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Significance of animal experimentation in biomedical research in the current era: Narrative review

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ABSTRACT

The last century has witnessed enormous advancements in the field of biomedical research. Although many factors can be attributed to this development, laboratory animals hold a significant stake in this journey. From disease modeling to finding its cure, in both academic and industry settings, animal studies are indispensable in every step currently. With the development of genetic engineering technologies, animals mimicking human conditions closely are being generated at a rapid race. However, lack of reproducibility, translation in clinical settings, and the growing objection toward morality of using animals in research has prompted the development of alternative sources as well. With advancements in computational technology and simulation-based studies, animal models are being attempted to be replaced by approaches like organs-on-chips, organoids, and artificial tissues. While most of these technologies are still in their infancy, there seems to be a long way ahead for complete replacement of animal studies by these techniques. Till then, the judicious use of animals in biomedical research seems to be the inherent part of advanced biomedical research.

INTRODUCTION

An investigation into the inception, development, and functioning of the living world can be encapsulated within the terminology of biomedical research. Malfunctioning in any stage of life of a living organism can be considered a disease, the understanding and cure of which can also be considered as biomedical research. In order to understand the mechanisms involved in each of the aforementioned aspects of life, physiology and pathophysiology, as well as targeting the same through natural or synthetic compounds, also constitute biomedical research (Jeffrey and Joseph, 2017; National Research Council, 2011). Thus, biomedical research can be considered the foundation of clinical research and development of life-saving medicines. The status of biomedical research is starkly different in developed and the developing nations. While nations with a robust healthcare program are at the forefront in carrying out and publishing the most relevant biomedical findings of this century, the developing and the underdeveloped countries still have a long way to go (Rahman *et al*., 2020).

Animal testing in biomedical research dates back to time of Alcmaeon and Aristotle, during the 4th and 5th century BCE, who performed exploratory surgeries on animals. Although considered a taboo during those times, many prominent personalities like Erassistratus and Herophilus performed dissections on convicted criminals on death row, thus paving the way for Romans and ancient Greek physicians to use live animals in various medical experiments. The major driving force behind such endeavors during those periods stemmed mainly from curiosity and an attempt toward understanding the nature of pain and deformation. However, a lack of knowledge usually rendered a majority of such observations error-prone, thus leading to the ultimate demise of such practices in the later centuries (Franco, 2013, Maehle and Trohler, 1987).

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The scenario evolved during the early renaissance period when more profound knowledge was developed from studying human cadavers; however, this was faced with strong opposition of such practices from powerful Catholic Churches. It was during the 15th century that Rene Descartes started thorough experiments on animals which would be rather gruesome from today's perspective owing to the fact that no animal or human anesthesia came into existence. It was William Harvey's experiments on live animals in the 15th century that led to the understanding of heart and blood circulation, a significant finding in medicine. It was Harvey who had established comparative anatomy of animals from different taxa, including fishes, amphibians, insects, and reptiles (Franco, 2013). By the 16th century, the Western and Chinese medicines were extensively using animals for obtaining knowledge of animal biology, which led to the development of evidence-based medicine. However, morality of using animals for scientific research began coming into question by the late 17th and early 18th century and has been in constant discussion until today. The problem became compounded when an exponential growth in animal experiments was being carried out for manipulating procedures rather than only observation. By 1980, the ethics behind animal experiments seriously began to be questioned and various acts related to animal protection, cruelty, and safety were established. The discussion is relevant today as much as during the past centuries with biomedical research garnering billions in funding, be it at the industrial or academic level, and is only set to grow rapidly with the recent healthcare crisis exposed by the ongoing pandemic (Geller *et al*., 2007; Von, 1989). The present review explores the sustained necessity of animal-based experimentation in the era of developing alternatives and their continuing importance in different avenues of biomedical research.

