Effect of Doxorubicin on the Uptake of 99mTc-DTPA in Rat kidneys

By
Tanzim Razaq
NUST201362100MSMME62413F

Supervisor
Dr. Adeeb Shehzad

Department of Biomedical Engineering & Sciences
School of Mechanical and Manufacturing Engineering
National University of Sciences and Technology
H-12 Islamabad, Pakistan 2016.

Effect of Doxorubicin on the Uptake of 99mTc-DTPA in rat Kidneys

A Thesis Submitted to the Department of Biomedical Engineering and Sciences in the partial fulfillment of the requirements for the degree of

Masters of Science(MS)

In

Biomedical Sciences

Submitted by

Tanzim Razaq

NUST201362100MSMME62413F

Supervised by: Dr. Adeeb Shehzad

School of Mechanical and Manufacturing Engineering(SMME)
National University of Sciences and Technology(NUST)
H-12 Islamabad, Pakistan 2016

Declaration

It is certified that the work presented in this thesis i.e. Effect of Doxorubicin on the uptake of 99mTc-DTPA is done during the scheduled period of study for the award of MS degree in biomedical sciences, National University of Sciences and Technology, H-12, Islamabad Pakistan under my supervision. I have gone through the Dissertation and have found it satisfactory.

Submitted through

Principal/Dean

Supervisor: Dr. Adeeb Shehzad

School of Mechanical and Manufacturing Engineering (SMME), NUST Islamabad.

Dedication

I dedicated this thesis to the sake of Almighty Allah, my creator; my beloved father, Muhammad Razaq Khan who never stop giving of himself in countless ways; to the loving memory of my mother, Makhmal Jan (late) who had been a constant source of encouragement and support in the challenges of life; my husband, Raja Raheel Ahmed Satti for his guidance and kindness at every step; and my beloved brothers and sisters who stands by me when things look bleak.

Acknowledgements

All Praise to Almighty Allah, the most merciful, the most beneficent, who is the source of light, help and provision in completion of this research work.

I am extremely thankful to my parents and husband for their moral and financial support in achieving my Masters degree which had been a challenge than ever.

I feel pride to show deepest sense of gratitude to my Supervisor Dr. Adeeb Shehzad and Cosupervisor Dr. Hasan Nisar for their help, advice, inspired supervision, unlimited support and compassionate attitude throughout the study. Their invaluable feedback and precious technical comments will always help me in my higher studies.

I am indebted to Dr. Sameena Roohi for granting me permission to work in Isotope Production division lab (IPD). I extend my thanks for her to help me in each and every step of the chemical analysis.

Special gratitude to my committee members, especially Dr. Nabeel Anwar (HOD Department of biomedical Engineering and Sciences) for allowing me to do research in PINSTECH and Dr. Umer Ansari for his assistance.

I would like to thank all employees of IPD for their continuous help and assistance.

I am grateful to the whole organization of PINSTECH for providing me the excellent consign to carry out my research.

I forfeit my regards to my colleagues who help me throughout my research.

Table of Contents

List of Tables

List of Figures

List of Acronyms and Abbreviation

IPD	Isotope production division
DOX	Doxorubicin
DTPA	Diethylenetriaminepentaaceticacid
Tc	Technetium
99mTcDTPA	Diethylenetriaminepentaaceticacid labeled with Technetium-99m
TRD	Total retained dose
BFCA	Bifunctional chelating agent
GFR	Glomerular filtration rate
PINSTECH	Pakistan Institute of Nuclear Sciences and Technology

Abstract

Diethylenetriaminepenta-acetic acid (DTPA) is a chelating agent used as a radiopharmaceutical compound, 99mTc-DTPA, for renography. Doxorubicin (DOX) on the other hand is an anthracycline antibiotic frequently used in treatment of various solid malignancies. Both 99mTc-DTPA and DOX may be used in close succession in patients undergoing DOX based chemotherapy to evaluate renal function. Here, effects of DOX on99mTc-DTPA uptake in normal Sprague Dawley rat kidneys have been explored. The study was divided in two arms; a control group (n=10) where 99mTc-DTPA alone and the experimental group (n=30) where DOX was injected prior to 99mTc-DTPA administration. The experimental group was further divided into six subgroups (n=5 each) based on the time intervals (4, 8, 18, 36, 72, 96 hours) between DOX and 99mTc-DTPA administration. In each group, the subjects were sacrificed 2 hours post 99mTc-DTPA injection, the organs isolated and counted for radioactivity. Results revealed that the percentage total retained dose (%TRD) significantly decreased in urinary tract while increased in liver and biliary tree in the experimental group. These results put the accuracy of renal scintigraphy in question in patients receiving DOX based chemotherapy. However, human studies are proposed for validity of results with regards to clinical practice.

Keywords:

Doxorubicin, 99mTc-DTPA, radiopharmaceutical, renography, total retained dose

1. Introduction

1.1. Radiopharmaceuticals

Radiopharmaceuticals are labeled compounds belong to a vast group of pharmaceutical drugs containing radioactivity. Radioactive properties help in making them used as diagnostic and therapeutic purpose. Radiopharmaceuticals incorporate into normal biological processes and eliminates via renal route naturally when it enters in the body of the subject. The radioactive material in the radiopharmaceutical absorbed by a specific organ emits gamma radiation that can be externally imaged through gamma camera and is then decayed soon after imaging is completed to observe the organ functioning. While Santos-Oliveira (2008) described two basic purposes of radiopharmaceuticals, first and the important purpose is to find the abnormal alterations in the body while secondly it is used as a tracer to find out the biochemical process in the body. With the advent of the 19^{th} century the field of nuclear medicine started on using radiopharmaceuticals in battling against the human diseases. Nuclear medicine made the diagnosis of diseases very easy and efficient, where such diagnosis took months and years nuclear medicine reduce the process to no time (Mettler Jr, Huda, Yoshizumi, & Mahesh, 2008).In these processes radioactive tracers are used which are chemical compounds in which the atoms are exchanged either one or more than one by a radio isotope where a decay process will start by exploring the chemical reaction and tracing the pathway of the mechanism.

Similarly, radioactive tracers are deployed in the human body, it may be injected in blood flow or the metabolism and sometimes induced in to the morphology of an organ (Gomes, Bezerral, & Bernardo-Filho, 2001). This will help in identifying the probable chemical reactions and these isotopes give whole information of the reaction i.e. from the reactants to the final product. The radioisotopes of iodine, carbon, hydrogen, sulphur and phosphorus are mainly used for location reactions. With the advent of such radiopharmaceuticals in the field of medicine it has brought medicine to another level. The radioactive properties help to provide clear images of the problem and so have made the diagnosis easy and efficient. They are administered according to the need and timing of the diagnostic imaging process.

1.2. Scintigraphy

Scintigraphy is a diagnostic test in which anatomy and physiology of a specific organ or tissue are assessed by the formation of two dimensional image detected by gamma camera using gamma radiations emitted by radiopharmaceutical.

Renalscintigraphy is conducted to evaluate

- Renal perfusion and function
- Urinary tract obstruction
- Infection
- Presurgical quantitation
- Renal transplantation
- Congenital anomalies

1.3. Technetium

Technetium having atomic number 43, half life of 215000 years, an artificially created atom from the nuclear reactors by the fission reaction belonged to most profuse member of the family of long lasting fission yield is so unstable atom that it does not occur in nature.

1.4. Technetium-99m

Technitium-99m is a widely used radiotracer isotope in nuclear medicine being involved in 80% of scintigraphies. It has a physical half life of 0.25 and biological half life of 1 so it is clears from the body very rapidly after an imaging. Gamma energy not accompanied by beta emission permits more precise alignment of imaging detectors. Technitium-99m is an excited state of technetium which subsists for several hours before returning into its ground state. Researchers use technetium 99m in their experiments because of half life of this compound which is 6 hours, these nucleuses is made use by researchers. Technetium-99m nuclei emit 140keV characteristic gamma rays in the transition from excited state to ground state. ^{99}mTc or technetium-99m is a gamma emitter with diethylenetriamine penta-acetic acid distribution of radioactivity which is labeled with the technetium-99m has bought a varied change in the nuclear medicine because of its imperative and numerous applications. These applications include the imaging of the vascular tumors (intramuscular hemangioma), hemorrhages of gastrointestinal tract and also circulatory system (cardiovascular system) (Al-Nahhas et al., 1988; Chandra & Rustgi, 1998; Santos-Oliveira, Smith, & Carneiro-Leão, 2008) Mostly when the blood cells are labeled with 99mTc stannous ions , reducing agents are required (Chandra & Rustgi, 1998; Sampson, 1993). Therefore, from its initiation in the clinical usage it has played a vital role in detecting the abnormalities specifically functional in the urinary tract (Al-Nahhas et al., 1988) as mainly because it is cleared by the process of glomerular filtration (Hilson, Maisey, Brown, Ogg, & Bewick, 1978).

1.5. Production of technetium-99m

When 98Mo are bombarded with the neutrons technetium-99m is produced. The decay rate of the resultant 99Mo is 66 hours which then resulted in the formation of technetium-99m followed by the production of metastable state of technetium. 99Mo can be separated from other fission products of 235U fission and then used to generate 99mTc.Technetium 99m is used as pertechnate (TcO_4^{-}) for medical purposes.

