INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

CURRENT PERSPECTIVES AND ALTERNATIVES TO ANIMAL RESEARCH

Dr. Sayyed Mateen*, Sinha Nishi, Ansari Farhan, Yadav Santosh, Sarode Vedika, Netke Vinit.
Oriental College of Pharmacy, Sanpada, Navi Mumbai-400705, India.

ARTICLE INFO

Article history
Received 02/06/2023
Available online
07/07/2023

Keywords
Four R' s,
Docking,
Computer Models,
Virtual Humans.

ABSTRACT

Following the advancement of healthcare sciences through investigation and development, their search done on animals has increased lately in the last 2-3 decades. Thousands of research animals are utilized annually all around the world. Since such large animals are being used for experiments, it became a debated issue for many years now, due to the fact that throughout research, animals go through discomfort, misery, and sometimes death. Attempting to resolve the issues concerning experiments on animals and avoid inhumane practices, a number of other possibilities have been proposed. When there are no other alternatives for scientific or educational analysis using animals is considered and it should follow the strategy of 3Rs (that is Reduction, Refinement, and Replacement) in laboratories. To successfully implement this strategy, the researchers use different approaches and alternative organisms. The objective of this review is to provide a brief overview of various options regarding the animal experiments, along with the benefits and restrictions related to them, using examples. Integrating such strategies could clarify whether using animals for experiments is suitable.

Corresponding author
Dr. Sayyed Mateen
Department of Pharmacology,
Oriental College of Pharmacy, Sanpada, Navi-Mumbai.
mateen.sayyed@ocp.edu.in
9923167787

*Please cite this article in press as **Dr. Sayyed Mateen** et al. Current Perspectives and Alternatives to Animal Research. Indo American Journal of Pharmaceutical Research.2023:13(07).*

INTRODUCTION

The R's principles such as "Reduce, Replace, Refine, Rehabilitation" are utilized in research or animal testing and these are commonly known as the 4R principles of animal testing. The aim is to prevent, or at least to minimize animal testing or experiments. For the purpose of limiting improper utilization of animals and reducing their miseries throughout research, many regulations and pieces of regulation had been put into place.

In the United Kingdom, a statute was created in 1876 to tackle the exploitation of animals, which was later followed by other countries like India-1960, France-1964, and the USA-1966. Guidelines for the care, feeding, breeding, transportation, and usage of animals for experimentation are supplied by organizations like the ICH (International Conference on Harmonization), CPCSEA (Committee for Purpose of Control and Supervision on Experiments on Animals), NIH (National Institutes of Health), and OECD. Therefore, for animal welfare, wherever possible, alternatives should be searched. There are ample of alternatives or strategies in this globe that can be employed in place of animals, which can prevent the inhumane killing of the animals used in experiments.

NEED FOR ALTERNATIVES TO ANIMAL STUDIES[1,2]

In India, there are lots of important measures which are needed to be taken to reduce the harm occurring during the procedure of animal toxicity studies. Not only in India but approximately more than 150 million animals in the whole world are also subjected to chemical or mechanical stress for determining the physicochemical properties of any chemical entity.

In order to determine the characteristics of drugs the poor animals unwillingly face the ill-treatment. For example:

1. **Draize test:** This test was carried out for determining the ocular toxicity of ophthalmic preparations.
2. **Sham Test:** The shamtestis often carried out on rabbits for the presence of pyrogen during the sterility testing of intravenous sterile solution. The Sham test is also referred to as the rabbit pyrogen test.
3. **Mutagenicity:** During the mutagenicity testing in animals, the chemical entity which is a mutagen is administered into the animal and then the animal is sacrificed to assess the nucleus of the cells in the animal' s bone marrow.
4. **Skin Penetration:** Ask in a penetration test is usually carried out in rats for evaluating the motion of the drug from the dermis to the blood plasma. After the administration of the test drug, the animal is sacrificed for the determination of the drug substance absorbed into the blood plasma.
5. **Neurotoxicity:** Neurotoxicity studies are often carried in hens or rats, out to determine if any chemical entity when administered shows any change in the nervous system or not and are assessed for a period of 21 days. At the end of the tests, the animal is sacrificed and evaluated for an indication of toxicity.
6. **Pharmacokinetic Studies (PK):** Animals are used in PK studies to determine the rate of absorption, metabolism, distribution, and excretion of any drug when introduced into the body. Animals are administered with test substances of single or multiple doses via various routes of administration (intravenous, skin penetration, inhalation, etc.), after administration of the drug the blood sample of animals is monitored for determining the rates of absorption metabolism distribution, and excretion. Animals are later sacrificed to evaluate the amount of the drug present in the particular organ.

