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Utilizing Nuclear Medicine Imaging Techniques in Preclinical Animal Models to Achieve the "3R" Concepts

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ABSTRACT

Laboratory animals are the most widely established species in numerous fields for studying and developing new drug molecules and toxicity testing. A disproportionate number of animals are euthanized in the drug development and research field for societal benefit. In order to reduce the number of laboratory animals, a precise in vivo assessment system is essential to obtain statistically significant data. The project was initiated to evaluate vital organ function by utilizing nuclear medicine imaging techniques and radiopharmaceuticals. In this study, six mature rabbits (8-9 months old) of both sexes were systematically evaluated for vital organs like the brain, thyroid gland, salivary gland, heart, lung, liver, kidney function, skeletal system, and lymphatic system using organ-specific radiopharmaceuticals with scintigraphy and PET-CT imaging techniques. A detailed assessment of the function of each organ in rabbits was undertaken. No abnormalities were observed in the study animals under investigation. The uptake and clearance pattern of radiopharmaceuticals determined the normal organ function of rabbits and was found to be comparable with humans. In vivo, analysis with data generation was conceivable without sacrificing animals. Henceforth, by applying the 3Rs principle, the reduction and refine procedure is accomplished with a significant amount of critical data generated against each time point. Therefore, applying imaging techniques with the "3RS" principle proves to be an efficient method for rational use of laboratory animals during the investigation.

Keywords: 3Rs, Rabbit, PET-CT, Scintigraphy imaging study, Biodistribution

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INTRODUCTION

Preclinical studies using animal models are crucial in most research investigations that bridge the discovery and clinical study milestones (Mukherjee et al., 2022). Using animal models has contributed significantly to advancing medical, biomedical, physiological, and behavioural science. Animal data has been used by two-thirds of Nobel laureates in physiology and development in the field of medicine and physiology (Rai and Kaushik., 2018).

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Thus, animal research's importance in human and animal healthcare development is undeniable. Laboratory rats, mice, and rabbits are extensively used in applied research, as they are lower in the phylogenetic classifications and cover protected animals in India (Sneddon et al., 2017, CPCSEA, India 2018 guideline). For different studies like biodistribution, behaviour science, biomechanics, toxicity, and pharmacokinetics, it is important to define the number of animals to generate statistically supported data for the approval and clearance of the drug (OECD guideline No. 408,433 and 417., Koziorowski et al., 2017; Schwarz et al., 2019; Taylor et al., 2008). To approve new radiopharmaceuticals, it is necessary to have preclinical data across time points to support and design clinical trials. This is essential for a radiopharmaceutical panel to support the project. (Schwarz et al., 2019). Prediction of risk assessment, suitability, and human hazard extrapolation of theranostic radiopharmaceuticals is a critical requirement. Therefore, the approving authorities expect well-designed in vivo pharmacokinetic/toxicokinetic studies in preclinical models to establish supportive data for clinical development from CROs and pharmaceutical companies.

The utilization of animals in drug development and related studies is increasing widely, but the number of clinical trials fails due to uncertainty in translating preclinical experiments. Therefore, animal usage is questioned by researchers, and animal protests have occurred (Van Norman, 2020). To explore new radiopharmaceuticals, it is necessary to use animals judiciously and develop well-thought-out study designs to overcome these issues.

Animals need to be handled humanely and should avoid their worthless killings. To address this issue, regulatory bodies across the globe are implementing several rules and regulations. As a result, many countries like Israel, the United States, and Europe have now limited and minimized animal studies in the investigation (Epstein and Leshem.,2002). In addition, alternative models like the in vitro organoid cell culture technique and non-vertebrate and lower vertebrate animal models have reduced and replaced animal usage to a certain extent (Park et al., 2024). However, it is equally important to understand that sacrificing animals in research investigation is considered ethical if used for animal welfare or human purposes justifiably (Shanks and Green, 2004., Daneshian et al., 2015). In certain research areas, using in vivo animal models to establish pre-clinical data is inevitable, and there is no substitute for the same (Mukherjee et al., 2022). Considering the scenario, it is important to focus on a better analysis system that provides us with maximum data with minimum requirements for animals and avoids unnecessary animal sacrifices. Considering this, a new concept of the "3Rs" principle has evolved in laboratory animal sciences. The 3Rs imply replacement, reduction, *and refinement* and are implemented in protected animals. Ethical consideration and human treatment of laboratory animals are now a global commitment (Sneddon et al., 2010).

