Original Research Article

A Comprehensive Analysis of Use of Small Laboratory Animals in Preclinical Research: A Five-Year Quantitative Descriptive Analysis

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Abstract

Background: Animal models play a critical role in biomedical research, yet comprehensive data on the types and distribution of animals used in different research contexts are sparse.
Objectives: This study aims to provide a detailed analysis of small laboratory animals used in preclinical research, highlighting the types of animal species used, study types, and disease systems targeted.
Materials and Methods: A total of 841 freely available full-text original research articles indexed in the PubMed database from January 2016 to January 2021 were analyzed. Variables recorded included a) type of animal species used for research, b) type of animal studies (*in vivo*, *in vitro*, mixed, transgenic, xenograft, others), c) distribution of animals across different study types, d) system and disease-wise distribution of studies, and e) type of animal used and type of studies conducted for different systems/diseases
Results: Mice were used in 75.98% of studies, followed by rats at 22.12%. Most studies were *in vivo* (35.43%), followed by mixed studies combining both *in vivo* and *in vitro* methods (24.49%). The gastrointestinal and genitourinary systems (GIT/GU: 21.04%) and the central nervous system (CNS: 15.33%) were the most studied. GIT/GU research focused on diseases like nonalcoholic steatohepatitis, colitis, inflammatory bowel disease, and various carcinomas. For CNS, studies predominantly addressed Parkinson's disease, Alzheimer's disease, and multiple sclerosis. In GIT/GU studies, mice were the most commonly used species (140/177), with *in vivo* (55/177), mixed (43/177), and xenograft (36/177) approaches. For CNS diseases, *in vivo* approaches (72/129) were prevalent, utilizing both mice (39/129) and rats (32/129).
Conclusion: The distribution of animal species in these studies validates that mice, followed by rats, are still very commonly used small animals for preclinical research. In disease areas like GIT/GU and CNS, there is a notable use of mixed and transgenic models, which offer a comprehensive understanding of disease mechanisms. The preference for mixed and transgenic mouse models in cancer research, particularly xenografts, underscores the shift towards more sophisticated and disease-relevant animal models.

Keywords

Transgenic, xenografts, *in vitro*, *in vivo*, rabbit, Guinea pig, mice, rat, small laboratory animals

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Introduction

Experimental animal studies are essential for advancing medical knowledge and improving patient outcomes. These studies are critical in developing new treatment methods and testing hypotheses, providing a foundation for future clinical research. The impact of experimental studies on clinical research is significant, resulting in the development of new drugs, medical devices, and treatment protocols. One fundamental way experimental studies in animals' impact clinical research is by identifying potential treatments and therapies for diseases.[1] Researchers conduct experiments on animals or cell cultures to test the effectiveness of new drugs

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or medical devices. These studies help researchers understand the mechanisms of action of these treatments and how they might work in humans.[2] Experimental animal studies also provide critical information on the safety of new treatments. Researchers conduct experiments to determine drug and medical device risks and side effects. This information is then used to design clinical trials that can evaluate the safety and effectiveness of these treatments in humans.[3]

Small labo*rat*ory animals like *rat*s, *mice*, rabbits, and *guinea pig*s are commonly preferred for research due to their anatomical and functional similarities to humans. The ideal animal model should exhibit similarities with human disease regarding pathophysiology, phenotypic and histopathological features, predictive biomarkers for disease prognosis, response to therapies, and drug safety and toxicity.[4] The main experimental approaches commonly used in preclinical research are disease induction models (*in vitro*, *in vivo*, mixed), xenograft models, and transgenic models.[5]

In vitro methods occur in laboratories and involve studying investigational drugs on microorganisms and human or animal cells. These methods only partially represent physiological conditions as they lack the complexity and interactions in living organisms, limiting their ability to. *In vivo* studies occur within a living organism, thus addressing the drawbacks of *in vitro* studies. However, *in vivo* studies face significant ethical concerns.[6] *Xenograft* models involve transplanting organs or tissues from one species into another. Inbred strains are genetically uniform animals that allow for the study of pathobiology with small sample sizes.[7] *Transgenic* models involve modifying the animal's genome to express or eliminate specific genes.[8]