ALTERNATIVES TO ANIMAL EXPERIMENTS

In order to address a few concerns about animal experiments and steer clear of inhumane practices, alternatives were suggested.

Four R' s

Reduction:

Reduction is referred to a decline in the number of animals employed during the creation of a particular amount and degree of accuracy. Example: 1) Invitro cell culture could be an excellent screening method for chemical substances in the initial phases. 2) Human hepatocyte culture offers knowledge concerning how the body might metabolize and eliminate medication.

Refinement:

The principle describes refinement as any reduction in the frequency or intensity of cruel practices carried out on animals that must nevertheless be utilized[3].Investigators ought to enhance the animal care facility to reduce discomfort and unhappiness experienced by animals throughout the investigation and experimental procedures. Example: Enrich and by improving or upgrade the living conditions of testing or research animals.

Replacement:

Principles of Humane Experimental Techniques describes it as using insentient material instead of aware distinct living beings[3].Replacementis of two types, namely;

- Relate Replacement– During the experiment, the animals are employed but not in any way distressed.
- Absolute Replacement– There shall be no animal testing throughout the entire experiment.

Example: Computerized simulations were used to study the manner in which the heart works in order to select potential treatment approaches[4].

Rehabilitation:

This can be defined as the method of taking care of research animals' post-experimentation. All the scientists and researchers concerned with animal experimentation have an ethical responsibility of taking care of animals before the experiment, during the experiment, and after the experiment. Rehabilitation of small animals is not necessary. The average cost of rehabilitation of different animal species was based on the actual average expenses incurred by various institutions.The average cost was for feed

and husbandry and not for the infrastructure or any other thing related to it.

ALTERNATIVE METHODS

Computer Models

Without dissecting the animal, the computer develops simulations that are utilized to forecast the biological activity and toxicity of a possible medicine. For in vivo tests, the compounds identified during the primary screening that exhibit promise is employed. For instance, an in-vivo study is essential to determine the receptor attachment location of a medication[5].Another prevalent type of program would be the Structure-Activity Relationship (SARs) computer program. It generates predictions about the biological implications of an experimental medication based on the existence of chemical groups connected with the primary substance. Quantitative Structure-Activity Relationships (QSARs) are mathematical models that show how a therapeutic agent's physical and chemical characteristics as well as biological effects are related[6].

Docking[7]

Currently, protein-ligand docking is a crucial tool in attempts at drug discovery and a vibrant area of study that has been the subject of significant advancements over the past ten years. These are well illustrated by the expanding selection of protein-ligand docking software programs, the increasing degree of sophistication of its most recent applications, and the expanding user base. Unlike the fundamental principles of protein-ligand docking, this review paper examines the development of this crucial area of study during the last ten years. The range of options for the protein-ligand docking software programs now available is given special consideration.