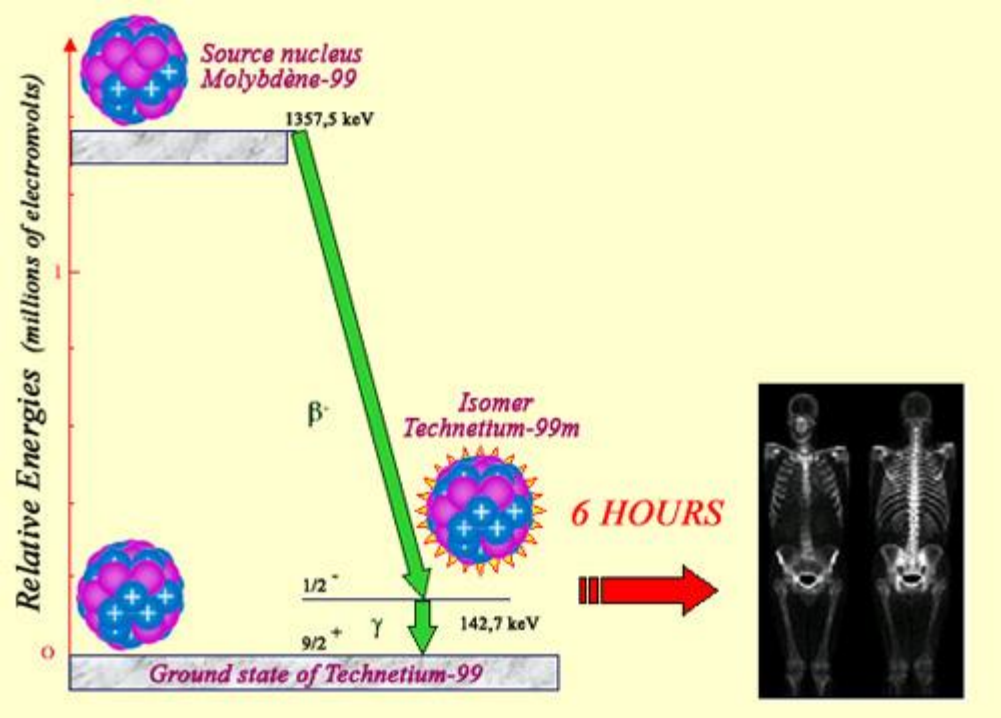


Fig1: Production of Technetium-99m

Technetium-99m radiopharmaceuticals are used in several diagnostic procedures, where Doctors employ pertechnetate for thyroid uptake and even the usage of 99mTc-octreotide derivatives to image neuroendocrine tumours. One of the factors for the development of Radiopharmaceuticals is for the reason that 99mTc has versatile chemistry. 99mTc multiple oxidation state made it possible to produce a variety of complexes with specific desired characteristics. Approximately 30 of the Technetium-99m radiopharmaceuticals are used in clinical studies.

The development of 99Mo/99mTc generator in the early 1960 at the Brookhaven National Laboratory brought a significant change in the field of Nuclear Medicine from where research on 99mTc radiopharmaceuticals began.

Technetium-99m radiopharmaceuticals have been divided into three levels depending on their complexity.

1.6. Classification of Technitium-99m Radiopharmaceuticals

1.6.1. First generation Technetium-99m radiopharmaceuticals

They are based on biological properties of 99mTc complexes such as absorption, distribution, metabolism and excretion. The first generation 99mTc radiopharmaceuticals are used to evaluate the function of thyroid, liver, bone and kidney and the radiopharmaceuticals used for these procedures were 99mTcO4–, 99mTc-colloids, 99mTc-phosphonate and 99mTc-DTPA respectively.

1.6.2. Second generation Technetium-99m radiopharmaceuticals

The molecular structure of coordination compounds and biological behavior of 99mTc agents were determined using the techniques of nuclear magnetic resonance spectroscopy, mass spectroscopy and X ray diffraction. Some common used second generation radiopharmaceuticals are 99mTc-MIBI, 99mTc-tetrofosmin, 99mTc-HMPAO and 99mTc-ECD for use in cardiac imaging and brain imaging respectively.

1.6.3. Third generation Technetium-99m radiopharmaceuticals

In this category, special biomolecules were used as a carrier vector to deliver specific amount of radioactivity to more precise targets such as receptors. For that purpose, labeling is employed in such a manner that the radionuclides do not disturb the biological activity. Bifunctional chelating agent (BFCA), Tc-tricarbonyl, Tc-nitrido, Tc-HYNIC and mixed ligand complexes have helped to achieve that objective. The 99mTc-HYNICEDDA-TOC for neuroendocrine imaging used for receptor studies is the best example of third generation 99mTc radiopharmaceuticals.

Diethylenetriaminepentaacetic acid (DTPA) is a polycarboxylic acid consisting of a Diethylenetriamine with five carboxymethyl groups that acts as chelating agent for heavy metals and as decorporation agent for radionuclides.

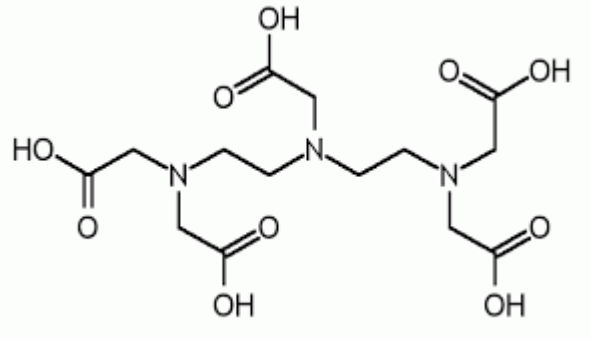


Fig 2: Molecular Structure of DTPA

1.7. Diethylenetriaminepentaaceticacid complexes

The DTPA has been widely used with different complexes for different purposes. For example stomastatinscintigraphy employs the complex of this compound [111In-DTPA-DPhe1] for diagnostic purpose. The Diethylenetriaminepenta acetic acid neolactosyl human serum albumin labeled with technetium-99m (99mTc-DTPA-LSA kit) is used for the imaging of hepatic receptor. The DTPA works as an effective and efficient compound when it is radio labeled with radioisotope for therapeutic purposes such as ^{90}Y or ^{177}Lu. The ^{99m}Tc-DTPA is the most frequently used as radiopharmaceutical compound for renography (GATES, 1983). When ^{99m}Tc-DTPA is used for renal scans it provides information of excretion, rate of renal blood flow, renal functioning, Glomerular Filtration Rate (GFR). This can help to study different renal problems like presence or extent of renal dysfunction and renal obstruction; working differences and comparisons of two kidneys of the same or different individuals (differential function).

1.8. Mechanism of Action of DTPA

The exclusive renal mode of clearance for Tc99m-DTPA facilitates its use in radioscintigraphy of kidneys. Specifically, the administered radiopharmaceutical is filtered by the renal glomeruli thereby allowing to measure glomerular filtration rate (GFR). Subject to single injection or continuous infusion, DTPA binds to plasma proteins in variable percentage which may decrease its glomerular filtration rate and hence lead to a measured clearance rate lower than the actual value. Amongst the various indications of Tc99m-DTPA for clinical use, GFR is measured in cancer patients prior to or following chemotherapy to assess renal function/ toxicity of the chemotherapy drug with suspected nephrotoxicity.

1.9. Doxorubicin

The Doxorubicin is a semi-synthesis (bacterial species) drug, this medication is commonly known under a trade name of Adriamycin (Ouyang, Wang, & Tang, 2014). This drug is commonly used in the treatment of cancer patients mostly those who are suffering from hematological malignancies/blood cancers which include leukemia and lymphoma, compact tumors and also helps in treating other soft tissue sarcomas. This drug usage is well known because of its inculcating nature with DNA.

DOX is widely distributed throughout the body compartments, binds extensively to plasma proteins and mainly metabolized in the liver. Moreover, DOX is primarily eliminated through bile in the stool with less than 10% cleared through the kidneys.

1.10. Mechanism of Action of Doxorubicin

Its mechanism of action includes generation of reactive oxygen species (ROS) that subsequently induce DNA injury in rapidly proliferating cells. The Quinone ring in its structure can be reversibly reduced in vivo by the action of FLAVA containing enzymes, producing oxidative damage in nearby cellular components in the process. DOX induced free radical damage is particularly amplified in the presence of iron. The drug potentiates its own cytotoxic effect by not only leaching out iron stored in Ferric form in the body as Ferritin but also by releasing the iron (Fe3+) bound to its carrier plasma protein, namely Transferrin. The ability of DOX to increase free iron plasma concentration is ascribed to its metal chelating properties.

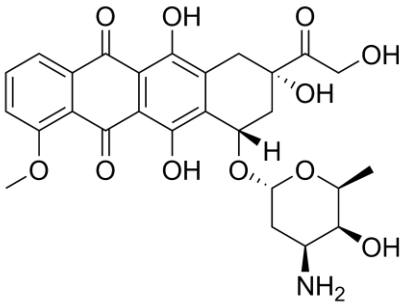


Fig 3: Molecular Structure Doxorubicin

1.11. Drug Radiopharmaceutical interaction

Concurrent administration of chemotherapy drugs and radiopharmaceuticals pose significant risk of interactions between them besides other, on account of their pharmacodynamics and pharmacokinetic properties. The radiopharmaceuticals drug interaction is a complex study especially because with the increase usage of radiopharmaceuticals and medication(Al-Nahhas et al., 1988). As, this interaction can create a state of disorder such as the drugs which are used for the acceleration of metabolism it can speed up the radiopharmaceutical hence, increase the processes of clearance however if it is for repeating purpose it will leads to negative consequences (Santos-Oliveira et al., 2008).

The biodistribution of radiopharmaceutical involves the following processes:

- Absorption
- Distribution
- Metabolism
- Excretion

It has been reported that iodine uptake and imaging are altered with all type of radiotracers.