Necessity of animal testing

Animal studies allow us to study mechanisms involved in the birth, progress, and systemic manifestations of diseased states. It further gives insight into the underlying genetics of the diseased state and the hereditary pattern of the same. Obtaining such enormous information about the plethora of diseases that haunt human survival from human subjects would be technically not feasible with serious questions of morality and equality. As such, using warm blooded mammals seems to be a rather easier approach. It is mostly rodents that are utilized in biomedical research and it is through the contribution of rodent-based studies that we have access to life-saving drugs and treatment approaches for multiple diseases that otherwise have the capacity to wipe out the human race from this planet. While questioning the use of animals in biomedical research may come from high moral ground, one has to keep in mind the fact that alternatives to animal studies can barely provide the information we obtain from animal studies (Hajar, 2011; Rall, 1979). Of course, with the evolution of alternate sources, like computer simulations, mathematical modeling, enormous data bases of diseases and treatments, cellular and organoid-based studies, biosimulations, labs–on-chips, etc., we have come a long way to reduce animal usage in research, but the fundamental question still persists, are these tools a better alternative to *in vivo* studies? If so, then why does the use of animals still persists even in nations holding themselves with high moral standards, be it for inventing vaccines or the latest cutting-edge medication (Culabbr, 1988). The fact is that as researchers, it is difficult to emulate the complications inherent in living animals with the tools we have at hand and hence there needs to be more rigorous research in this sphere so that a day comes when no animals would be harmed for any scientific discovery. Till then, the focus remains to develop better standards for handling and testing animals and to minimize their usage at least in the trial and error stages as much as possible. Until then, it seems the only way to avoid more of the 'elixir sulfanilamide' or the thalidomide crisis that resulted in severe human tragedies (Hajar, 2011).

ANIMAL MODELS

Any nonhuman species that helps to study the appearance, progression, and manifestation of the disease state as in humans can be considered an animal model. Sometimes diseases in humans, at both the genetic or subsequent levels, can be replicated in animals artificially. Such disease models in animals can also be referred as xenograft models. Animal models are highly important in understanding rare diseases prognosis and treatment and is the only way to study complicated diseases at present. Such rare disorders or disease variants would be otherwise almost impossible to study due to its rare occurrence in humans as well as the high ethical standards associated with human experimentations. Common animal models include drosophila, yeasts, zebrafish, and mice (Doke and Dhawale, 2015).

Rodent model

Rodent models include mainly mice and rats for studying various kinds of diseases. Most rodent models have provided deep insight into the study of cancer, diabetes, wound healing, Alzheimer's, and other behavioral and neurological diseases. Rodent models are popular in biomedical research due to the similarity in disease manifestation as in humans, an ease of availability at lower costs, comparatively liberal animal ethics guidelines, ease of housing and handling, a faster reproductive cycle, etc. Moreover, their shorter life span makes it easier to study disease inheritance, developmental biology, and effects of genetic engineering. Apart from mice and rats, hamsters and guinea pigs are also widely used as animal models (Elizabeth, 2013). Apart from wild types, genetically engineered models are widespread in biomedical research and can be procured based on experimental design. Such genetically engineered models are more suited to study variable states of human diseases. Many agencies like the Rat Resource and Research Center as well as Mutant Mouse Regional Resource Centers are leading repositories for preservation and distribution of rodent models. Rodents share almost 95% similarity to humans in terms of their genetic make-up and hence hold immense importance in biomedical research (Khorramizadeh and Saadat, 2020).

Nonrodent model

Among nonrodent species, rabbits, zebrafish, and fruit flies are most popular in biomedical research. Among rabbits, the New Zealand white rabbit is most popular along with the Watanabe heritable hyperlipidemic and its myocardial infarction prone counterpart. Rabbits are routinely used for studies of atherosclerosis, immunology, reproduction, ocular diseases, and osteoporosis (Rappuoli, 2014; Shiomi *et al*., 2003). It is also

widely used for the production of polyclonal antibiotics. Zebrafish is extensively used in research related to developmental biology, biochemistry, and molecular biology (Peterson *et al*., 2008). Due to the presence of orthologous human genes, rapid development, large clutches of egg-laying capacity, and shorter life span, the use of zebrafish as animal models has grown exponentially over the years. Other nonrodent species, like reptiles and amphibians, are frequently used in studies related to evolution and ecology (Hickman *et al*., 2017).