Cells and Tissue Culture

In vitro cell and tissue, preparations are used to cultivate cells independent of the human body under a controlled environment.In-vitro cultivation involves isolating either human or animal cells and growing them in one thin film on the outer surface of a plate used for culture or flasks[5]. To test new therapeutic molecules or compounds for toxicity and effectiveness, these approaches or processes are first applied. All cosmetic products and ingredients are checked by this method. Cells and tissue cultures are also used for skin corrosion and skin irritation. The "Organization for Economic Co-operation and Development" (OECD) released test guideline 439 in August 2010, outlining a new procedure for in-vitro hazard detection of irritating compounds. OECD also approves the methods for tissue culture which are used for the measurements of the rate of epidermal absorption of chemicals or drugs[8].As a fungal model for abnormality and the breakdown of drugs, both cell and tissue preparations are frequently employed.In order to study the microbiology of mammals and their drug breakdown, fungal organisms such as Cunninghamellaelgans are often utilized[9– 11]. This technique is also used for phototoxicity and organoids that are 3D cell cultures or mini-organs. Organoids develop in a culture dish or on scaffolds. Organoids are made from 3 distinct kinds of human or animal cells: adult somatic stem cells (ASCs), induced pluripotent stem cells (iPSCs), and embryonic stem cells that are pluripotent.John Hopkins University investigators were able to construct a minuscule brain to study the manner in which COVID-19 might affect the function of the brain[12].

Danio rerio

A little freshwater fish, measuring between 2-4 cm, Danio rerio, iscommonly called a Zebrafish. Its body is practically translucent in its early stages of growth which gives access to watch its internal anatomy with the naked eye. Because of its optical clarity, it enables immediate examination of embryonic phases, detecting phenotypic characteristics during genetic mutation, prompt detection, assessment of toxicological tests, as well as direct observation of expression of genes employing light microscopy[5].By choosing a D. rerio instead of an animal for the experiment, less workspace, and money will be spent on lab supplies, test chemicals, and labor overall. Developing larvae and embryos, it may be used to assess material inside cell culture plates as well as petri dishes. The complete genome arrangement of the zebrafish has made it a desirable option to support genetic and molecular research. While it is used for a wide range of different purposes between early years and maturity, its primary use involves the determination of a number of toxicological examinations of compounds and drugs. Thereexist numerous possibilities for researching mutagenesis and problems with the development of organs brought on by testing substances, including research into cardiovascular disorders, neurological difficulties, behavioral disease, and cancer[5].

Drosophila[13]

Drosophila is considered a good model organism because its use is not fraught with ethical problems.

- The life span of drosophila is short and it is taken as an advantage because then a large number of small flies are producible in a short amount of time.
- Within 24 hours of fertilization, an embryo forms, and it takes just 10 days for a fly to mature into an adult.
- As they are small so they are easily maintained and due to this reason, even though it lacks the resources of time, space, and money, a tiny laboratory can test Drosophila.
- This species is a good animal model because of genetics.

The Drosophila animal model is used for cancer, microbiome research, food, and nutrition research, Alzheimer’ s disease,

to study nanotoxicity, the system for neurotransmitter measurements, and exercise research.

Microorganism

Microorganisms like E. coli, Bacillus substilismodel for Molecular and genetic studies and cell differentiation studies. Because the rate of reproduction is so quick, it is simple to create a big population and study it. Understanding the fundamental characteristics of cell biology in neurodegenerative illnesses is beneficial.

Fungi

For investigations on the circadian rhythm, metabolism, and genetics, fungi like Neurospora crassa are used as models. Despite being under-utilized, fungi make good substitute organisms for experiments with animals. The fungus "Aspergillus Terreus" produces lovastatin, a medication used to decrease cholesterol.Every year, new fungal-produced substances are discovered, but only a small portion of all known fungi have been thoroughly studied, according to researcher JelmerHoeksma[14].Some Alternatives methods for Standard toxicity tests are tabulated in Table 1.

Table 1: Alternatives for Standard Toxicity Test.

Endpoint	*Animal test*	*Alternative tests*	*Guidelines*
Skin Absorption	*Rats are murdered the next day after having the drug applied to their shaved backs (OECD TG 427).*	*The amount of material that flows through excised skin is measured in ex vivo skin-based testing.*	*OECD TG 428*
Eye Irritation/ Corrosion	*Live rabbits that have substances injected into their eyes are observed for up to three weeks (OECC TG 405).*	*Ex vivo eye removal from animals slaughtered for food allows for the detection of both mild and severe irritants.*	*OECD TG 437 and 438*
Skin Sensitization	*The substance is applied onto the guinea pigs' hairless skin, whose subjective allergy status is evaluated (Buehler or guinea pig maximization test; GPMT; OECD TG 406), or mice's ears have been daubed with it, who're eventually eliminated 6 days following this to assess the immunological response (local lymph node assay).*	*Human Cell Line Study*	*OECD TG 442C*

RECENT TRENDS

Virtual Humans[15]

In order to anticipate medication metabolism and metabolite interactions with any organ, virtual people are human computational models. This computational model shows higher accuracy than the animal models. The advantages of these models are –

1] Early on in the drug testing process, they employ fewer animal trials.
2] By lowering patient risk during clinical studies, they increase medication safety.
3] They help by increasing the developmental process of medicines.