1.12. Classification of Drug Radiopharmaceutical interaction

Drug Radiopharmaceutical interactions are classified as

- Pharmacologic
- Pharmacokinetic
- Pharmaceutical
- Interactions due to tissue toxicity
- Unknown

Drug interactions may arise from a variety of factors including a particular drug's pharmacological action, physicochemical interactions between drugs and radiotracers, age, stress, smoking and many other factors. A family of isoenzymes called cytochrome p450 in which some drugs induce and some inhibits p450 isoenzyme.

Indeed, considerable body of evidence on such drug radiopharmaceutical interactions (DRIs) supports this hypothesis. The extensive literature review has helped to find out that past research carried out in this area is limited to specific drugs and their effect on 99m Tc conjugated with different pharmaceutical complexes (Bernardo-Filho et al., 2005)

It has been demonstrated in an experimental study that ^{201}TI uptake increases in hearts of doxorubicin treated rats and resulted in a slow washout from the myocardium (Miyagawa et al. 1991, Yurekli et al. 2005). The authors Latham et al. (1992), state that dipyridamole increases or, depending on the concentration may decrease, the excretion of ^{99}mTc DTPA by the kidney.

The chemotherapy drug appears capable for altering the chemical identity of the radiopharmaceutical or the physiological status of the given organ and may result in false negative or false positive evaluation of the clinical radio-tracer study, respectively. Consequently, investigation of DRI is essentially required towards clear understanding of clinical radiopharmaceutical-based studies. To this end, the interaction of DOX with 99mTc-DTPA has been explored.

1.13. Literature Gap

The extensive literature review has helped to find out that past research carried out in this area is limited to specific drugs and their effect on Technetium-99m conjugated with different pharmaceutical complexes (Bernardo-Filho et al., 2005). The effect of Doxorubicin on Technetium-99m DTPA is not directly studied in the literature. However, the following study is to provide a consolidated result of radiopharmaceutical drug interaction. With such a broad scope of the study we have confined our research with the usage of Doxorubicin on Technetium-

99mDTPA uptake on renal scanning. This project will solely focus on this issue, which arises when the drugs used for treatment of the patients may affect the uptake or bio distribution of these compounds and the effective relationship between the drug "Doxorubicin" and 99m Tc-DTPA uptake.

1.14. Problem statement

The review of several studies indicated that 99mTc-DTPAis used in renal scan of the patients. This will help in getting information about Glomerular Filtration Rate (GFR). After its GFR it can be decided that whether the patient is fit for further treatment, especially chemotherapy. It also helps in monitoring renal functioning during and after chemotherapy of the patient with the help of renal scan. But the problem arises in the scan, when the drug which is administered to the patient for his treatment, will affect the 99mTc-DTPA uptake. This will ultimately affect the renal scan.

1.15. Research Questions

This research will help to find out answers of various questions like:

1. Is Technetium-99m DTPA effectively used for renal scan?
2. Is there any effect of drugs used for treatment of patients, on the uptake of Technetium-99m DTPA?
3. How much the increase or decrease in the uptake of Technetium-99m DTPA will occur with the passage of time?
4. The TRD% will be measured in different organs of mice so it will also be studied that, is there any difference in Total Retained Dose (TRD %) between the organs of the rats?

1.16. Aims and objectives

1. To study any changings in the uptake of 99mTc-DTPA, after the administration of Doxorubicin in rats.
2. To compare the results of control group (99mTc-DTPA administered only) with other experimental group of mice in which both the drug Doxorubicin and 99mTc-DTPA will be administered
3. To study if drug interaction (which may causes increase or decrease in 99mTc-DTPA uptake) have any impact on the specificity and sensitivity of 99mTc-DTPA renal scan.

2. Literature Review

The complex anatomy of human body is very difficult not only to understand but also the identification of the diseases, their right type and severity level is difficult to identify. In the field of medical sciences, scientists and professionals have done enormous progresses but still with every subsequent day more complex problems emerge. To cure any ailment the fore most phase is to identify the nature and cause of the problem. To diagnose the problem referring to the medical science is to ascertain the cause and effect of the problem(Mettler Jr et al., 2008). Even though after proper diagnosis of the diseases next step is to give treatment of the disease and it should be in an environment that is safe for the patients.

2.1 Nuclear Medicine:

Nuclear medicine is one of the emerging fields in the medical sciences. It has created marvels in this field, from its radioactive substances it can serve both the issues, at one end it helps in diagnosis and on the other end it also helps in the proper treatment of the diseases (Love, Tomas, Marwin, Pugliese, & Palestro, 2001). However, it is not only confined to this but it also facilitates by providing information before, during and after the surgical procedures. In diagnosis of such problems they identify the type and kind of the problem. And few of these procedures help to give treatment options that are effective, efficient and painless. Before these radioactive procedures diagnosis was a lengthy process but now due to nuclear medicine these procedures are quick and accurate with a minimal chance of inaccuracy(Jurisson & Lydon, 1999).

The procedures of nuclear medicine involve the usage of radionuclides, it can demonstrate both the functional as well as structural variations. It has a special feature i.e. decay process to achieve stability, its decay at a constant rate. This constant rate of decay is also known as its half-life, during the process it emits radiation, and these emissions are electromagnetic while they are observed through radiation detectors (Moyes et al., 2008). The radionuclides become radioactive with the change of its one or more than one atom than it is bound to another chemical compound. As to localize any organ each chemical has its own effects, therefore for each organ a specific chemical compound is used. This combination of radionuclide with the chemical compound is known as radiopharmaceutical.

Radiopharmacology deals with the radiopharmaceuticals for diagnostic and therapy of certain diseases. Radiopharmaceuticals uses lays on the principle called as tracer, which was invented in 1912 by a chemist known as Georg von Hevesy (1885-1966). He gave the idea that radioactive nuclides have identical properties than those of non-radionuclide, than they are used as “tracer”

of different psychological behaviors and biochemical integration in the human body (Bernardo-Filho et al., 2005). The basic purpose of this “drug” is to inspect a disease process by observing the images of the tracer and with the images abnormal bio-distributions interactions will be immediately noticed and such that diagnosis of various diseases will be done. These “drugs” reveal the altered state that might be because of any reason such as if the physiology of the organ system, duct blockage and any of diseases process thus, the radiopharmaceutical helps in reflecting the abnormality.

3. Methods and Materials

Freeze dried kits of DTPA were prepared by Isotope Production Division (IPD) of Pakistan Institute of Nuclear Science and Technology (PINSTECH). Tc-99m was obtained from 99Mo/99mTc generators, manufactured by IPD, PINSTECH. Platform for radio labeling of DTPA kits with 99mTc, the quality control of the radiopharmaceutical and bio-distribution of 99mTc-DTPA in animals was also provided by IPD, PINSTECH. Sprague-Dawley male rats with a mean age of 6 weeks were acquired from National Institute of Health (NIH), Pakistan for the study. Doxorubicin Hydrochloride was purchased from Pfizer, Pakistan in injectable form and the reference dose for rats was calculated by finding a dose equivalent to 50 mg/m2 in humans with the help of "Equivalent Surface Area Dosage Conversion Factors. The km factor given by Freireich, EJ, et al for rat is 5.9. The dose to be administered came out to be 9.46mg/kg. The mean weight of the rats used in the study was 55g so that the actual administered dose to each rat was 0.5 mg.

3.1 Labeling of DTPA with Tc-99m

For the purpose of the study, Tc-99m was freshly eluted from Mo-99/Tc-99m generator in the form of sodium pertechnetate (Na99mTcO4) in 2mL solution (activity≈ 370 MBq=10 mCi in 2 ml).

A freeze-dried vial of DTPA was reconstituted with the above mentioned solution. The vial was stirred for 2 min for complete dissolution of DTPA in the Tc-99m solution. This was followed by an incubation period of 10 minutes at room temperature to ensure proper labeling. The 99mTc-DTPA labeled in this manner was utilized for our experiments well within 4 hours of labeling.

3.2 Quality Control of Tc-99m -DTPA Radiochemical

3.2.1 Stability

The average amount of stannous chloride in the kit was ascertained using iodometric analysis. Stannous salts are used as reducing agents for converting technetium in the +7 oxidation state in the pertechnetate to a desired lower oxidation state, which complexes with the ligand to form the desired radiopharmaceutical. It is crucial to maintain a minimum Sn(II) level, as very low amounts of Sn(II) will result in inadequate oxidation of technetium, while high amounts will damage the compound formed.

The DTPA kit was reconstituted with 5 mL of saline; 1 mL of 1.5N HCl and two drops of starch indicator were added to the kit vial. Simultaneously, a blank (control) was set up with 5 ml of saline. The solution was titrated against 0.0015M iodine solution until the blue color

persisted. One millilitre of iodine solution corresponds to 0.564 mg of SnCl2.2H2O. The average amount of stannous chloride in the kit was ascertained to be at least 50% of the expected value of 0.16 mg/ml.

Fig 4: Weighing Balance

3.2.2 Purity

Quality control analyses of the reconstituted kit containing Tc99m-DTPA were performed in accordance with institutional (i.e. PINSTECH) protocols by ascending paper chromatography.

Free technetium as 99mTcO4-, in the radiochemical solution was determined using Whatman paper No.3 strip as a stationary phase and acetone as a mobile phase (system-1). Strip measuring 14 ×2 cm was spotted with 5µL of the prepared sample and placed in a jar containing the mobile phase. When solvent reached the solvent front, the strip was scanned using a 2π scanner for radio-chromatography. The Tc99m-DTPA and reduced/hydrolyzed Tc-99m remained at the origin, (Rf=0) while free 99mTcO4- moved towards the solvent front (Rf =1).