There are several issues with experimental studies in animals that can impact their effectiveness. These issues include variations in drug dosing schedules and regimens of uncertain relevance to the human condition and the choice of comparison therapy (none, placebo, vehicle) varying between studies. Nuances in laboratory techniques that may influence results, such as methods for blinding investigators, are often not recognized or reported. Another issue is the selection of outcome measures, which may be surrogates or precursors of disease and are of uncertain relevance to the human clinical condition. Finally, the variable duration of follow-up may not correspond to disease latency in humans.[9] However, selecting animal species and an appropriate study approach is essential for effective preclinical research. Improper selection of the animal species and research approach leads to reduced translational value of animal research to human diseases. A thorough and succinct experimental research study can prevent redundancy and reduce the number of animals used in testing, provide specific information about research questions, and save time and resources.[10]

Therefore, this study aims to explore the current status of researchers using animals for experiments. We have focused on the a) type of animal species used for research, b) type of animal studies (*in vivo*, *in vitro*, mixed, transgenic, xenograft), c) distribution of animals across different study types, d) system and disease-wise distribution of studies, and e) type of animals used and type of studies conducted for different systems/diseases.

Materials and Methods

The researchers conducted descriptive analysis using data from published literature available in the public domain. Before it commenced, the study received an exemption of review from the Institutional Ethics Committee (EC/OA-188/2021). We searched the PubMed database (http://pubmed.ncbi.nlm.nih.gov/) between January 2016 and January 2021. Keywords used were rat, mice, rabbit, guinea pig, *in vivo, in vitro,* xenograft, transgenic, and disease models. We used filters like last year, original article, Journal article, English language, and journal: MEDLINE to narrow down the research. All the abstracts were analyzed, and articles publishing original research on *rat*s, *mice*, rabbits, and *guinea pig*s were included in the study. Articles using *in vivo, in vitro,* transgenic, xenograft, or other methods for inducing the disease models for the abovementioned species were included. Short communications, research letters, letters to editors, case reports, review articles, meta-analyses, and systemic reviews were excluded. Free full texts available for the articles included were further downloaded for analysis. The data collection for this study involved two authors who worked collabo*rat*ively but independently to ensure a thorough examination of each report, focusing on assessing and evaluating key information regarding animal models. The researchers then analyzed each article in detail and collected the necessary data. If any discrepancies were found, they were resolved by reanalyzing the studies and by discussion among researchers.

The variables recorded from the studies included the a) type of preclinical studies (*in vivo, in vitro*, mixed, transgenic, xenograft) b) type of animal species used for research c) distribution of animals across different study types, and d) System & disease-wise distribution of studies, and e) type of animals used and type of studies conducted for different systems/ diseases. Study results are presented as numbers and percentages using Microsoft Excel.

Results

A total of 841 free full-text original research articles selected from 2062 abstracts spanning January 2016 and January 2021 were analyzed (Figure 1).

a. Type of preclinical studies conducted and b. Type of animal species used.

Mice were the most frequently used animal species for preclinical research (75.98%) followed by rats (22. 12%). *In vivo* research approach is very commonly used by researchers (35.43%) followed by mixed research (24.49%) (Table 1).