Animal imaging by advanced imaging techniques is gaining growing attention from researchers in cancer, new drug discovery, and metabolic or infectious diseases (Yitbarek & Dagnaw, 2022). Earlier imaging systems were primarily confined to X-rays and ultrasound techniques to visualize the anatomical location in *in vivo* systems. The imaging field has witnessed advanced techniques like CT, MRI, SPECT, and PET systems, which provide more detailed visual data in soft and hard tissue contrast with organ-specific radiopharmaceuticals. In this investigation, we utilized the nuclear imaging technique to investigate the functionality of different organs in an *in vivo* system by eliminating the need to sacrifice the animals. In this well-designed study protocol, we primarily focused on the "reduction and refinement" principle by employing organ-specific radiopharmaceuticals to understand the organ functions and its standard pharmacokinetic profile in *in vivo* animal systems using nuclear imaging systems. Generally, studies conducted to derive this kind of information necessitate animal sacrifice to a large extent. The use of organ-specific radiopharmaceutical and nuclear imaging techniques proves to be a promising tool to help reduce the number of animals and avoid unnecessary killing. These nuclear imaging techniques pave the way for the judicious use of animals in the research field and help abide by the desired goal of the "3Rs" principle to improve animal welfare.

MATERIALS & METHODS

Scintigraphic and Positron emission tomographic animal imaging was done using gamma and PET-CT cameras. Organ-specific radiopharmaceuticals labeled with ^{99m}Technetium and ^{18}Fluorine isotopes were used.

Animal Studies: The animal experiments were executed in agreement with the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), India. This project was approved by BARC, Animal Ethics Committee, Mumbai Institutional Animal Ethics Committee Project No. (BAEC/14/2017). A healthy New Zealand White Rabbit (Body Weight 3-3.5 kg, Male, n=6) was used for an in vivo biodistribution study using scintigraphic imaging techniques. The rabbits were housed individually in stainless steel cages, fed on a carrot, sprouted pulses and Lucerne grass, and had free access to feed and water. The animals were maintained at a controlled temperature of 23 ± 1°C and humidity of 60 ± 5 % in a 12 h light/12 h dark cycle.

Animal PET-CT imaging: The rabbit PET-CT imaging studies were performed under the GEMINI TF 16 slice

PET-CT camera. Animal restraining was done by using Ketamine (22 mg/kg body weight) and Xylazine (5 mg/kg Body Weight) by intramuscular route for fast recovery of the animals. In the study, imaging of the animal was kept for 6 hrs fasting, and imaging was done using a standard acquisition protocol (Serview: 90 KV, 20 mA, CT: 120 KV, 80 mA, PET: 1 min emission/bed) by using radiopharmaceutical [^{18} F] fluorodeoxyglucose (FDG). Around 1-2 mCi radioactivity was injected by intravenous route. The 1-hour post-injection imaging was conducted. Data were reconstructed using the RAMLA reconstruction algorithm, and images of different sections like coronal, sagittal, and transverse were analysed qualitatively (Pawar et al.,2019).

Animal Scintigraphy Imaging: Healthy New Zealand white rabbit (body weight 3.0- 3.5 kg, Male, n = 14 times) was used. Animal restraining was done by using Ketamine (22 mg/kg body weight) and Xylazine (5 mg/kg body weight) by intramuscular route. The animal was placed in a supine or prone position as per the study protocol under a GE dual head gamma camera and 99mTc labeled radiopharmaceutical like DTPA, MDP, HIDA, MAA, MIBI, DMSA and labelled with Technetium-99m administered by ear vein at a dose of 3 mCi. Sequential static or dynamic imaging was performed at 0, 5, 20 min, 1hr, 2hr, and 3hr post-injection points per the study protocol (Table No.1). Analysis was done with the help of image analysis software in Xeleris workstation (Saha,2004).

RESULT

PET & SPECT imaging system was used for the evaluation of organ function

Brain and Whole-Body PET-CT scan:^{18}F-FDG scan performed in the (n=3) rabbits showed no abnormal changes in the whole-body scan and brain of the rabbits. [^{18}F] The FDG study showed the maximum and minimum uptake values of 2.26±0.29% and 1.83±0.23% in the brain at 30 minutes post-injection. Apart from these, no intense uptake was noted in the bone marrow at 1 hr and 2 hrs. No other focal or diffuse uptake was seen in other body organs **(Fig.1A and Table No.1).**

Lung function was done with ^{99m}Tc-MAA, and the right and left lobes were seen immediately after the injection. Uniform distribution of the radioactivity was observed in all lobes. No obstruction or focal uptake was seen **(Fig.1B).**

Heart Function was done with a ^{99m}Tc-MIBI scan. Uptake was observed in the heart, and no cold spot was observed. However, due to rapid cardiac activity, it was challenging to perform further deep analysis using existing software(1hr) **(Fig.1C).**