Reduced/ hydrolyzed 99mTc was also determined using Whatman paper 3 strips (14 × 2 cm) as stationary phase but this time, saline (0.9%) was used as mobile phase (system–2). The remaining procedure was same as described earlier. The reduced/ hydrolyzed 99mTc remained at the origin (Rf=0) while the Tc99m-DTPA and free 99mTcO4 moved towards the solvent front (Rf=1).

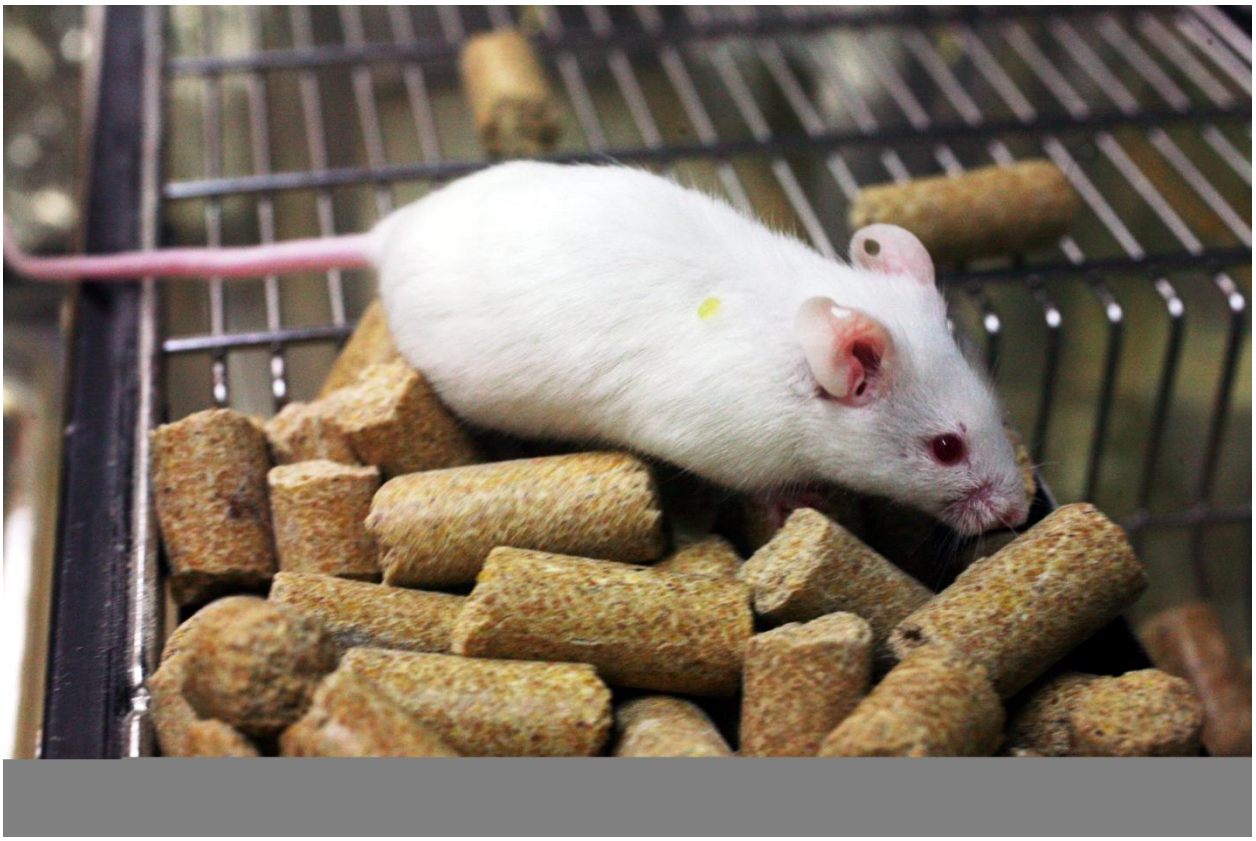


Fig 5: 3 weeks old Sprague Dawley rats

3.3. Bio-distribution Study

Male Sprague Dawley rats with a mean weight of 55 g were used for the bio-distribution study of DOX and 99mTc-DTPA. A prior approval was taken from the ethics committee of the institute for the use of animals in these experiments which were performed in accordance with the guidelines provided by the committee.

The study was divided in two arms; a control group (n=10) where99mTc-DTPA (0.1mCi by taking 0.02ml of the prepared Tc-99m activity) alone was injected intravenously in the tail vein of the rats and the experimental group (n=30) where 0.5 mg DOX was injected (0.5 mg by taking 0.25mL of the prepared drug solution) prior to 99mTc-DTPA (0.1mCi) administration.

Fig 6: Intravenous injection of DXR through tail vein of rats

The experimental group was further divided into six subgroups (n=5 each) based on the time intervals (4, 8, 18, 36, 72, 96 hours) between DOX and 99mTc-DTPA administration.

In each group, the subjects were sacrificed 2 hours post 99mTc-DTPAinjection; the organs isolated were liver, spleen, stomach, intestines, lungs kidneys, femur, bladder, heart, and carcass. The organs were washed in saline, dried on filter paper, weighed on a weighing balance and counted for radioactivity using a well type gamma counter. The weight per gram of each organ was found by the formula,

Weight per gram=%TRD of each organ /weight of each organ.

Fig 7: Dissection and isolation of different organs

The urine was also collected in a filter paper. The results of bio-distribution were expressed as percent of administered dose per gram of tissue (organ) and percentage of total activity in organ. The percent total urinary tract activity was calculated as the sum of percent total activity of kidney, bladder and urine. The average percent total activity of both control and experimental groups was then compared for assessment of possible effects of the DOX on the renal function and uptake of 99mTc-DTPA in kidneys.

The Total retained dose percentage for each organ was calculated from the data. In order to find out the relevance of the results for test groups in comparison to the control, the total retained dose percentage (%TRD) was calculated for each organ using the formula,

TRD=A/B*100

where A and B are the counts in the given organ and counts in all organs except tail respectively.

4. Results

4.1. Statistical Analysis

The results from the two groups were analyzed and compared for statistical significance using paired two-tail t test. The commercial software package SPSS-19 was used for this purpose.

The results of bio-distribution study in various isolated organs of rats with particular emphasis on urinary tract and liver for both control and experimental groups are presented herein.

The administered radiopharmaceutical 99mTc-DTPA is primarily taken up by the kidneys, rapidly passed on to the ureters and bladder and finally eliminated from the body through urine.

Consequently, %TRD was calculated for the entire urinary tract plus urine rather than kidneys alone; the results are summarized below which reveals an initial increase followed by progressive reduction in uptake of 99mTc-DTPA as the time span between the drug and radiopharmaceutical administration is increased.

Table 1: Mean %TRD of kidney, urine and bladder in control (n=10) and test group (n=5) injected with 0.5mg DXR/0.25 ml of saline prior to the injection of 99mTc-DTPA.

Groups and Subgroups	**Mean% activity of kidney, urine and bladder**	**Standard deviation**	**p-value**	**Significance/non significance at confidence level of 0.05**
Control(n=10)	88.016	± 1.94		
4 hour(n=5)	89.3875	± 5.89	0.6393	Not significant
18 hours(n=5)	93.52	± 3.96	0.0367	Significant
24 hours(n=5)	80.253333	± 7.27	0.0576	Not quite significant
48 hours(n=5)	63.563333	± 5.53	<0.0001	Extremely significant
72 hours(n=5)	17.606666	± 2.10	<0.001	Extremely significant
96 hours(n=5)	34.29	± 29.77	0.0096	Very significant