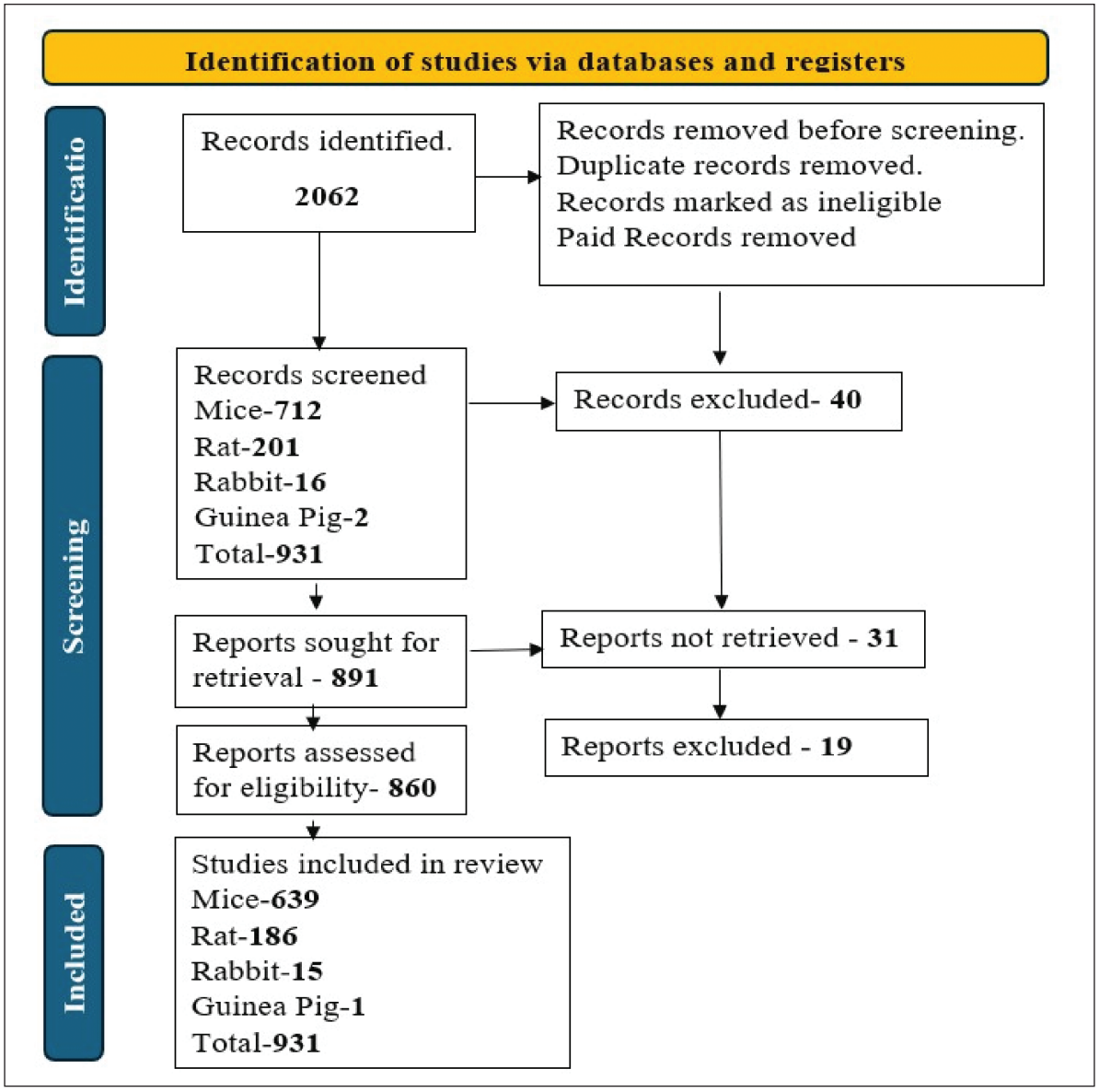

Figure 1. Flowchart for the Screening of Experimental Studies Conducted from January 2016 to January 2021.

Table 1. Type of Preclinical Studies Conducted from January 2016 to January 2021 and Type of Animal Species Used for Research.

Sl. No.	Type of Study	Rat (*N*)	Mice (*N*)	Guinea pig (*N*)	Rabbit (*N*)	Total (*N*) (%)
1	*In vivo* studies	90	201	1	6	298 (35.43%)
2	*In vitro* studies	66	39	0	4	109 (12.96%)
3	Mixed studies	26	175	0	5	206 (24.49%)
4	Transgenic	1	92	0	0	93 (11.06%)
5	Xenograft	1	94	0	0	95 (11.30%)
6	Others[a]	2	38	0	0	40 (4.76%)
7	Total studies	186 (22.12%)	639 (75.98%)	1 (0.12%)	15 (1.78%)	841

[a]Others include the studies which do not come under other above category or the ones whose type is not specified in the article.

N = Number of studies.

Distribution of Animals Across Different Study Types

Analyzing these animals' use in various research types revealed that mice are preferred for *in vivo* (201/298, 67.45%) and mixed studies (175/206, 84.95%). *Mice* are also exclusively used for *transgenic* and *xenograft* research. The *rat*s were the second choice of animals for *in vivo* (90/298, 30.20%) and mixed types (26/206, 12.62%) studies. However, its use for *in vitro* studies is more than that of mice (rats: 66/109, 60.55% vs. mice: 39/109, 35.77%) (Table 1).

