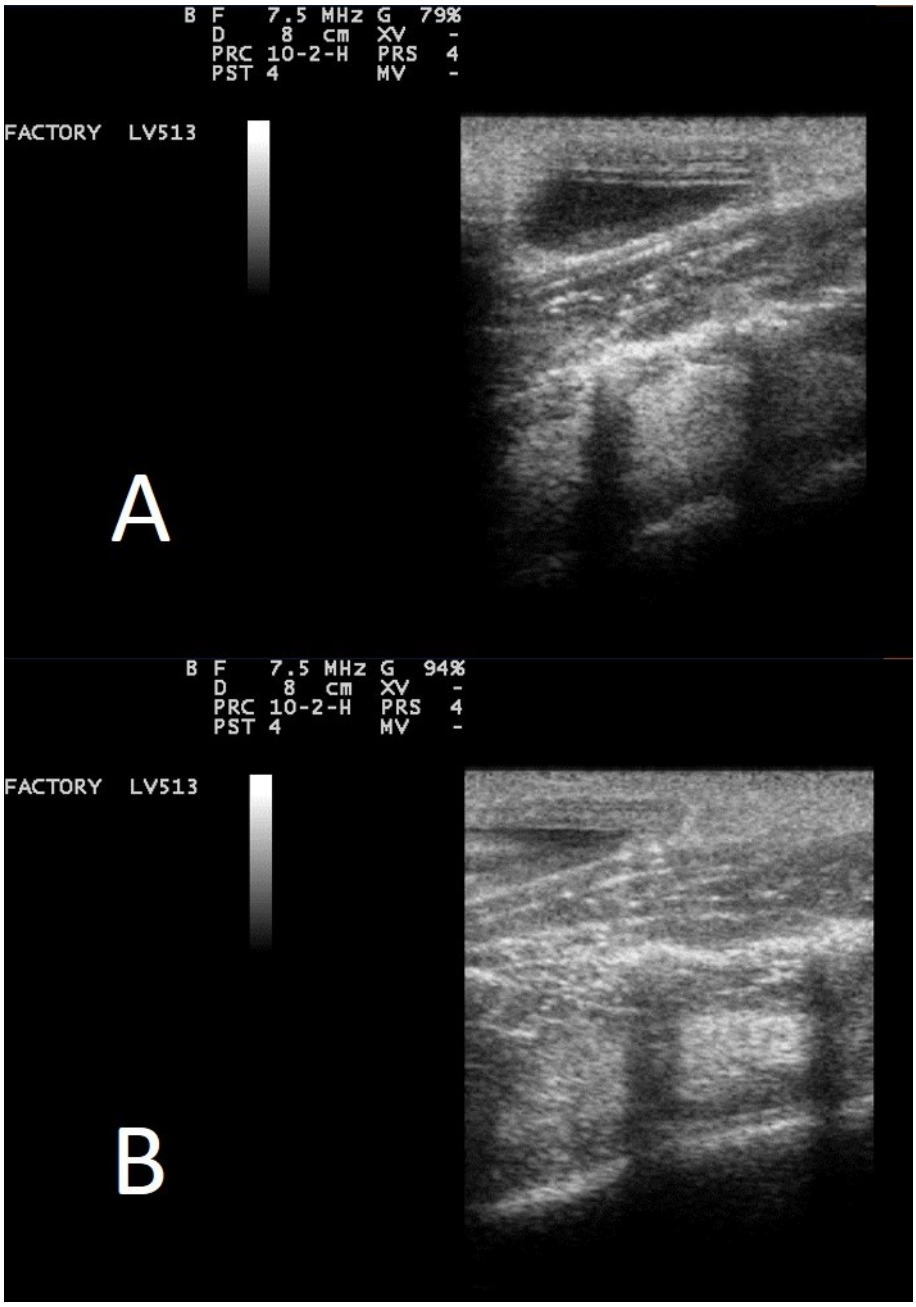

Fig. 10-11: Ultrasonography images of rabbits treated with CBP-UDNVs formulation A) after 2 weeks B) after 4 weeks