ROLE OF ANIMAL TESTING IN THE BIOMEDICAL FIELD

Pharmaceutical drug development

The importance of animal models in medicine can be gauged from the fact that 186 Nobel recipients of 222 from 1901 to 2020 have used animal models in their research (Baptista *et al*., 2021). Animals ranging from mouse, sea urchins, frogs, nematodes, pigs, protozoan, flies, hamsters, and chimpanzees have been used extensively in their research. The various drugs and pharmaceutics developed in the field of pharmacology for different diseases include animal testing signifying the importance of the same. From Robert Koch's usage of cow, sheep, rabbits, and mice for developing the treatment of tuberculosis in 1905 to Allison and Honjo's use of mice to study negative immune regulation of cancer therapy in 2018, animal studies have largely contributed to their success in treating such complicated disorders. A pharmaceutical drug development initiative is a huge undertaking in terms of money, manpower, and time involved. With more and more instances of drugs getting banned post-market arrival, there is a lot of risk and anxiety involved in developing new therapies today (Kumar *et al*., 2000). A lot is at stake for a private organization or industry when spending millions of dollars and 10–15 years of time to develop a pharmaceutical drug formulation only to fail during clinical trials. Rigorous stages of clinical trials, as well as the risk involved in using human volunteers in treatment and the cost involved in carrying out the same, makes development of novel pharmaceutical formulations an arduous task to undertake. As such, most organizations have to rely on elaborate animal studies, including *ex-vivo* studies with animal tissues to develop a confidence toward performance of their product. Thus, developing a drug for a disease without animal testing will be a risky endeavor. Of course, animal studies are largely debated in terms of poor translation in humans and lack of proper reporting. However, a methodological planning with high-quality protocols in adherence to the 3Rs (replacement, reduction, and refinement) policy should help in improving the quality of animal studies reported in scientific studies from both academia and industry. In the pharmaceutical industry, preclinical animal models are still mericanice for predicting clinical outcomes. However, the reporting has to be robust, reproducible, and interactive to obtain maximum engagement (Everitt, 2015; Kumar *et al*., 2000).

Tissue engineering

Although the development of 3D bioprinting and technologies pertaining to modern day bioreactors have reduced animal usage in tissue engineering-related studies, animal models and animal tissue explants still hold a central role in tissue engineering. Animals used in tissue engineering can either be wild type or a particular disease model. Sometimes animals are wounded surgically or topically to study critical aspects of wound healing or skin cell regeneration. Many animal models may be induced with bone size defects to check for osteo-repair and regeneration (Dehkordi *et al*., 2019). Through working on various kinds of animal models, ranging from rats to horses, a range of treatment has been developed related to biomechanics, spinal cord injuries, stem cell delivery, etc. Pigs are among the most sought after animals in the field of tissue engineering because of similarity to human physiology, ease of availability, and size. Pigs have served as an excellent model for wound healing, as well as dermal and transdermal treatments. They have also contributed toward research related to musculoskeletal engineering (Ribitsch *et al*., 2020). While choosing animals for tissue engineering applications, one has to be considerate of the size and efficacy of treatment, and the aim needs to be toward reaching a consensus of single animal models and obtaining as much data as possible. This will not only help in reducing the number of animals harmed but will also allow downregulating the cost of research and development (Piedrahita and Williams, 2017). Further disease models are mericanice because of strict regulations against using and obtaining human tissue explants which are also not viable in terms of long-term culture conditions and may fail to recapitulate the complex tissue micro-environment and dynamics as observed in *in-vivo* experiments. However, the scenario may improve somewhat with the development of sophisticated disease models of human organs and tissues which may allow research into specific areas of the field like vascularity, immunity, geometry, and dynamic and biomechanical forces (Healy, 2018).