System/Disease-Wise Distribution of Studies

The analysis system-wise distribution of studies revealed that research was maximum in the gastrointestinal and genitourinary system (GIT/GU: 177, 21.04%), followed by the central nervous system (CNS:129, 15.33%). Key disease areas covered among these two major systems included acute pancreatitis, liver cancer, and chronic kidney disease in the GIT/GU system. In the CNS, there was a strong focus on Alzheimer's disease, Parkinson's disease, and multiple sclerosis. Detailed distribution of other systems and their disease areas is provided in Table 2.

Type of Animal Used and Type of Studies Conducted for Different Systems/Diseases

Mice were commonly used in disease areas within the GIT system (140/177). The *in vivo* (55/177), mixed (43/177), and xenograft (36/177) approaches of research were common for the GIT system. CNS research used mice (78/129) and rats (50/129). For CNS disease, the *in vivo* (72/129) approach using both mice (39/129) and rats (32/129) was common. Transgenic studies were notably significant, comprising 14.73% (19/129) of CNS-related studies. Xenograft models were prominent in cancer-related studies, particularly in the GIT/GU and circulatory systems, where they accounted for 20.34 (36/177) and 33.33% (18/54) of studies, respectively (Table 2).

Table 2. System/Disease-Wise Distribution for Animals Used As Well As Type of Studies.

Sl. No.	Organ-System *N* = 841 (N/%)	Type of Study (*N*/%)	Name of Animal (*N*/%)	Key Study Areas
1	Gastro-intestinal and genito-urinary system (177/21.04)	*In vivo* (55/177, 31.07)	Mice (39/177, 22.03)	Colitis, non-alcoholic steato-hepatitis, steatohepatitis. Gastric ulcer, acute kidney injury, diabetic nephropathy, chronic kidney disease, others.
			Rat (16/177, 9.04)	Hepatocellular carcinoma, nonalcoholic steatohepatitis, colorectal cancer, inflammatory bowel disease, others.
		Mixed (43/177, 24.29)	Mice (35/177, 19.77) Rat (8/177, 4.52)	Pancreatic cancer, Sjogren's syndrome, inflammatory bowel disease, pancreatic cancer, acute kidney injury, chronic kidney disease, others.
		Xenograft (36/177, 20.34)	Mice (36/177, 20.34)	Colorectal cancer, hepatocellular carcinoma, pancreatic cancer, bladder cancer, prostate cancer, others.
		In vitro (25/177, 14.12)	Mice (12/177, 6.78)	Acute pancreatitis, liver cancer, esophageal cancer, cholangiocarcinoma, chronic kidney disease, prostate cancer.
			Rat (12/6177, .78)	Fatty liver, renal ischemia–reperfusion injury, colorectal cancer.
			Rabbit (1/177, 0.56)	Renal impairment.
		Transgenic (18/177, 10.17)	Mice (18/177, 10.17)	Nonalcoholic steatohepatitis, hepatitis, gastric neoplasia, nephropathy, acute kidney injury, others.

(Table 2 continued)

(Table 2 continued)