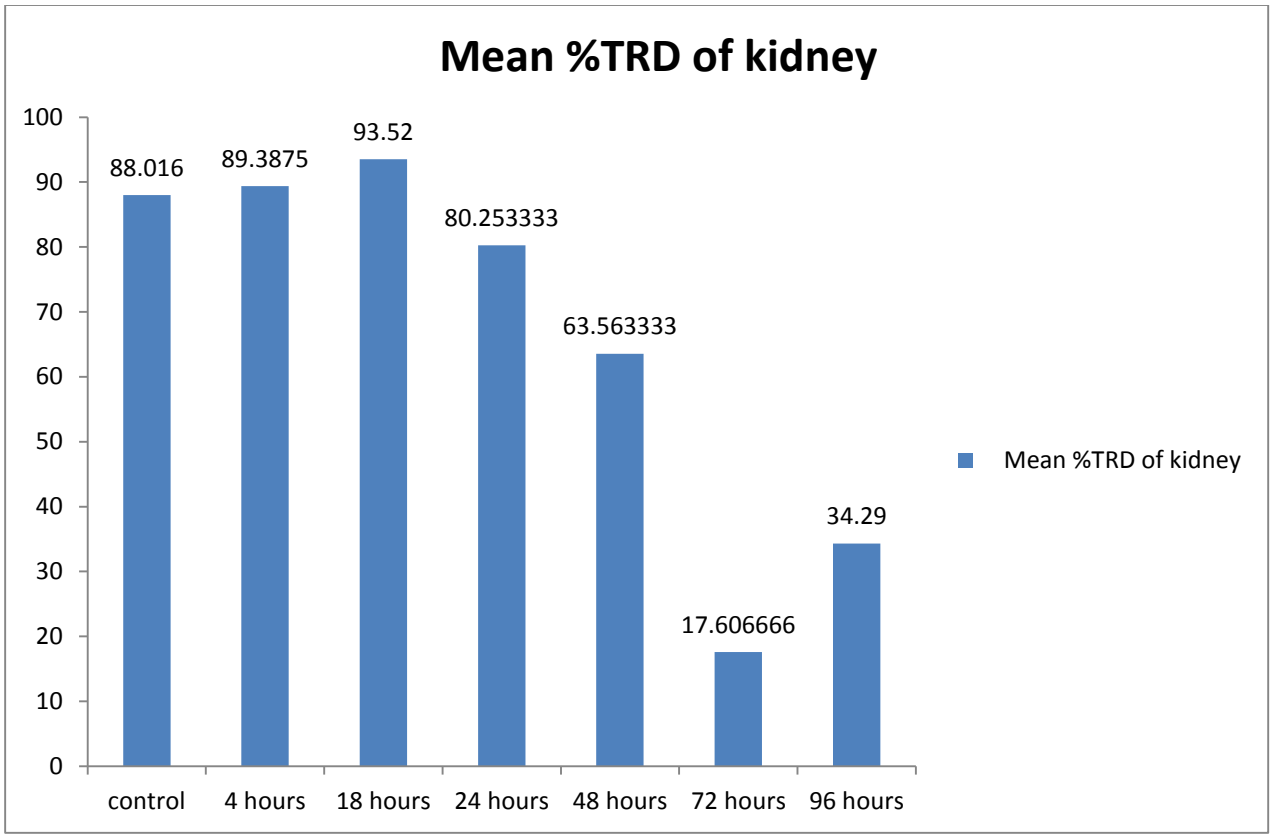


Fig 8: Cumulative mean %TRD of kidney, urine and bladder in Control (n=10) and Experimental group (n=5 each). Time (in hours) on x-axis shows the gap between administration of DOX (0.5mg DOX/0.25 ml of saline) and 99mTc-DTPA.

Liver has been believed as the major body compartment for DOX accumulation; thereby liver constitutes an interesting target for % TRD calculations in our study. The results of %TRD in liver are illustrated in Fig 9. Contrary to urinary tract, the %TRD in liver increases with the time interval between the drug and radiopharmaceutical administration. In addition, the %TRD for liver is significantly lower as compared to urinary tract.

Table 2: Mean %TRD of liver in control (n=10) and test group (n=5) injected with 0.5mg DXR/0.25 ml of saline prior to the injection of 99mTc-DTPA.

Groups and Subgroups	**Mean% activity of Liver**	**Standard Deviation**	**P-value**	**Significance/nonsignificance at confidence level of 0.05**
Control	0.848			
4 hours prior to 99mTc-DTPA	0.31	± 0.07594	0.0008	Extremely significant
18 hours prior to 99mTc-DTPA	0.3233	± 0.10969	0.0042	Very significant
24 hours prior to 99mTc-DTPA	0.4166	± 0.10969	0.1020	Significant
48 hours prior to 99mTc-DTPA	0.4066	± 0.3100	0.0402	Significant
72 hours prior to 99mTc-DTPA	8.98	± 2.7435	0.0004	Extremely significant
96 hours prior to 99mTc-DTPA	1.1893	± 0.5623	0.2377	Not significant

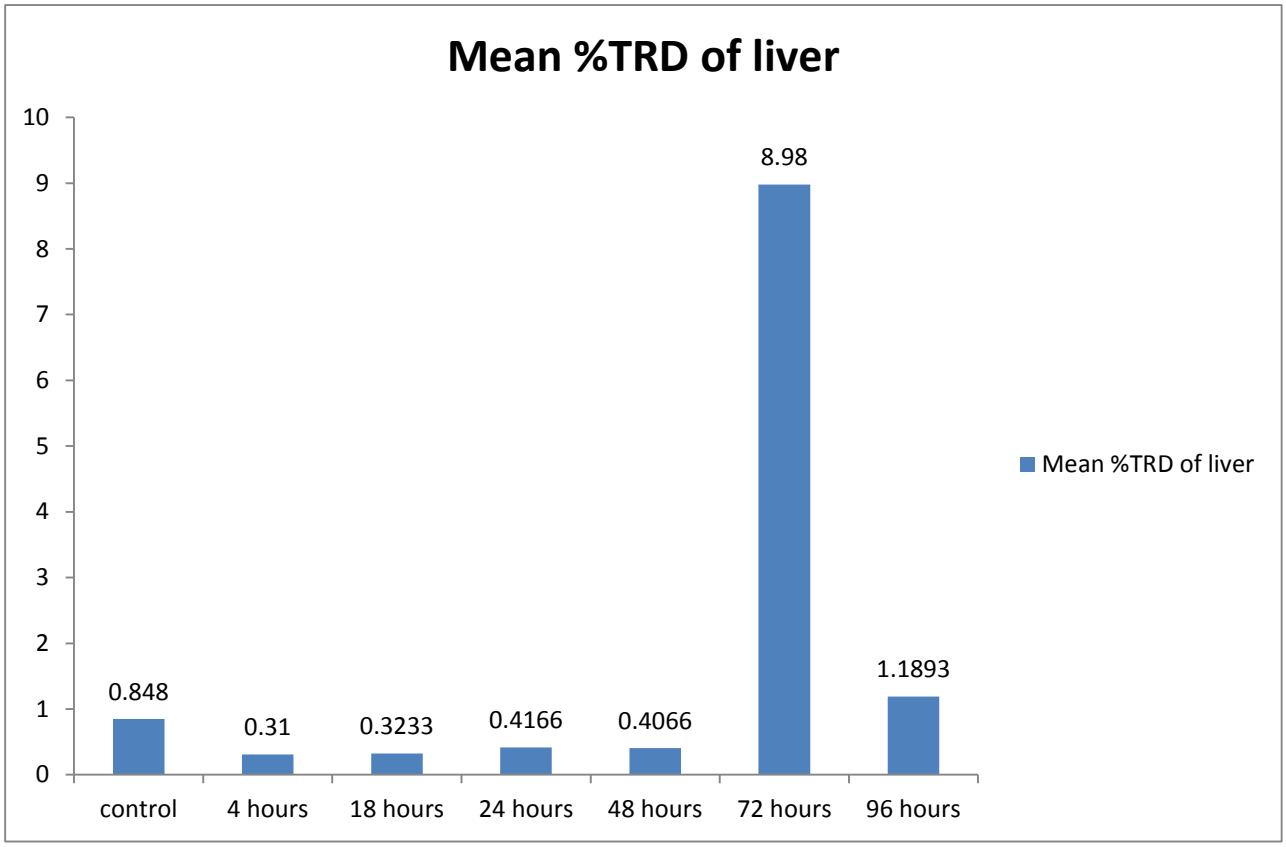


Fig 9: Mean %TRD of liver in Control (n=10) and Experimental group (n=5 each). Time (in hours) on x-axis shows the gap between administration of DOX (0.5mg DOX/0.25 ml of saline) and 99mTc-DTPA, calculated with two-tail unpaired t-test.

The overall activity being excreted via bile can be probed through the %TRD determined collectively for liver, intestines and stool. This may also complement the observed increasing trend in %TRD for liver alone.

Table 3: Mean %TRD of liver and intestines in control (n=10) and test group (n=5) injected with 0.5mg DXR/0.25 ml of saline prior to the injection of 99mTc-DTPA.

Groups and Subgroups	**Mean% activity of liver + intestines**	**Standard deviation**	**p-value**	**Significance/nonsignificance at confidence level of 0.05**
Control	4.038	± 2.9225		
4 hours prior to 99mTc-DTPA	4.4675	± 1.759	0.6269	Not significant
18 hours prior to 99mTc-DTPA	3.3633	± 3.4959	0.775	Not significant
24 hours prior to 99mTc-DTPA	1.9399	± 0.8204	0.2823	Not significant
48 hours prior to 99mTc-DTPA	2.0466	± 1.02787	0.3099	Not significant
72 hours prior to 99mTc-DTPA	16.6366	± 3.0122	0.0012	Very significant
96 hours prior to 99mTc-DTPA	10.0753	± 6.835	0.1232	Not significant