Medical device development

Animal models in medical device development specifically serve as preclinical models for screening the devices prepared in the laboratory before clinical trials. As per Food Drug Administration (FDA) recommendation of animal studies in the medical device development, Good Laboratory Practices should be strictly followed while carrying out the same. The chosen animal model should be backed with relevant scientific evidence for its utility (Victoria and Samuel, 2019). There has to be standard protocol maintenance for performance and handling and studies related to acceptance of the device in the biological system, as well as the corresponding response of the biological system toward the device has to be elaborately studied and reported (FDA Guideline, 2015). Preclinical animal models can be exploratory, explanatory, or predictive in nature, based on the aims to study critical hypotheses related to inherent biological functioning. The animal models in medical device development for diseases can be induced (or experimental), spontaneous, transgenic, negative, or orphan in nature. Later stages of animal studies in medical device development have to go through elaborate studies on design verification and validation of the multiple prototypes developed in the lab benches. Such studies give definite assessment and validation of the design of the medical device and lay the foundation of the development of production line from an industrial perspective. It is through the initial animal studies, which provide product design validation and assembly line set up, that the proper manufacturing cost, manpower, and time can be estimated. Thus,

animal studies are the fundamental source of initial information toward working or nonworking of a novel medical device under development (Cheng *et al*., 2021).

Other relevant fields

Besides these, animal studies are explored extensively in the cosmetic industry. However, the use of animal experiments in cosmetic industry is highly opposed based on the fact that suffering is induced in animals with the sole aim to develop products that make humans look better. Morally speaking, such an argument can be held at higher grounds, which has prompted the UK and states in the European Union to ban animal testing for cosmetic development. However, due to the risk of adverse reactions from novel cosmetic products, particularly those which are used invasively, toxicological assessment is required. Hence, many countries like China and the United States of America still use animals for experimentations related to development of cosmetic and other human aesthetic products. Mice, rats, rabbits, birds, and fishes are the mostly used animals for exploratory as well as regulatory testing of cosmetics around the world (Kabene and Baadel, 2019). Other fields include psychology involving diseases of addiction, posttraumatic stress disorders, behavioral sciences, evolution, etc.

ANIMAL MODELS IN DRUG DISCOVERY

Disease models

Disease models are disease pathophysiologies in nonhuman animal species that replicate the actual human diseases. Disease models are crucial toward understanding the complicated network of genetic, cellular, or subcellular processes underlying a diseased state that give rise to tissue-specific, organ-specific, or systemic manifestations or malfunctions. Disease models now exist for almost all major diseases and disorders that plague the human population along with inter- or intradisease variations in causation and manifestation. Disease models can be created through invasive surgeries, exposure to chemical agents, and genetic manipulations either in parents, embryonic states, or during developmental state. Genetically manipulated adult-stage disease models can also be designed. Disease models are mostly induced in nature (Denayer *et al*., 2014).

Spontaneous models

Spontaneous models are referred to diseases in animals that mimic that of humans but have generated naturally, i.e., without direct manipulation through surgical or nonsurgical measures. Spontaneous models are rarer when compared to induced models but provide precise information about the inception of a disease state naturally, followed by its progression and manifestation. Spontaneous models are useful while studying naturally occurring hereditary diseases or susceptibility to the same. For instance, the appearance of diabetes in nonobese diabetic (NOD) mice is spontaneous and hence an NOD mouse model can be considered a spontaneous model (Swearengen, 2018).

Genetically engineered models

Animal models which have been modified genetically for the expression of disease phenotypes can be considered genetically engineered animal models. Genetically engineered models are types of experimental models that are modified through mutations induced chemically, inbreeding or cross-breeding with disease models or through recombinant DNA technology. Such genetically modified animal models are also referred to as transgenic animals (Stokes and Marsman, 2014). Genetically modified animal models are high value in research related to genetic engineering, especially now with the development of the CRISPR Cas9 technology, such models are comparatively easier to design and also a large variety of disease states can be formulated. Animal research, including medical research and human gene therapy, as well as plant science research, notably for agricultural development, has been transformed by CRISPR/Cas9 technology. The generation of genomic knockout mutants is one of the most popular uses of CRISPR/Cas9 (Zhang and Showalter, 2020).