Sl. No.	Organ-System *N* = 841 (N/%)	Type of Study (*N*/%)	Name of Animal (*N*/%)	Key Study Areas
2	Central nervous system (129/15.34)	*In vivo* (72/129, 55.81)	Mice (39/129, 30.23)	Alzheimer's disease, Parkinson's disease, autoimmune encephalitis, Huntington's disease, others.
			Rat (32/129, 24.81)	Stroke, Parkinson's disease, Alzheimer's disease, epilepsy, other.
			Rabbit (1/129, 0.78)	Stroke.
		Transgenic (19/129, 14.73)	Mice (19/129, 14.73)	Alzheimer's disease, Parkinson's disease, autoimmune encephalomyelitis, others.
		Mixed (16/129, 12.41)	Mice (11129, /8.53)	Alzheimer's disease, Parkinson's disease, ischemic stroke, epilepsy, others.
			Rat (5/129,3.88)	Parkinson's disease, epilepsy, stroke, others.
		In vitro (16/129,12.40)	Mice (3/129,2.33)	Parkinson's disease, Alzheimer's disease, multiple sclerosis.
			Rat (13129,/10.08)	Parkinson's disease, glioblastoma, neuropathy, myasthenia gravis.
		Xenograft (6/129,4.65)	Mice (6/129,4.65)	Glioma, glioblastoma, others.
3	Musculoskeletal system (61/7.25%)	*In vivo* (34/61,55.73)	Mice (18/61,29.50)	Rheumatoid arthritis, osteoarthritis, hemarthrosis, others.
			Rat (16/61,26.22)	Rheumatoid arthritis, osteoarthritis, spondyloarthropathy, others.
		Mixed (20/61,32.78)	Mice (11/161, 8.03)	Rheumatoid arthritis, osteoarthritis gout.
			Rat (5/61, 8.19)	Osteoarthritis, rheumatoid arthritis.
			Rabbit (4/61, 6.55)	Osteoporosis, bone regeneration, osteoarthritis, anterior cruciate Ligament regeneration.
		In vitro (4/61, 6.55)	Rat (4/61, 6.55)	Diabetes, keratoconus retinalischemia, retinal ischemia reperfusion injury, others.
		Others (3/61, 4.91)	Mice (3/61, 4.91)	Joint disorders others.
4	Circulatory system (54/6.42%)	*Xenograft* (18/54, 33.33)	Mice (18/54, 33.33)	Acute myeloid leukemia, acute lymphoid leukemia, multiple myeloma, others.
		In vivo (10/54, 18.52)	Mice (10/54, 18.52)	Acute myeloid leukemia, hemophilia, myeloma, polycythemia vera, others.
		Mixed (8/54,14.81)	Mice (8/54,14.81)	Acute myeloid leukemia, multiple myeloma, vascular injury, atherosclerosis, others.
		In vitro (7/54,12.96)	Rat (4/54,7.41)	Abdominal aortic aneurysm, acute myeloid leukemia, hemophilia, artero-venous malformation.
			Mice (3/54,5.55)	Myeloma, acute myeloid leukemia, hemophilia, others.

(Table 2 continued)

(Table 2 continued)

Sl. No.	Organ-System *N* = 841 (N/%)	Type of Study (*N*/%)	Name of Animal (*N*/%)	Key Study Areas
		Transgenic (6/54,11.11)	Mice (6/54,11.11)	Sickle cell anemia, acute lymphoid leukemia, erythrocytosis, others.
		Others (5/9.26)	Mice (5/43,9.26)	Leukemia, acute myeloid leukemia, others.
5	Cardiovascular system (46/5.46%)	*In vivo* (17/46,36.95)	Mice (11/46,23.91)	Myocardial infarction, pulmonary hypertension, autoimmune myocarditis, others.
			Rat (6/46,13.04)	Myocardial infarction, infective endocarditis, pulmonary artery hypertension.
		Mixed (9/46,19.56)	Mice (6/46,13.04)	Myocardial infarction, aortic valve calcification, arrhythmia, others.
			Rat (2/46/4.35)	Pulmonary artery hypertension myocardial infarction.
			Rabbit (1/46,2.17)	Arrhythmia.
		Transgenic (8/46,17.39)	Mice (8/46,17.39)	Atherosclerosis, pulmonary artery hypertension, chronic heart failure, others.
		In vitro (7/46,15.21)	*Rat* (6/46,13.04)	Pulmonary artery hypertension, infective endocarditis, myocardial infarction, others.
			Rabbit (1/46,2.17)	Myocardial infarction.
		Others (5/10.87)	Mice (3/6.52)	Atrial fibrillation, pacemaker.
			Rat (2/4.35)	Cardiomyopathy.
6	Endocrine System and ophthalmic disorders (42/4.99%)	*In vivo* (24/42,57.14)	Mice (16/42,38.10)	Type 1 diabetes, Grave's disease, Hashimoto's thyroiditis.
				Diabetic retinopathy, retinitis pigmentosa, diabetic dry eye, others.
			Rat (6/42,14.28)	Type 1 and 2 diabetes.
			Rabbit (2/42,4.76)	Uveitis, retinoblastoma.
		In vitro (6/42,14.28)	Rat (4/42,9.52)	Diabetes keratoconus, retinal ischemia, retinal ischemia, reperfusion injury, others.
			Mice (1/42,2.38)	Diabetes
			Rabbit (1/42,2.38)	Glaucoma
		Mixed (6/42,14.28)	Mice (4/42,9.52)	Diabetes, hyperinsulinemia
			Rat (2/42, 4.76)	Diabetes, dry eye others
		Others (3/42,7.14)	Mice (3/42,7.14)	Retinopathy, corneal edema
		Xenograft (1/42,2.38)	Mice (1/42, 2.38)	Thyroid cancer
		Transgenic (2/42,4.76)	Mice (2/42,4.76)	Diabetes
7	Respi*rat*ory system (41/4.87%)	Mixed (15/41, 36.59)	Mice (14/41,34.15)	Lung cancer, pulmonary fibrosis, asthma, others
			Rat (1/41, 2.44)	
		In vivo (14/41, 34.15)	Mice (14/41, 34.15)	Asthma, pneumonia, lung cancer, others
		Xenograft (6/41, 14.63)	Mice (5/41, 12.19)	Lung cancer, asthma, others