Liver function was evaluated with a ^{99m}Tc-Mebrofenin (HIDA) scan. Dynamic imaging done for hepatobiliary function demonstrated the visualization of the liver within 2 min, 30 min complete liver was observed, and gall bladder at 45 min. No hold and abnormalities were observed in the liver. The hepatobiliary tract was seen very well **(Fig 1D).**

Kidney Function was performed with ^{99m}Tc-DTPA and ^{99m}Tc-EC renogram with 3mCi activity injected via ear vein (n=2). The mean GFR was 118 ml/min, and ERPF was 361.2 ml/min. The split function mean value for the right and left kidney was found to be 49.4 ±0.3% and 50.6±0.3%, respectively. ^{99m}Tc (III)-A DMSA scan was done to check kidney morphology. Showed that the right kidney (48.99%) and the left kidney (51.01%) showed split function, which is equivalent to normal kidney split function **(Fig 2A).**

Stomach/GE reflex function: Normal GE reflex and peristalsis were seen in rabbits, no stasis or impaction was seen, and activity clearance was seen with time **(Fig. 2B).**

Thyroid gland function: Thyroid gland function was done using 99mTc-sodium pertechnetate scan (n=6), and the mean uptake value was 1.63 ±0.09 %. Both males and females showed similar uptake values. Thyroid uptake in humans is typical for an individual (mean: 0.4- 4.5%). The uptake value for rabbits and humans is in the similar range at (25-30min). No cold or hot spot was seen in the thyroid gland **(Fig.2C).**

Salivary Gland Function: Salivary Gland function was done with ^{99m}Technetium-sodium pertechnetate. The distribution of radioactivity was observed predominantly in the thyroid, followed by salivary glands. Total uptake values for the parotid gland showed (0.165 ± 0.11), and salivary gland parenchyma was seen in both the right and left parotid gland at 10 min **(Fig. 2D).**

Lymph node: lymphoscintigram done with ^{99m}Tc-HSA nano colloid. The whole body showed a prominent injection site with intestinal mesenteric and inguinal lymph nodes overlapping with the injection site. The spleen was prominent, with minimal liver tissue. Thymus and mediastinal lymph nodes were prominent, followed by cervical lymph nodes **(Fig. 3A).**

Skeleton System: Skeleton analysis and associated deformities were done. A 99mTc-MDP scan showed the normal symmetric activity distribution in the axial and appendicular skeleton. Intense uptake was seen in the kidney, urinary bladder, facial bone, thoracic vertebra, lumbar vertebra, pelvic bone, and physis of long bone **(Fig. 3B).**