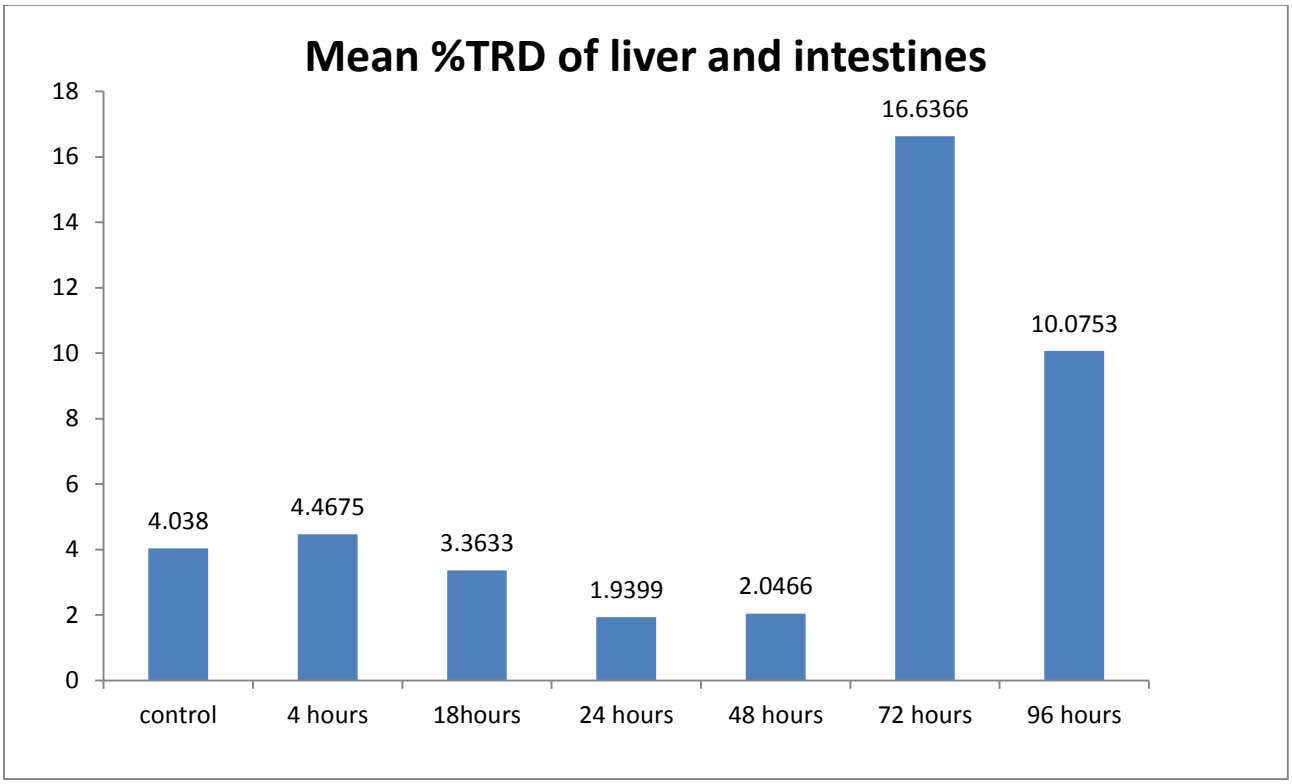


Fig 10: Cumulative mean %TRD of liver and intestines in Control (n=10) and Experimental group (n=5 each). Time (in hours) on x-axis shows the gap between administrations of DOX (0.5mg DOX/0.25 ml of saline) and99mTc-DTPA.

5. Discussion

Doxorubicin has a mean terminal half-life of more than 30 hours, while Tc-99m has an effective half-life of about 4 hours in humans. A major reason for the longer terminal half-life attributed to DOX is its extensive binding to plasma proteins (about 70%). On the other hand, only about 10% of DTPA- Tc99m is recognized to bind with plasma proteins. Doxorubicin being lipophilic is widely distributed in tissues by passive diffusion as opposed to DTPA-Tc99m which is hydrophilic and requires carrier mediated transport into cells. Furthermore, DOX is metabolized heavily in the liver particularly by the CYP3A4 subfamily of cytochrome P450 liver oxidases.

This results in active and inactive metabolite which are partly eliminated via the kidneys but the major pathway for elimination of DOX and its metabolites remains bile unloading into stool.

DTPA-Tc99m is not metabolized in the body and quickly gets filtered by the renal glomeruli to be eliminated from the body via urine.

Both DOX and DTPA-Tc99m show similar pharmacokinetics and pharmacodynamics in rats as compared to humans. However, overall time for drug clearance in rats is expected to be faster presumably due to smaller body surface area, faster heart rate and thereby faster blood flow. Therefore, it is expected that the choice of using Sprague Dawley for our study may result in fairly accurate insight into the behavior of these drugs in humans.

In our study, the % TRD in urinary tract (Figure 8) is different in all experimental subgroups as compared to the control group. These results show that the renal clearance of DTPA-Tc99m changes when DOX is administered prior to the radiopharmaceutical. Particularly, when the time interval between the two drugs was 48, 72 and 96 hours, the renal clearance of DTPA-Tc99m was less as compared to the control group and this decline in renal clearance was statistically significant at p value < 0.05.

Furthermore, Figure 9 shows a reciprocal relationship of liver %TRD compared to that of the urinary tract. Maximum %TRD in the liver is seen at an interval of 72 hrs (8.98 ± 2.74) which is also the time interval at which there is maximum decline in urinary tract %TRD. The liver % TRD increase is statistically significant at p value < 0.05, for 48 and 72 hours time intervals.

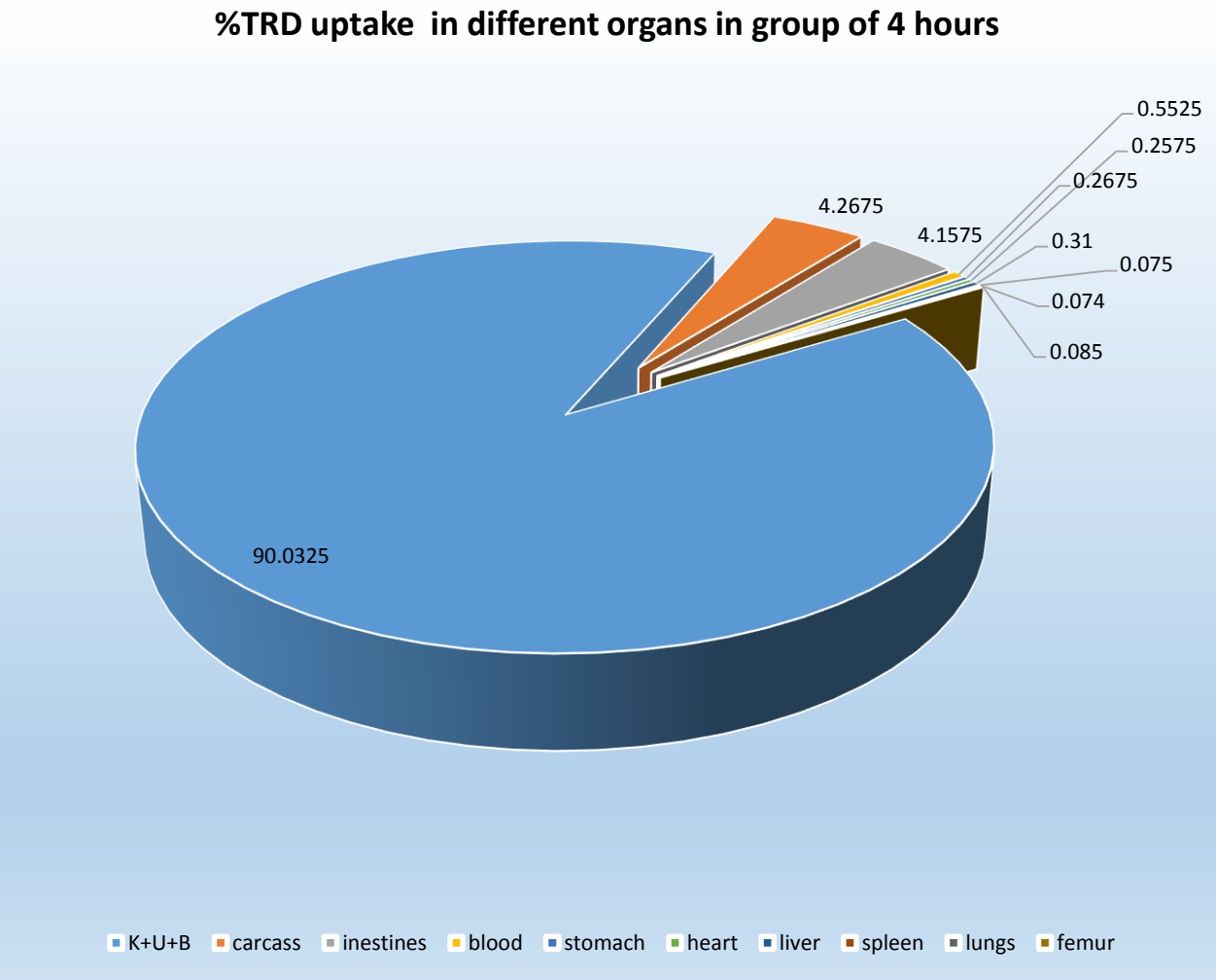
These findings (if also holds for humans) indicate that a similar DRI between DOX and DTPA-Tc99m as seen in rats may result in loss of accuracy of DTPATc99m based renography due to a decline in renal clearance of the radiopharmaceutical.