(Table 2 continued)

(Table 2 continued)

Sl. No.	Organ-System *N* = 841 (N/%)	Type of Study (*N*/%)	Name of Animal (*N*/%)	Key Study Areas
			Rat (1/41, 2.44)	Asthma
		Transgenic (3/41, 7.32)	Mice (3/41, 7.32)	Lung injury, adenocarcinoma, asthma, others.
		Others (3/41, 7.32)	Mice (3/41, 7.32)	Cancer airway, inflammation, others.
8	Others (291/34.60)	Mixed (89/291, 30.58)	Mice (86/291, 29.55)	*Staphylococcus aureus* infection, Leishmaniasis, *Candida albicans* infection, dengue, melanoma, psoriasis, autoimmunity, endometriosis.
			Rat (3/291, 1.03)	Breast cancer
		In vivo (72/291, 24.74)	Mice (54/291, 18.55)	Chlamydial infection, tuberculosis, human papillomavirus infection, atopic dermatitis, psoriasis, breast cancer, cancer immunotherapy, melanoma, others.
			Rat (14/291, 4.81)	Breast cancer, lymphedema, erectile dysfunction, others.
			Rabbit (3/291, 1.03)	Osteoporosis, osteoarthritis, deep vein thrombosis.
			Guinea pig (1/291, 0.34)	Herpes simplex virus infection
		In vitro (44/291, 15.12)	Mice (20/291, 6.87)	Leishmaniasis, Japanese encephalitis, tuberculosis herpes, simples virus infection, melanoma, breast cancer. autoimmune disease, Gaucher's disease, others.
			Rat (23/291, 7.90)	Psoriasis, breast cancer, causing bone metastasis, reperfusion injury, others.
			Rabbit (1/291, 0.34)	Bone marrow defect
		Transgenic (37/291, 12.71)	Mice (36/291, 12.37)	Atopic dermatitis, autoimmunity cancers, others.
			Rat (1/291, 0.34)	Spondyloarthritis
		Xenograft (28/291, 9.62)	Mice (28/291, 9.62)	Autoimmunity, breast cancer, cancers others.
		Others (21/291,7.21)	Mice (21/291, 7.21)	Autoimmunity, allergy, cancers others.
		Total	841	

Discussion

Using animals for research has contributed significantly to important discoveries in medicine. However, specific segments of the scientific community have questioned the use of animals in research due to ethical aspects, translational value, and the validity of the research being conducted.[11] Our analysis demonstrates that using animals in research is still quite common.

The distribution of animal species in these studies validates that *mice* and *rat*s are still very commonly used small animals for preclinical research. In an animal statistical report released in 2023 by Great Britain, mice were the most frequently used species of rodents, and they were involved in 71% of all experimental procedures in 2022. As per this report, the use of *rat*s has declined over the period.[12] These findings were again repeated in the analysis conducted by Domínguez-Oliva et al., which states that rodents are the predominant animal species used for research. The authors of this study also stated that nonmammal use is trending, and the use of animals in research depends on rules and regulations in that particular country.[13] Guinea pigs and rabbits were less widely employed, possibly indicating their usefulness only for specific research areas. Mice, followed by rats, are highly

regarded as excellent animal models for experimental research, mainly due to their genetic, physiological, and anatomical similarities to humans, which offer valuable insights into human health across various organ systems.[14] Moreover, these rodents exhibit ease of handling and a docile temperament, which is highly convenient for conducting experiments and behavioral studies.