Fig.1A

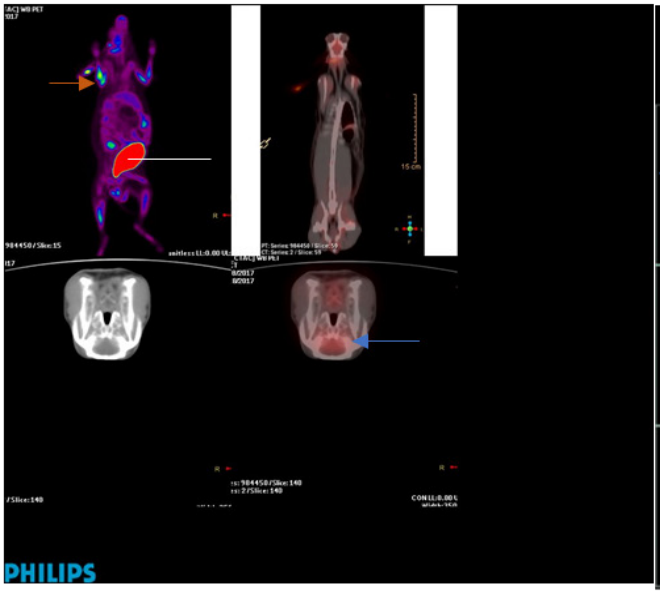


Fig.1B

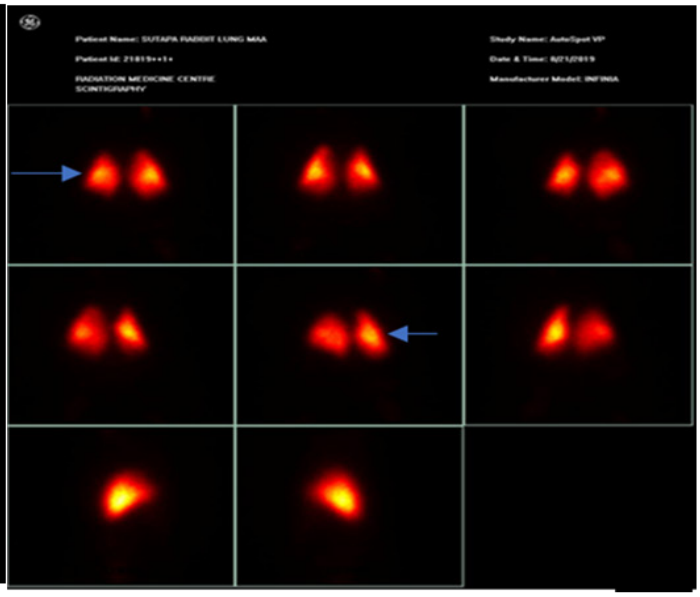


Fig.1C

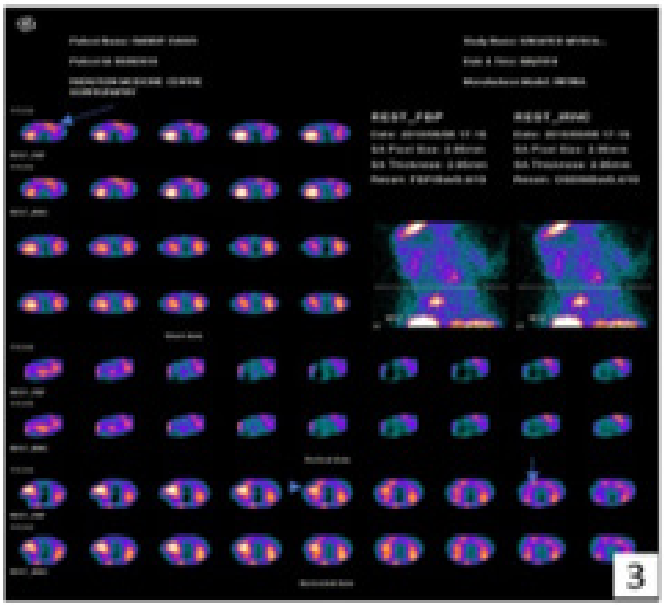


Fig.1D

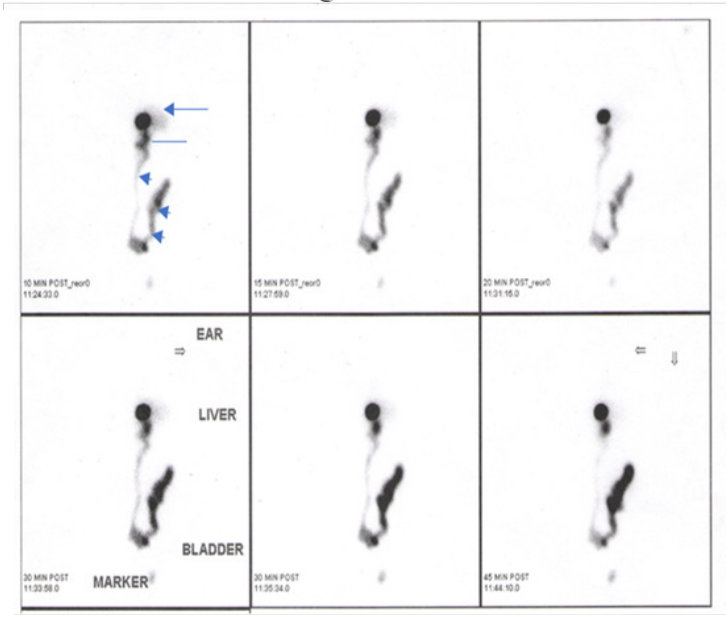


Fig.1A: Brain & Whole body 18F-FDG PET-CT scan: A:18F-FDG brain (blue arrow), skeletal muscle uptake (brown arrow), excreted in the urinary bladder (white line) at 30min. B: 18F-FDG brain (blue arrow), skeletal muscle uptake (brawn arrow), and whole-body scan at one hr. in the rabbit.

Fig.1B: Lung 99mTc-MAA SPECT scan: Dorsal and lateral right (arrow) and left lobe (arrow) lungs with normal uniform uptake in all lobes at 5min, 15min, 30min in rabbit.

Fig.1C: Transverse 99mTc-MIBI SPECT of myocardial scan: The short axis of the heart is normal at the apex region (arrow), the vertical axis found normal marked with (arrow), the horizontal axis with normal perfusion from the left and right side of the heart (arrow tip) & apex (arrow) in the rabbit.