ANIMAL WELFARE

Animals plays a vital role in the biomedical research, therefore it is the responsibility of all the researchers that the welfare of animals shall not be impacted. Animal welfare policies and laws have been implemented by different countries in order to minimize or avoid pain and/or distress to the animals by using pain-relieving drugs, humane endpoints, supportive veterinary care, husbandry, acclimatization, and consideration of replacement, reduction, and refinement alternatives (Stokes and Marsman, 2014).

Ethical guidelines

For research involving animal use in biomedical sciences, the 3Rs concept (replacement, reduction, and refinement) needs to be followed (Ranganatha and Kuppast, 2012). As per the European Directive 2010/63, the primary aim has to be replacement. It tends to minimize animal usage by being replaced with nonanimal methods. Provided that animal use seems unavoidable, the aim is to reduce the number of animals as much as possible to obtain a valid output. Finally, special care needs to be provided for refinement of animal welfare and valid uses so as to maximize the data obtained from animal studies. Animal studies should be ensured only with quality planning, the state of animal facility, the suitability of the particular species in the studies planned, and a trained team of specialized personnel involved in animal handling, upkeep, and dose administrations (Doke and Dhawale, 2015). All animal studies that are reported for publication must be in compliance with the Animal Research: Reporting of *In-Vivo* Experiments (ARRIVE) guidelines (Percie du Sert *et al*., 2020). The Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines must further be followed before conducting animal studies. The PREPARE guidelines contain checklists and reminders that should be followed prior to and during carrying out animal studies (Smith *et al*., 2018). The organizations including the Committee for Purpose of Control and Supervision on Experiments on Animal, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), National Institute of Health, and Organization for Economic Cooperation and Development provide guidelines for the animal welfare like animal house maintenance, breeding, feeding, transportation, and mainly for their use in scientific experimentation (Rollin, 2003).

CHALLENGES IN ANIMAL TESTING

Selection of a relevant animal model

Choosing the right animal model for a particular study involves an awareness of multiple factors like advantages of the chosen species as well as its disadvantages. Besides, the similarities and dissimilarities of the animal from human physiology and anatomy should also be taken into consideration. Other factors like research objectives, disease progression in different animal species, reviewing of previous animal use literature for similar research objectives, preparing a detailed biological information matrix, etc. should also be taken into account while designing animal studies (Fontana, 2021).

Translation of knowledge from preclinical studies to the clinic

The biggest challenge in animal model use or animal testing is its translation in human subjects. One has to keep in mind that at best an animal is an imitation of the human physiology and hence it is almost impossible to extrapolate all data obtained from animal studies to human subjects (Swearengen, 2018). Irreproducibility of data from animal studies is another aspect that many researchers complain about, thus questioning the utility of animal studies in its entirety. Irreproducibility or lack of translation from animal to humans has led to the loss of billions of dollars, effort, and time, and is the currently the biggest challenge that animal studies in biomedical research encounter.

Humane end point

Humane end points are cut-off points at which the experiment or use of a particular animal should be stopped to avoid further suffering (Fry, 2014). The lack of understanding of a 'humane end point" may lead to unnecessary infliction of pain and suffering to animals during animal studies (Stokes and Marsman, 2014).

Housing condition and management

The design of animal facilities and management are essential contributors to animal well-being, which not only affect the behavior of the animals but also the experimental results. Enrichment and refinement procedures in the facilities can help in reducing the stress of animals in a particular environment (Balcombe *et al*., 2004).

Molecular and genetic differences in the species

Understanding the differences in molecular and genetic makeup from species to species is important but beyond that an understanding of the habitat, behavior, geography, and environmental conditions of different species which may have an impact on disease manifestation or treatment outcome is required. This wide variability makes the assessment of treatment outcome as well as extrapolation of experimental results from species to species a cumbersome, or sometimes, error-prone effort (Barré-Sinoussi and Montagutelli, 2015).