In vivo and *in vitro* are common research approaches. However, as evident from study findings, a *mixed* method, that is, using *in vitro* followed by *in vivo*, transgenic, and xenograft models, is a prevalent approach. *In vitro* studies, besides offering advantages in terms of ethical consider*rat*ions, are less expensive. Conducting *in vitro* studies before *in vivo* studies will reduce the number of animals and help in a focused approach for further *in vivo*, transgenic, or xenograft studies.[15]

Our study findings suggest that the GIT/GU and CNS have received considerable attention in research among the organ systems. In an animal statistical report released in 2023, within basic research, the single largest category in 2022 was a study of the nervous system, followed by the immune system and oncology.[12] This finding is different from the results of our study. It is important to note that the need for more attention may depend on factors such as disease prevalence, impact on public health, and available treatment options. A report published by IQVIA in February 2023 also showed that the most explored therapeutic area is oncology, followed by neurology.[16] We have not categorized oncology as a separate system. However, in our study, as well as under every system, cancer remains a common disease pattern.

As per our study for diseases in the GIT/GU system, *mice* were utilized more using an *in vivo* and mixed approach. Transgenic and xenograft approaches were also common in this system because most diseases in these systems were carcinomas.[17] Mice can be genetically engineered to imitate almost any human disease or condition since they share 95% of human genes. Mice also show a significantly high acceptance rate for xenografts, making them an ideal implement for the *in vivo* culture of human tumors. The immune system of mice is also similar to humans. Hence, mouse xenograft models have provided an indispensable tool for studying tumor initiation, maintenance, progression, and response to treatment.[16]

Whereas CNS systems have used *rat*s and *mice*, primarily *in vivo*, as shown by our results. These results are similar to those stated in the literature regarding animal use for research in this area.[18] These findings are again correlated with disease patterns shown in this system, that is, Parkinson's disease, Alzheimer's disease, and multiple sclerosis. These models are traditionally developed by chemical induction.[19] However, even in CNS, due to advanced research in identifying genes responsible for various CNS disorders, *transgenic* animal models are commonly used next to *in vivo* animal models.[20]

Animal research in other systems, such as musculoskeletal, endocrine, cardiovascular, circulatory, and infectious diseases, is also common, as our study shows. These were, again, key therapeutic areas discussed by Domínguez-Oliva et al. their study where they have analyzed animal research conducted between 2019 and 2023.[13]

This study's limitation was that it relied on data collected from previously published lite*rat*ure. We did not verify the quality and correctness of the published articles, which may have introduced the possibility of bias and inconsistency. Furthermore, there is a chance that selection bias resulted from the study's inclusion criteria, which restricted the findings' applicability by only including freely accessible public domain articles that were listed on the PubMed database.

Conclusion

Our study findings give an idea about the current use of animal species for different research areas, and research approaches depending on the disease area. For conducting research in GIT/GU diseases such as nonalcoholic steatohepatitis and colitis, for research in CNS diseases such as Parkinson's disease and Alzheimer's disease, and musculoskeletal diseases like OA and RA, instead of separate *in vitro* and *in vivo rat*/*mice* models, *mixed* models and transgenic models would be most suitable. Whereas for cancer research, mice xenograft models would be most appropriate. It should be emphasized once more that cost and feasibility play an essential role in the choice of species and research approach for a particular disease area.

Abbreviations

GIT/ GU: Gastrointestinal and genitourinary system; **IQVIA: IQVIA:** I (IMS Health), Q (Quintiles), and VIA (by way of); **OA:** Osteoarthritis; **RA:** Rheumatoid arthritis; **CNS:** Central nervous system.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

The study has received an exemption of review from the Institutional Ethics Committee (EC/OA- 188/2021).

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Informed Consent

Not applicable.

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