Fig.1D: Liver HIDA scan: Normal Liver (arrow) with hepatobiliary channel (line) at 10 min. Further clearance in the intestine (short arrow) in rabbit

Fig2 A

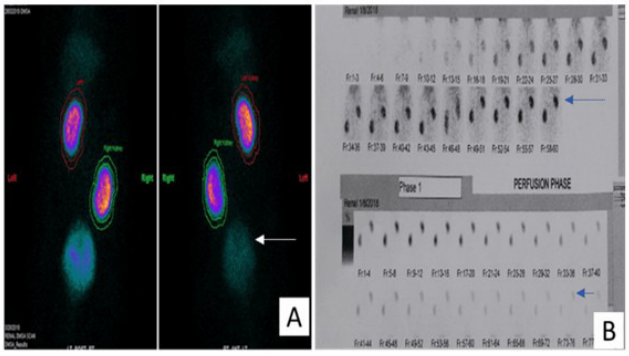


Fig.2B

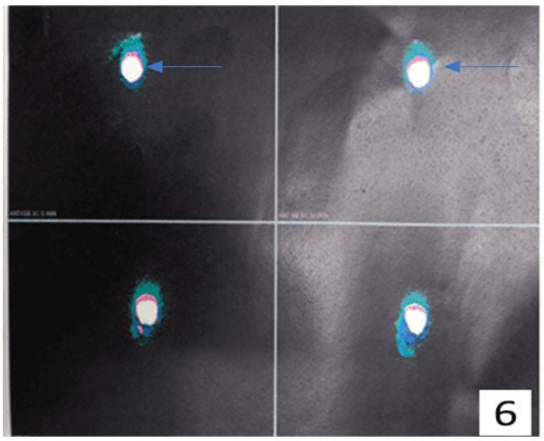


Fig2C

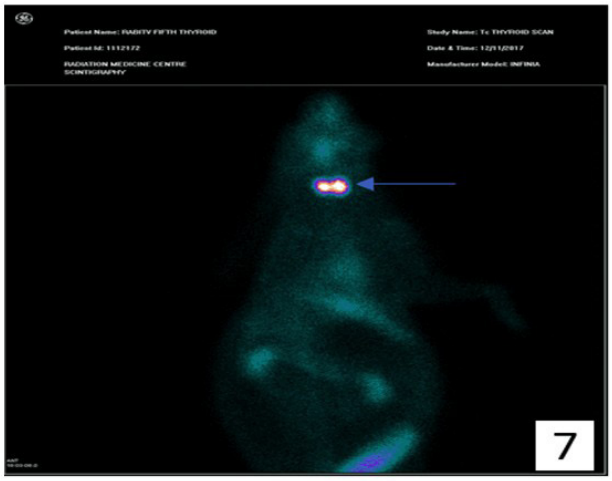


Fig.2D

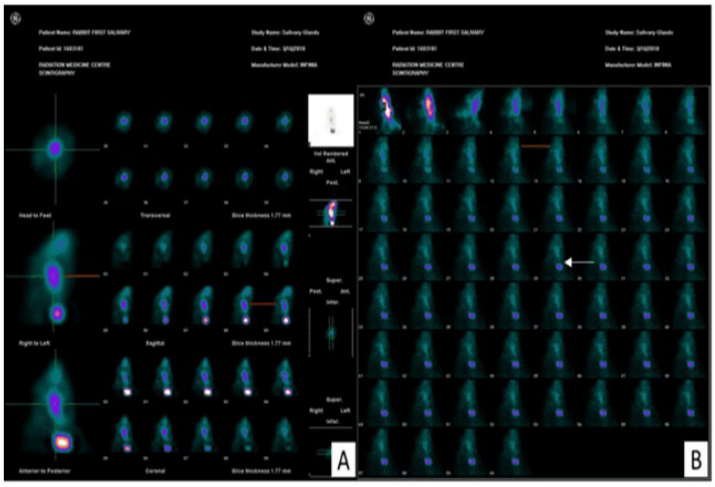


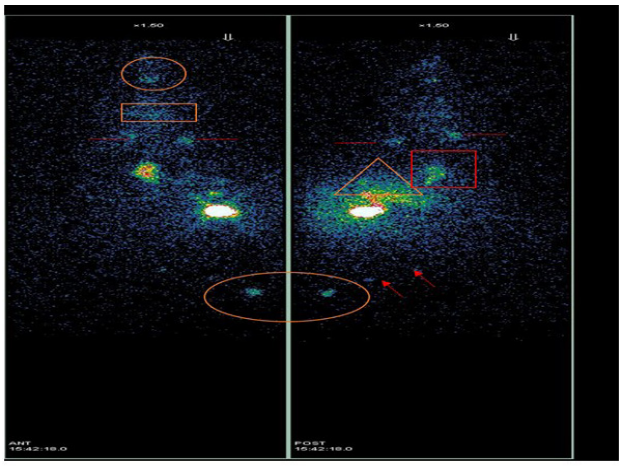
Fig.2A: Kidney scan: A: Kidney DMSA scan: Normal diffuse uptake in right (green circle) and left (red circle) kidney and clearance in the bladder (arrow), B: Kidney DTPA scan: Normal renography for right and left kidney (long arrow) and clearance from the kidney (short arrow) in the rabbit.