Statistics

Often there is a requirement of calculating appropriate sample sizes for obtaining statistically significant outcomes. A lack of knowledge of formulation of appropriate sample sizes for control and treatment groups may result in the inaccurate reporting of results (Singh *et al*., 2016). Lastly, rising instances of legal challenges empowering animal rights activist will only make carrying out animal studies far more challenging in future, especially in developed nations.

SELECTION OF AN APPROPRIATE ANIMAL MODEL IN PHARMACOLOGY RESEARCH

Among the various criteria for selecting the appropriate animal model, few are important for accurate extrapolation of data, as well as continuation of research with the same models. The disease state or the fundamental physiology must mimic human conditions. The animal should not be too rare so that other researchers can continue their research on the same models. The animal should be easy to export, produce a large number of offspring, and be of sufficient size so that convenient sampling of blood or other body fluids can be carried out. Animals selected for survival studies for cancer or other life-threatening diseases must have an ideal longevity so that prolonged experimentation can be carried out. For most studies, animals should be able to be segregated based on their genetics (Swearengen, 2018).

Different disease models can be constructed in many ways. For instance, animal models of diabetes can be constructed through pancreatomy, beta-cytotoxicity, diabetogenic nutrients, anti-insulin serum, etc. Some popular animal models for diabetes include BioBreeding Diabetes-Prone (BB) rat, LEW.1AR1/-IDDM rat, NOD mouse, AKITA mouse, etc. (Pandey and Dvorakova, 2020). Animal models for arthritis pathogenesis mainly involve studying pro-inflammatory cytokines, autoantibodies, crystallizable fragment (Fc) gamma receptors (FcγRs), etc. The CIA mouse models which include strains like DBA/1, B10.Q, and B10.RII DBA/1 are prepared through immunization against collagen (CII) in complete Freund's adjuvant. Apart from the collagen antibody-induced mouse model, human Tumor necrosis factor (TNF)-α-transgenic mice are spontaneous models of arthritis. Il-1 receptor knockout mice and K/BxN are other examples of spontaneous models of arthritis (Lewis and Branch, 2020). Different kinds of genetically engineered mouse models are also available for diseases like inflammatory bowel disease (IBD), psoriasis, and cancer. For IBD, majority of the animal models have been designed to express Th-1-dominant immune responses. Knockout mice models [TNF (ARE), CBI-b, IL-2, Atg5, etc.], transgenic mice models (LIGHT, B7.2, STAT4, NCAD, etc.), congenic mice models (SAMP and C3H/HeJ), chemically induced model (OXZ, TNBS, and DSS), and cell transfer models (CD45RB) are few examples of animal models for IBD research (Mizoguchi and Mizoguchi, 2010). Modeling psoriasis is complicated as it does not occur naturally in most laboratory animals. Examples of few rodent models of psoriasis include HLA-B27 transgenic rats, Fsn mice, Ab mice, basal expression of IL-17A in mice, and CARD-14 deficient mice. Many of these mice models are transgenic mice models (except Ab and Fsn which are spontaneous models) (Schön *et al*., 2021). Due to homogeneity of the mouse genome with human genome, mice are used commonly for studying various stages of cancer (generation, development, and metastasis). Mouse cancer models can be chemically induced, cell-line or patient-derived xenografts, or genetically modified. Among these, the chemically induced model is the simplest but requires many weeks for appearance of

disease phenotypes. As such, xenograft-derived models are mostly preferred for cancer research. The genetically derived models give much insight into the process of tumorigenesis. Some examples of cancer models include Hu-BLT Model, Hu-HSC Model, Hu-PBL Model, zebrafish cancer model, and zebrafish melanoma model. Large animal models may include canines, pigs, and nonhuman primates (Li *et al*., 2021).