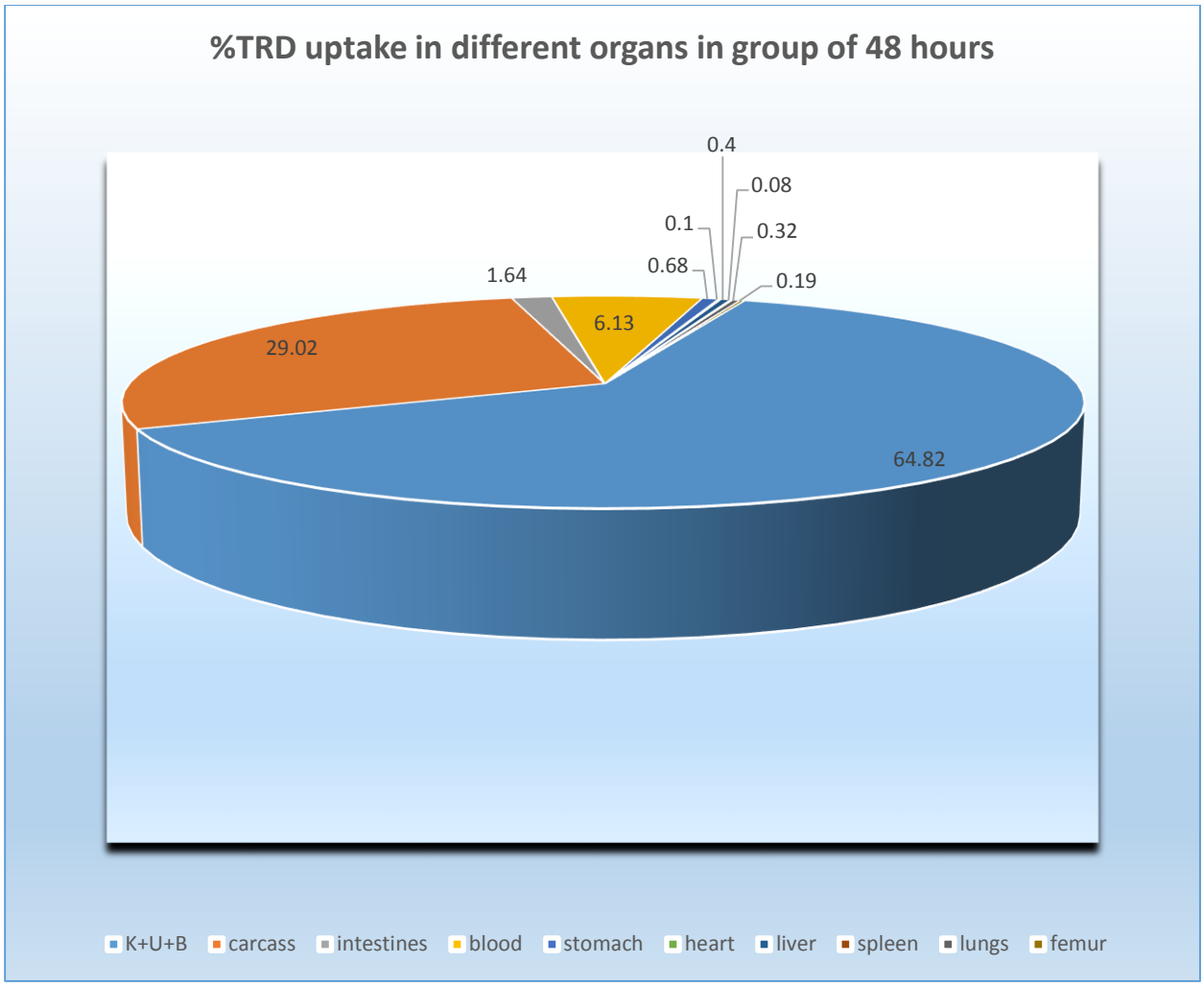
We have analyzed the observed DRI on the basis of the fact that both DOX and DTPA are metal chelators. Consequently, some quantity of 99mTc may be chelated by DOX and directed away from the kidney system resulting in a %TRD decline for urinary tract as depicted in Figure 8.

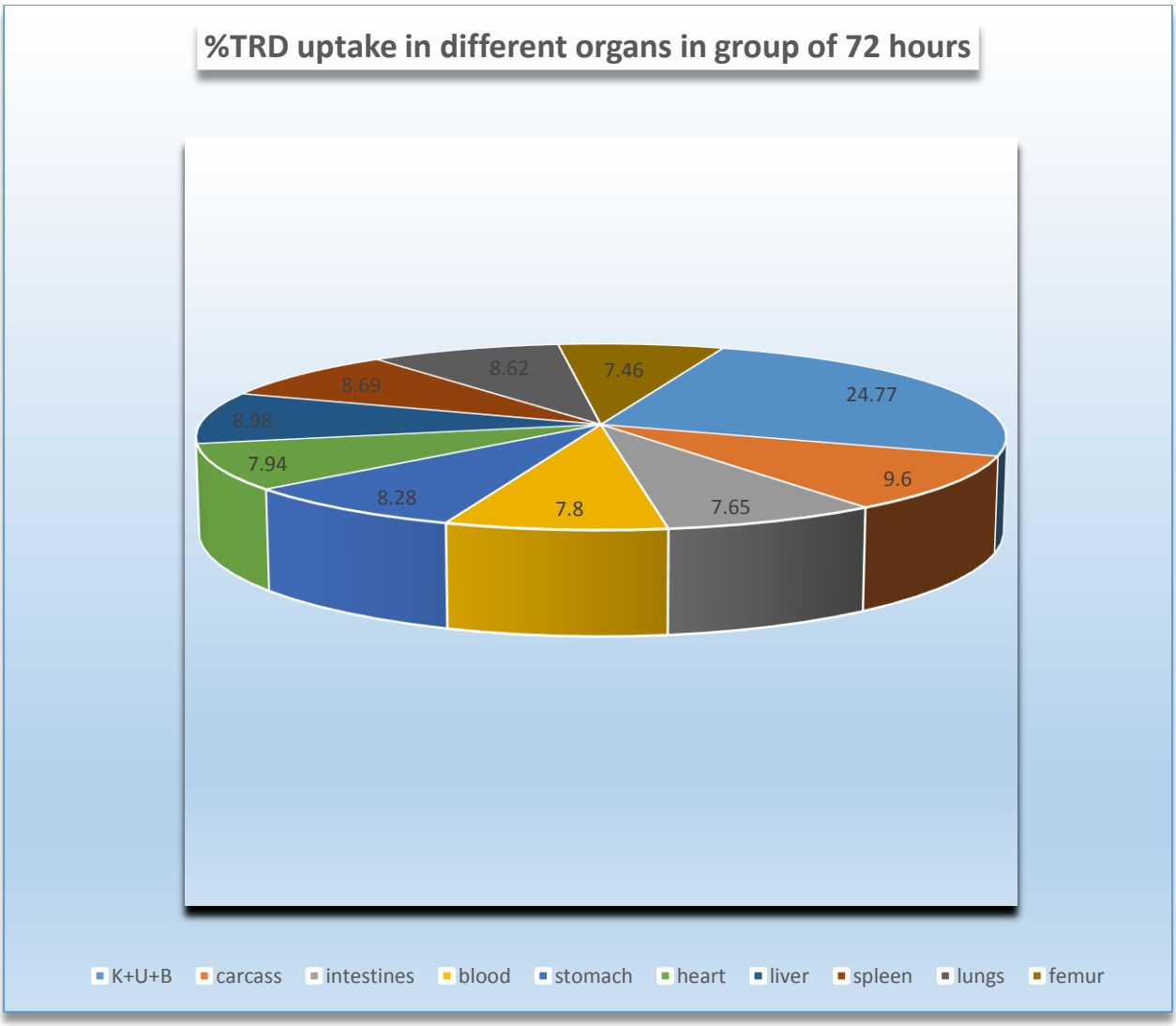
Similarly, increased activity in liver may be explained on the basis of chelation of 99mTc with DOX that draws it in the liver in greater concentrations than the control group.

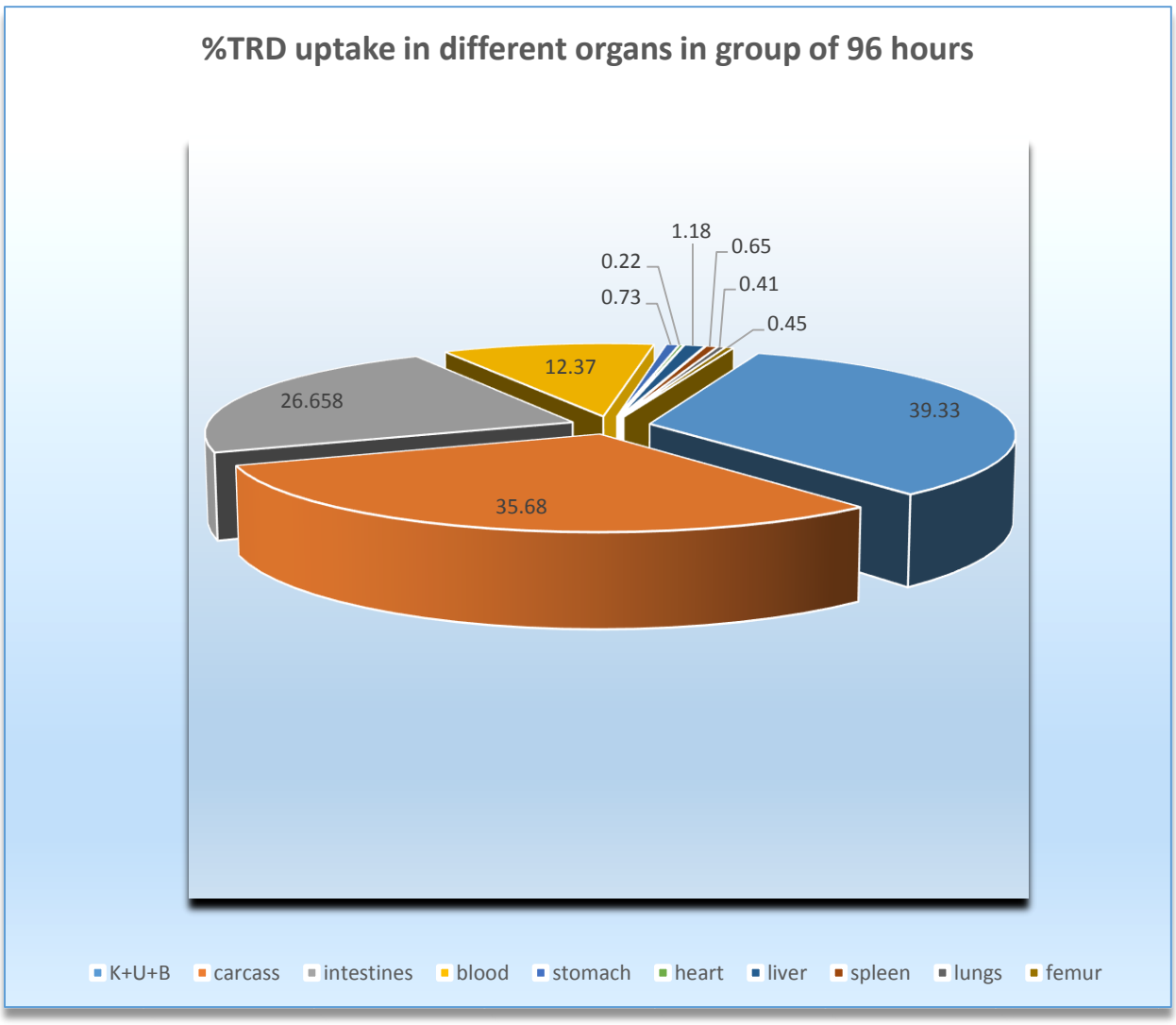
Subsequently, increased excretion of 99mTc in bile is supported by the statistically significant relative increase in %TRD observed in the 72 hours interval subgroup (16.64±3.01) as shown in Figure 10.