Fig.2B: Gastric emptying: 99mTc-MAA gastric emptying at 5 min, 1 hr, 2 hrs & 4 hrs in rabbit

Fig.2C: Thyroid 99mTc-pertechnetate scan: Thyroid lobes with homogenous normal uptake at 5 mins. in rabbit.

Fig.2D: Salivary gland 99mTc-pertechnetate scan: Lateral view of the parotid gland with normal uptake (red line), and thyroid gland (white arrow). In Ventral view, paired parotid gland (yellow line) at 5min scan in rabbit.

3A



3B

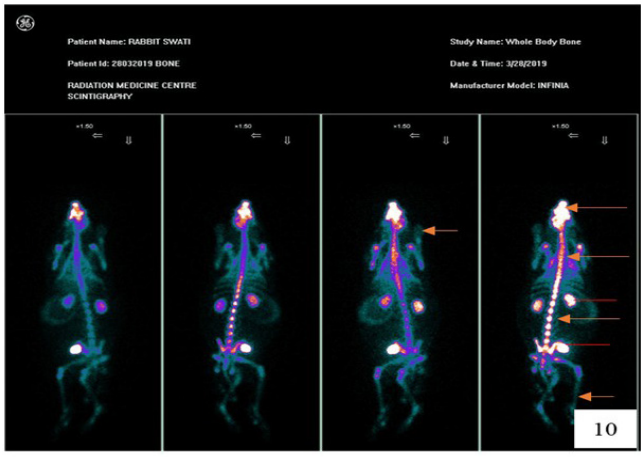


Fig.3A: Lymph Node 99mTc-HSA Nano colloid scan: Parotid nodes (circle), Pre-scapular lymph node (arrow), Thymus gland (rectangles), Axillary nodes with thoracic lymph duct (line), Spleen (square), Mesenteric nodes (oval shape), Hepatic & Gastric Lymph node (small triangle), Superficial Inguinal nodes (Triangle), Popliteal Lymph node (circle).

Fig.3B: Transverse 99mTc-MDP SPECT bone scintigraphy: Dorsoventral position with the vertical image. The skull area, Thoracic vertebra, and Lumbar vertebra were seen with normal physiological uptake (long arrow), and the forelimb and hind limb were marked with a short arrow. Kidney and bladder image (red line) excretory organ for 99mTc-MDP in rabbit.

Table No.1: Organ-Specific Radiopharmaceutical Studies in New Zealand White rabbit

Sr. No	Organ Function	Mechanism	PET-CT Imaging	Scintigraphy Imaging	Qualitative Interpretations
1	Brain	It enters inside the cell as glucose enters. It acts as deoxyglucose and does not take part in the further synthesis process. Trapped inside the cells. Based on physiological/ pathological conditions, the uptake is varied.	[^{18}F]FDG-fluorodeoxyglucose		Brain uptake usual as per the standard study protocol There was no abnormal increased metabolic focal uptake in the rest of the body
2	Lung	radiopharmaceutical is stuck in the lung capillaries due to its particle size, permitting visualization of blood flow and detection of abnormalities		^{99m}Tc-MAA *macro aggregated albumin*	In vivo Biodistribution was normal
3	Liver	Quickly taken up by hepatocytes via organic anion-transporting polypeptide (OATP) transporters and then excreted into the biliary system through multidrug resistance protein 2 (MDRP2) transporter		^{99m}Tc-Mebrofenin (HIDA) scan	Liver clearance was faster indicating the normal liver parenchyma. Gall bladder and bile duct were observed, suggesting no obstruction in the hepatobiliary path.
4	Kidney	collects in the kidney cortex through a process of glomerular filtration, binding to α1-microglobulin, and subsequent endocytosis by receptors in the proximal tubules		^{99m}Tc-DMSA Dimercaptosuccinic acid Cortical Defect	Normal couture and no focal abnormalities or cold area Split kidney function Right:51.08 % Left :48.99%