ANIMAL TESTING IN TOXICOLOGY RESEARCH

The study of toxicology involves detection, evaluation, and prevention of any component or compound that humans may be or are getting exposed to. Toxicology research plays a significant role in drug development as it is carried out extensively to check for potential side effects of drugs which are in preclinical stages. Toxicology also plays a vital role during clinical trials of drugs so as to estimate that the drug of choice does not cause more harm than intended benefit (Stokes, 2015).

REGULATORY REQUIREMENT FOR TOXICITY TESTING

The FDA guidelines for toxicity assessment include assessment of safety of pharmacological drugs on cardiovascular, central nervous, and respiratory systems before exposure to humans. Further established guidelines are intended to investigate the mode of action/effects of substances toward its therapeutic targets. These studies include toxicokinetic and pharmacokinetic studies, acute toxicity studies, clinical development trials, marketing authorization, local tolerance studies, gentoxicity studies, carcinogenicity studies, reproduction toxicity studies, trials in pediatric population, immunotoxicity, nonclinical abuse liability, purity of ingredients, combination drug toxicity studies, improving harmonization of timing of nonclinical safety studies, etc. (Guideline ICH, 2009). The ICH guidelines are elaborately provided for testing each of the aforementioned aspects of drugs. The current regulatory guidelines involve the assessment of the effects of chemical or cosmetic ingredients on human health as well as the integration of nonanimal methods in current regulatory practices. There are established criteria for assessing skin corrosion and irritation along with serious eye damage or irritation for cosmetic products, photo-induced toxicity assessment, mutagenicity, genotoxicity, acute systemic toxicity, repeated dose toxicity, carcinogenicity, developmental immunotoxicity, endocrine disruptions, mixture risk assessments, and finally implementation of the 3Rs for animal use in research in most countries (Pistollato *et al*., 2021).

LIMITATIONS OF ANIMAL USAGE IN BIOMEDICAL RESEARCH

Lack of an appropriate animal model for particular human disease

Although science have evolved to generate animal models for even the rarest of animals, it is still difficult to reproduce certain symptoms which are complicated to manifest or understand in humans. The various states of mind or multitude of emotions that humans experience during a plethora of physical or mental illness often make animal models limiting in illustrating the same with accuracy. For instance, development of models of infectious diseases or rare genetic disorders is often difficult to replicate in animals. Viruses like the Ebola virus can only be manifested in laboratory animals after serious passages and may not be considered an exact replica of the virus that affects humans. The chances of genetic drifting in microorganisms is much higher when regular passaging is carried out in animals, thus rendering the genetic makeup almost nonidentical to the human disease causing counterpart (Swearengen, 2018). Besides, intrinsic factors which determine the interaction of the disease-causing agent with the host and the extrinsic factors which further affect such interactions may cause significant change to disease pathophysiology. Incoherency in understanding of these factors among researchers and fundamental differences from species to species or even within species may lead to error-prone observations and differences in opinion among different research groups.

Difference in animal and human genetics

Irrespective of similarities between human genome and those of laboratory animals, many laboratory animals do not contract human genetic diseases. Most rodent genomes are smaller than the human genome. Humans have 23 pairs while mice and rats have 20 and 21 pairs of chromosomes, respectively. Besides, the number of divergences and rearrangements in rodent genomes are higher than that of primate genomes. As a result of the inherent differences in genetic makeup and disease state expression, multiple methods have been developed to induce disease-causing mutations or mutations that make animals susceptible to human diseases. Some of the prevalent techniques involve transgenesis, gene knockouts, knock-ins, chromosomal rearrangement, conditional gene modifications, etc. (Simmons, 2008).