Decreased renal clearance and increased biliary clearance of Tc-99m is not seen at shorter time intervals (e.g. 4 hrs) between administration of DOX and 99mTc-DTPA. This could simply be due to an inability to observe the DRI in these subgroups due to smaller number of experimental animals in the subgroups. Moreover, for shorter time gaps DOX and DTPA-Tc99m, DOX extensively binds to plasma protein with limited distribution; the bound DOX would not be able to interact with Tc-99m.


%TRD uptake in different organs in group of 4 hours
0.5525
0.2575
0.2675
4.2675
4.1575
0.31
0.075
0.074
0.085
90.0325
K+U+B
carcass
inestines
blood
stomach
heart
liver
spleen
lungs
femur


%TRD uptake in different organs in group of 48 hours
0.4
0.08
0.1
0.32
0.68
0.19
1.64
6.13
29.02
64.82
K+U+B
carcass
intestines
blood
stomach
heart
liver
spleen
lungs
femur


%TRD uptake in different organs in group of 72 hours
24.77
9.6
7.65
7.8
8.28
7.94
8.98
8.69
8.62
7.46
K+U+B
carcass
intestines
blood
stomach
heart
liver
spleen
lungs
femur


%TRD uptake in different organs in group of 96 hours
1.18
0.65
0.22
0.41
0.73
0.45
12.37
26.658
39.33
35.68
K+U+B
carcass
intestines
blood
stomach
heart
liver
spleen
lungs
femur

6. Conclusion

The present work has been designed to study the effects of DOX on the uptake of 99mTc-DTPA in rats by administering a DOX dose of 0.06mg prior to injecting 99mTc-DTPA (0.1mC). It was observed that the mean activity in rat urinary system decreased while activity uptake in liver increased. The altered behavior was more obvious when the time interval between sequentially injected drug and radiopharmaceutical was 72 hours. These findings indicate that the scintigraphic study of renal scan using 99mTc-DTPAmay be complicated in patients receiving DOX based chemotherapy and demands caution for proper assessment. Furthermore, there is a need to thoroughly explore Drug-Radiopharmaceutical interactions by investigating the behavior of other chemotherapy drugs and radiopharmaceuticals that may be used concurrently or in close conjunction to one another in clinical practice.

References

1. A.Aktas and M. Haberal, “Classification of Tc-99m DTPA renograms based on the relationship between uptake and perfusion pattern,” *Transplant. Proc.*, vol. 37, pp. 4259–4265, 2005.
2. H. Kaiserova, T. Šimůnek, W. J. F. Van Der Vijgh, A. Bast, and E. Kvasni, “Flavonoids as protectors against doxorubicin cardiotoxicity : Role of iron chelation , antioxidant activity and inhibition of carbonyl reductase,” *Biochim. Biophys. Acta*, vol. 1772, pp. 1065–1074, 2007.
3. M. L. Gomes, D. M. M. De Mattos, R. D. S. Freitas, R. J. Bezerral, and M. Bernardo-Filho, “Study of the toxicological effect of mitomycin C in mice : alteration on the biodistribution of radiopharmaceuticals used for renal evaluations,” *Hum. Exp. Toxicol.*, vol. 20, pp. 193–197, 2001.
4. O. Article, B. B. Demirel, T. A. Balci, B. Tasdemir, and Z. P. Koc, “Comparison of DTPA and MAG3 renal scintigraphies in terms of differential renal function based on DMSA renal scintigraphy,” *Pakistan J. Med. Sci.*, vol. 28, no. 5, pp. 795–799, 2012
5. P. Gibson, A. Shammas, M. Cada, C. Licht, and A. A. Gupta, “The role of Tc-99m-DTPA nuclear medicine GFR studies in pediatric solid tumor patients,” *J. Pediatr. Hematol. Oncol.*, vol. 35, no. 2, pp. 108–111, 2013.
6. P. J. Mountford, F. M. Hall, C. P. Wells, and J. Coakley, “Breast milk radioactivity after aTc-99m DTPA aerosol / Tc-99m MAA lung study,” *J. Nucl. Med.*, vol. 25, pp. 1108–1110, 1984.
7. R. S. Benjamin, C. E. Riggs, and N. R. Bachur, “Plasma pharmacokinetics of adriamycin and its metabolites in humans with normal hepatic and renal function,” *Cancer Res.*, vol. 37, pp. 1416–1420, 1977.
8. S. R. F. Moreno, A. L. C. Silva, G. Dire, H. Honeycut, J. J. Carvalho, A. L. Nascimento, M. Pereira, E. K. Rocha, M. Oliveira-Timoteo, A. Arnobio, B. Olej, M. Bernardo-Filho, and L. Q. A. Caldas, “Effect of oral ingestion of an extract of the herb Uncaria tomentosa on the biodistribution of sodium pertechnetate in rats,” *Brazilian J. Med. Biol. Res.*, vol. 40, no. 1, pp. 77–80, 2007.
9. S. Budnitz, D. A. Pollock, K. N. Weidenbach, A. B. Mendelsohn, T. J. Schroeder, and J.L.Annest, “National surveillance of emergency department visits for outpatient adverse drug events,” *J. Am. Med. Assoc.*, vol. 296, no. 15, 2006.

10. S. R. F. Moreno, J. J. Carvalho, A. L. Nascimentoc, M. Pereira, E. K. Rocha, B. Olej, L.Q. A. Caldas, and M. Bernardo-Filho, "Experimental model to assess possible medicinal herb interaction with a radiobiocomplex : Qualitative and quantitative analysis of kidney , liver and duodenum isolated from treated rats," *Food Chem. Toxicol.*, vol. 45, pp. 19–23, 2007.
11. S. Zhou, B. Zhang, E. Sturm, D. l Teagarden, C. Schoneich, P. Kolhe, L. M. Lewis, B. K. Muralidhara, and S. K. Singh, "Comparative evaluation of disodium edetate and diethylenetriaminepentaacetic acid as iron chelators to prevent metal –catalyzed destabilization of a therapeutic monoclonal antibody," *J. Pharm. Sci.*, vol. 99, no. 10, pp.4239–4250, 2010.
12. Veilleux-lemieux, A. Castel, D. Carrier, F. Beaudry, and P. Vachon, "Pharmacokinetics of ketamine and xylazine in young and old sprague – dawley rats," *J. Am. Assoc. Lab.Anim. Sci.*, vol. 52, no. 5, pp. 567–570, 2013.
13. X. Xu, R. Sutak, and D. R. Richardson, "Iron chelation by clinically relevant anthracyclines : alteration in expression of iron-regulated genes and atypical changes in intracellular iron distribution and trafficking," *Mol. Pharmacol.*, vol. 73, no. 3, pp. 833–844, 2008.
14. Y. Cho, R. R. Lomeda, S. Ryu, J. Lee, J. H. Beattie, and I. Kwun, "Cellular Zn depletion by metal ion chelators (TPEN , DTPA and chelex resin) and its application to osteoblastic MC3T3 E1 cells," *Nutr. Res. Pract.*, vol. 1, pp. 29–35, 2007.
15. Y. Fan, N. Lin, L. Luo, L. Fang, Z. Huang, H. Yu, and F. Wu, "Pharmacodynamic and pharmacokinetic study of pegylated liposomal doxorubicin combination (CCOP) chemotherapy in patients with peripheral T-cell lymphomas," *Acta Pharmacol. Sin.*, vol. 32, no. 3, pp. 408–414, 2011.
16. Y. Ichikawa, M. Ghanefar, M. Bayeva, R. Wu, A. Khechaduri, S. V. N. Prasad, R. K. Mutharasan, T. J. Naik, and H. Ardehali, "Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation," *J. Clin. Invest.*, vol. 124, no. 2, pp. 617–630, 2014.
17. Y. Kim, S. Ha, Y. So, and W. W. Lee, "Improved measurement of the glomerular filtration rate from Tc-99m DTPA scintigraphy in patients following nephrectomy," *Eur. Radiol.*, vol. 24, pp. 413–422, 2014.

18. Y. Yurekli, P. Unak, T. Ertay, Z. Biber, I. Medine, and S. Teksoz, “Radiopharmaceutical model using 99mTc-MIBI to evaluate amifostine protection against doxorubicin cardiotoxicity in rats,” *Ann. Nucl. Med.*, vol. 19, no. 3, pp. 197–200, 2005.
19. Zhang, G. Luo, X. Ding, and C. Lu, “Preclinical experimental models of drug metabolism and disposition in drug discovery and development,” *Acta Pharm. Sin. B*, vol.2, no. 6, pp. 549–561, 2012.