Rabbits of group III and IV were monitored for tumour regression after initiation of the treatment by scanning the uterine horns and carefully observing the clearance of the lumen filled with neoplasia using ultrasonography. After second week of treatment in both the groups, noticeable reduction in the uterine horn lumen size and clearance of

the filled tissue mass was detected during ultrasonography. **Fig. 10-10** A) and **Fig. 10-11** A) shows the ultrasonography of the rabbits treated with PTX and CBP formulation respectively after second week of the treatment. The rabbits were continued to be monitored further for assessment of regression in tumour mass. **Fig. 10-10** B) and **Fig. 10-11** B) shows the ultrasonography of the rabbits treated with PTX and CBP formulation respectively after fourth week of the treatment. After four weeks of the treatment, the lumens of the uterine horns in both the groups of rabbits were observed clear of cellular mass. The acoustic images generated by the echoes reflected from the lumen cavity clearly indicated that the proliferation of the endometrial tissue was greatly inhibited by the treatment after four week and the uterine horn lumens of both the group rabbits started recovering from the neoplastic stage. The appearance of the lumen was seen comparable to that of normal rabbit uterine horns since the dark echoes reflected from the centre of the horns represents clearance of the tissue mass from the cavity. The results of the study indicates efficacy of the developed formulations in vivo for the treatment of endometrial carcinoma. Conclusively, the findings of the investigation serve as the basis of successful utilization of the intravaginal route for targeting the uterus by designing the formulation and delivery device which can improve patient compliance.

References

1. Faqi, A.S., *A comprehensive guide to toxicology in nonclinical drug development*. 2016: Academic Press.
2. Walsh, S.A., Z.-X. Luo, and P.M. Barrett, *Modern imaging techniques as a window to prehistoric auditory worlds*, in *Insights from comparative hearing research*. 2013, Springer. p. 227-261.
3. Ying, X. and T.M. Monticello, *Modern imaging technologies in toxicologic pathology: An overview.* Toxicologic pathology, 2006. **34**(7): p. 815-826.
4. Li, C. and M. Tian, *Drug Delivery Applications of Noninvasive Imaging: Validation from Biodistribution to Sites of Action*. 2013: John Wiley & Sons.

5. Willmann, J.K., et al., *Molecular imaging in drug development.* Nature reviews Drug discovery, 2008. **7**(7): p. 591-607.
6. Imaging, N.R.C.C.o.R.C.t.A.C., *Imaging Techniques: State of the Art and Future Potential*, in *Visualizing Chemistry: The Progress and Promise of Advanced Chemical Imaging*. 2006, National Academies Press (US).
7. Hill, T., *Conducting Phase IV clinical studies: a moral imperative?* Ecancermedicalscience, 2012. **6**.
8. Katze, M.G., et al., *Viral Pathogenesis: From Basics to Systems Biology*. 2015: Academic Press.
9. Monteiro, L.O., et al., *Technetium-99 m radiolabeled paclitaxel as an imaging probe for breast cancer in vivo.* Biomedicine & Pharmacotherapy, 2017. **89**: p. 146-151.
10. Jain, V., et al., *Paclitaxel loaded PEGylated gleceryl monooleate based nanoparticulate carriers in chemotherapy.* Biomaterials, 2012. **33**(29): p. 7206-7220.
11. Kaul, A., et al., *Preliminary evaluation of technetium-99m-labeled ceftriaxone: infection imaging agent for the clinical diagnosis of orthopedic infection.* International Journal of Infectious Diseases, 2013. **17**(4): p. e263-e270.
12. Nagaoka, T., et al., *Experimental induction of uterine adenocarcinoma in rats by estrogen and N-methyl-N-nitrosourea.* In vivo, 1993. **7**(6A): p. 525-530.
13. Vollmer, G., *Endometrial cancer: experimental models useful for studies on molecular aspects of endometrial cancer and carcinogenesis.* Endocrine-related cancer, 2003. **10**(1): p. 23-42.
14. Niwa, K., et al., *Rapid induction of endometrial carcinoma in ICR mice treated with N-methyl-N-nitrosourea and 17β-estradiol.* Japanese journal of cancer research, 1991. **82**(12): p. 1391-1396.
15. Niwa, K., et al., *Enhancing effects of estrogens on endometrial carcinogenesis initiated by N-methyl-N-nitrosourea in ICR mice.* Japanese journal of cancer research, 1993. **84**(9): p. 951-955.
16. Rao, K.M., K.S.K. Rao, and C.-S. Ha, *Functional stimuli-responsive polymeric network nanogels as cargo systems for targeted drug delivery and gene delivery in cancer cells*, in *Design of Nanostructures for Theranostics Applications*. 2018, Elsevier. p. 243-275.
17. Maso, K., et al., *Molecular platforms for targeted drug delivery.* International review of cell and molecular biology, 2019. **346**: p. 1-50.
18. Alexander, N.J., et al., *Why consider vaginal drug administration?* Fertility and sterility, 2004. **82**(1): p. 1-12.

19. Cicinelli, E., et al., *"First uterine pass effect" is observed when estradiol is placed in the upper but not lower third of the vagina.* Fertility and sterility, 2004. **81**(5): p. 1414-1416.