Nonreplication of animal data in clinical trials

The lack of applicability of animal models to clinical settings stems from the fact that the timing of administration of drugs usually differs in both the settings. In laboratory animals, drug administration is done either prophylactically or in early stages of disease expression. Most clinical trials involve human subjects with diseases in different stages and hence the treatment outcome may differ significantly (Pound and Ritskes-Hoitinga, 2018). It has been suggested that the majority of failure of novel drugs in clinical trials is due to human toxicity which was otherwise not accurately predicted in animal studies. In fact, many drugs which have been deemed safe in animal studies, as well as clinical trials, had to be withdrawn even after being in the market in years. This has led to tremendous loss of life and resources in the past. The opposite has also been true. Many drugs which had been abandoned due to supposed toxicity in animals may have been lost to potential patients (Bracken, 2009; Mignini and Khan, 2006). As most animal studies are termed error-prone, chances of such occurrences seem plausible, if not too high. The root of these problems is lack of reproducibility among animal studies, even within the same species and experiments carried out in rigorously controlled environments. Multiple factors like sensitivity, specificity, and positive and negative predictive value have predicted that animal test outcomes may be heterogeneous in nature. A lack of satisfactory methodological quality, standard data presentation, and choice of proper sample sizes for control and test groups have raised questions over preclinical animal studies

in the past and continues to be of primary concern in academia and industry alike (Van Norman, 2019).

RECENT ADVANCES IN ANIMAL TESTING

Animal models have evolved significantly over the years. With technologies like clustered regularly interspaced short palindromic repeats (CRISPR) Cas9 and other breeding technologies, disease-specific models and humanoid models can be developed. The humanoid strategy is used in Phase 0 trials to reduce medication failure rates by evaluating candidates in multidimensional human organoid-based illness models (MacDonald and Lansdowne, 2021). These models not only possess human mutations, but they are housed and fed so as to mimic human conditions. With technologies to study neuronal activities, animal behavior and psychology can also be studied and hence more information of animal's physical and mental state can be acquired as per requirement. Furthermore, sequencing techniques and whole organ analysis helps in obtaining enormous information from a single animal, thus reducing the number of animals that are otherwise required (Doke and Dhawale, 2015; Khwatenge and Nahashon, 2021).

ALTERNATIVE TO ANIMAL TESTING

Due to limitations of traditional *in-vitro* studies and experience of working with animal models for decades, it appears that animal studies are still going to be around for some time now. However, an increasing awareness of its inability to mimic human conditions at the molecular and cellular levels has led to development of alternate methods of testing. Of course, an increasing opposition toward the morality to use animals in research is also a driving force behind such developments. With the development of human-specific therapeutic interventions like CRISPR ribonucleoprotein particles (RNPS) and vaccine responses, it is becoming imperative to model human organs-on-chips or in form of organoids and laboratory grown model organs/tissues in bioreactors (Horejs, 2021). Apart from this, the development of *in-silico* platforms and computerized models has already begun to replace live animal studies in colleges and universities. Integrated *in vitro* models along with *in-silico* models help in predicting toxicity and have potential to replace animal studies. However, many such alternatives are still in its developmental stage and may require more years for completely replacing animal studies. In fact, drug studies on organs-on-chips suffer from drawbacks, including lack of data about its metabolism or biodistribution, which are of utmost importance in assessing dosage and adverse effects (Doke and Dhawale, 2015, Freires *et al*., 2017).

CONCLUSION

Animal studies have been an inherent part of biomedical research for centuries and have contributed toward the development of most life-saving drugs and remedies for illnesses that challenged human existence from time to time. Animal studies have evolved from being exploratory to the latest humanized models that can mimic almost every vital disease that plagues our society. However, rising concerns of indiscriminate animal usage without proper planning or improper reporting coupled with lack of translation and reproducibility has significantly hampered the dependency of the scientific community on animal studies. Also, the development of powerful computing tools, simulation-based studies, organs-on-chip technology, and 3D printing of live organs and tissues has led to a reduction in the dependency mentioned earlier. As of now, both animal studies and the research into its proposed alternatives will be carried out in parallel so that both refinement of practices and data presentation in both spheres can be attained. With proper regulations and an attempt to minimize animal usage, animal studies will continue to be an important part of biomedical research, alongside evolving alternatives.

AUTHORS' CONTRIBUTIONS

All authors have made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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The authors declare no conflict of interest.

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All data generated and analyzed are included within this research article.

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