		cleared by the kidneys solely through glomerular filtration, without tubular secretion or reabsorption		^{99m}Tc-DTPA Diethylenetriamine pentaacetate GFR alteration	Normal couture and no focal abnormalities or cold area
5	Stomach	Intact radiolabelled formulation trapped in the stomach and linking of the		^{99m}Tc-MAA *Macroaggregated albumin*	Gastric retention seen till 4hr
6	Skeletal System scan	Accumulates in bone through chemisorption to hydroxyapatite crystals		^{99m}Tc-MDP Methylene diphosphonate	Bone and other skeletal parts were normal
7	Lymph Node Analysis	Nano colloid engulfed by macrophages and other histiomonocytic cells in the lymph nodes, particularly in the subcapsular sinus, allowing for visualization		^{99m}Tc-HSA nano colloid	No lymphatic obstruction seen
8	Thyroid	trapped by the thyroid gland similarly to iodide, but unlike iodide, it's not incorporated into thyroid hormones		^{99m}Tc-pertechnetate	Normal uptake
9	Salivary Gland	Enters through sodium/iodide symporter (NIS)		^{99m}Tc-pertechnetate	Salivary gland uptake and clearance were normal
10	Heart	Lipophilic, cationic radiotracer used for myocardial perfusion imaging, taken up by cardiomyocytes proportionally to myocardial blood flow and concentrated in mitochondria due to their negative membrane potential		^{99m}Tc-MIBI Methoxy isobutyl isonitrile	Normal perfusion seen Due to the rapid cardiac activity, it was unable to process by using available software.

DISCUSSION

Different organ-specific radiopharmaceuticals are routinely used in humans to evaluate organ function and altered pathophysiological changes in the organs as diagnostic agents *in vivo*. Similarly, the use of radiopharmaceuticals to evaluate organ function by exploring imaging techniques in experimental laboratory animals has been extended. However, hardly any references were available to explore these methods in experimental animals. Hence, different vital organs were evaluated in rabbits using nuclear imaging techniques to mimic humans and better understand the rabbit as a model.

[^{18}F] FDG is an ideal agent for evaluating brain function or the whole body and associated pathological lesions developed by chemicals or drugs. The uptake intensity increases if inflammation or proliferative lesions form in the brain tissue or whole-body parts, which this agent can detect. The uptake mechanism is based on cell metabolism. If cell metabolism increases, then glucose requirement at the site also increases. As a result, intense uptake can be seen at affected parts of the organs or the whole body (Subtirelu et al., 2023). Application of these types of brain imaging studies can be seen in neurotoxicity and spontaneous Alzheimer model evaluation in vivo in lab animals. Hence, toxicity effects can be judged using [^{18}F] FDG radiopharmaceuticals. In our study, normal uptake was seen, and no hot lesions were found anywhere, indicative of a normal brain scan. Animal imaging was performed in real-time, and related information was obtained without sacrificing the animals (Tremoleda J., 2022). [^{18}F] FDG showed normal brain, heart, and facial bone uptake similar to humans. However, muscle uptake was also observed, and it varied among animals. Specific artifactual uptake within skeletal muscle can occur if the animal is physically active post-administration before the scan. Hence, muscle uptake is considered normal. In contrast, whole-body uptake was comparable to humans. Predominant bone marrow uptake indicates active bone marrow (Saha., 2004).

Lung perfusion was done with ^{99m}Tc-MAA. The uniform distribution of the activity observed in all lobes suggests normal perfusion and no evidence of embolism or altered pathological changes in the lung (Wu et al., 2012). The mechanism of lung attachment is based on the number of particles (50,000) and size (10-30 μm), which go into the capillaries and produce the image (Silberstein et al., 1996). Altered lung perfusion can be spotted in the animals, either increased or decreased.

The 99mTc-MIBI scan measured heart perfusion. The myocardium is another primary site for toxicity damage and plays an important role in the circulatory and associated pathological changes. MIBI is characterized as lipophilic and cationic. Distribution and delivery of MIBI depends on regional blood flow, mitochondrial membrane potential, and mitochondrial content in the cells. Significantly negative transmembrane potential allows sestamibi to undergo passive diffusion into mitochondria. Lack of uptake would be seen in the loss of perfusion and lack of mitochondrial activity (Vanaja et al., 2000).

The liver is the critical organ where all kinds of toxic metabolites are detoxified and converted from one form to another. The hepatobiliary function was evaluated using a ^{99m}Tc-HIDA scan, which showed a normal liver with gallbladder (GB) and cleared from the organ over time. Indicating no retention or obstruction in the liver or gallbladder. The activity retention indicates hepatomegaly, abscess, and no further clearance from the liver to GB, indicative of biliary obstructions (Saha, 2004). Over-observed values fall in the normal range. The uptake of radiopharmaceuticals based on lipophilic anions is similar to bilirubin by active transport method. This organ function is evaluated *in vivo* in the animals, and the study can be repeated in the same animals.

Kidney function was evaluated functionally. The kidney is the second organ, followed by the liver in the excretion of products followed by liver. ^{99m}Tc-DTPA gives an idea of the glomerular filtration rate. Hence, the affected kidney has compromised renal function, as measured in terms of GFR rate. Quantitative and qualitative scintigraphic measurement will allow unilateral or bilateral assessment of renal function, which is most important in animals (Meyer-Lindenberg et al.,1996). This radiopharmaceutical is hydrophilic with a molecular weight of less than 5000 Daltons, which filters only through glomeruli. Hence, we have estimated that GFR for both kidneys is normal. The renal tubular function was evaluated by 99mTc-EC renogram; tubular function for both kidneys was normal, equal, and comparable.

Kidney morphology was done with ^{99m}Tc (III)-DMSA; renal uptake is based on affinity to proteins in renal proximal tubules. Damaged kidneys fail to excrete the radiopharmaceutical, and the hold of activity can be checked by imaging, and a follow-up case is straightforward. Hence, both the radiopharmaceuticals provide the key information about the kidney.

The thyroid gland is one of the main endocrine organs that regulates the basal metabolic rate by controlling the synthesis of thyroid hormones. Homogeneous distribution of radioactivity was seen within the gland. Thyroid uptake observed in the rabbit was normal, similar to the earlier reports (Pawar et al., 2019; Kintzer et al., 1994).

This qualitative scintigraphic imaging is helpful to determine unilateral or bilateral involvement, alterations in the position of thyroid lobes, and Cold or hot spots related to hypo and hyper-functioning of glands indicated by hypothyroidism and hyperthyroidism. This finding is crucial in defining the toxicity and alteration of thyroid gland functions. Imaging provides these findings within 10 mins in test animals *in vivo*. Like the thyroid gland, salivary gland functioning can be assessed using ^{99m}Tc radiopharmaceuticals, its uptake mechanism based on Na^+/I^- symporters. Pertechnetate ion, with a size and charge similar to that of iodine, is trapped by the thyroid gland. Therefore, uptake can be done simultaneously in both glands. Our finding for salivary glands was similar to earlier scientific reports in veterinary practices (Pawar et al., 2021).

No lymphoid obstructions or hold-ups were seen, indicative of no lymphadenopathy seen either due to inflammation or spontaneous infection or inflammation observed in the rabbit (Ergun et al., 2002).

The parotid gland is the primary functional salivary gland and is key in digitation. Salivary glands are also the target organs for many agents and are excreted through salivary glands. Hence, the altered function will directly impact oral cavity health. ^{99m}Technetium uptake was homogeneous in both the glands, suggestive of normal glands. Uptake of ^{99m}Technetium occurred due to active transport by Na^+/Iodide$^-$ symporters present in the glands (Beck et al., 1985).

A whole-body bone scan was done to estimate rabbits' skeletal patterns and associated abnormalities. ^{99m}Tc-MDP uptake will be uniform in typical cases. Identifying focal or multifocal uptake indicates bone metastasis, bone infection, trauma, localized bone metabolic activity, and inflammatory changes (Vanaja et al., 2000) and the exchanges of the phosphonates with hydroxyapatite of bone by chemisorption mechanism.

Different anatomical structure changes within the joint and bone can be diagnosed. The bone scan is important for identifying various primary and secondary lesions in the bone (Peremans et al., 2011). Different organ evaluation was possible with nuclear imaging techniques in laboratory rabbits.

CONCLUSION

Preclinical animal studies are often mandatory to generate the safety data in one rodent and non-rodent species and to understand the diagnostics and therapeutic potential of novel radiolabeled drugs and radiopharmaceuticals for regulatory approval. The generation of preclinical *in vivo* biodistribution data using laboratory rabbit models is possible through radio-tracer studies and PET/CT and Scintigraphy imaging techniques. A reduction and refinement principle can be achieved through radio-tracer studies such as PET/CT and scintigraphy imaging techniques in *vivo* in the animals. Traditional methods fail to achieve animal welfare; hence, adopting new *in vivo* imaging techniques, judicious, admissible utilization, and adapting new regulatory policies are required with time.

Authors Contribution: Dr. Yogita Shete: study conception and design, data collection, analysis and interpretation of results, drafting of manuscript., Sutapa Rakshit: data collection, analysis, Swati Satamkar: data collection, analysis, Nabar Swapna: data collection, analysis, and manuscript drafting Ashok Chandak: data collection, analysis, and radiopharmaceutical preparation, manuscript drafting., Avik Chakraborty: manuscript drafting and analysis, Dr. Sandip Basu: Preparation and scientific inputs in manuscript drafting.

Animal Ethics approval: The project sanction No. BAEC/14/2017.

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Conflict of Interest: Authors declare no conflicting interest.

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