High Throughput Screening (HTS)

HTS is a technique for the delivery of early-stage drugs. It rapidly tests a large number of compounds for their ability to modify the properties of a selected biological target[16]. Applications: 1] Used in drug discovery. 2]In a systemic study of Mitochondrial toxicity of environmental chemicals. 3] To screen all kinds of novel biologically act*ive* compounds (which may include natural products and combinatorial libraries) 4] To screen microarrays (DNA chips, RNA chips, Protein chips).

Virtual Screening (VS)

It is a computerized approach for searching libraries of small compounds to find the structures most likely to connect with the pharmacological target, which is often an enzyme or protein receptor[17,18].

Combinatorial Chemistry

It is a chemical synthetic method that prepares several different chemicals (which may be 10, 1000, or millions) throughout a single procedure. These compounds can be as a mixture, as a set of individual compounds, or as chemical structures generated by computer software[19].

Molecular Modelling

These theoretical approaches and computational tools imitate the behavior of molecules and molecular systems.

Microfluidic Chips

Microfluidic chips are devices that help in processing and or visualizing a very small amount of liquid. These chips are transparent and generally have 2 to 10 cm of length with 0.5 mm to 5mm thickness. The tiny chambers on this tiny device each contain a sample of tissues coming from a different part of the body. Microchannels connecting these compartments carry blood replacement through them. The test substance is infused into the artificial blood and circulates around the apparatus. This chip's detectors provide feedback data for additional computational analysis.

TABLE 2: Software Used for Recent Trends.

RECENT TRENDS	***SOFTWARE***
Virtual humans	*DAZ 3D*
	CLO 3D
	Maya LT
Virtual Screening	*PyRx*
	MSLDOCK
	FWAVina
	vsFilt
	molar
High Throughput Screening (HTS)	*Magellan-data analysis software by Tecan*
	MARS data analysis software
	Gen5 Microplate reader and imager software by Biotek Instruments, Inc.
	Metaxpress high-content image acquisition and analysis software.
Combinatorial Chemistry	*smiLib*
	CLEVER
	GLARE
	ChemT
Molecular Modelling	*APBS*
	Avogadro
	BALL
	Cn3d
Microfluidic Chip	*3DμF*
	COMSOL

BENEFITS OF ALTERNATIVE TO ANIMAL STUDIES[20– 22]

Earlier there was no alternative and as animals were easily available and they also have a similar system to humans so they were used for testing any newly invented drug or product.

Alternative methods of testing may be more accurate.

Researchers carried out one test by two different methods, the first method by using human skin cells which were developed in the lab (Invitro technique), and the second method by using a rabbit. Invitro testing has correctly identified all the chemical irritants while tests on rabbits failed 40% of the time.

Alternative methods of testing save the lives of animals.

Animal testing results dying of animals either during the testing process or after the process. The best example to explain this is Lethal Dose 50 (LD 50) in which animals are made to ingest toxic substances until the half popular of animals involved in testing die, while the other half of the population of animals were killed later. Dr. Bjorn Ekwall (Swedish researcher) replaced this with donated human tissues. This replacement test can target specific human organs and reveal certain features of toxicity. This method is more accurate as this can measure toxicity 85% of the time whereas LD 50 shows 60% to 65% accuracy.

Alternative methods of testing may be faster.

Using synthetic skin rather than animal and many other alternative methods give information in few minutes to few hours while in an animal testing method that often takes weeks to months to give the result of the information. In simple words, at the same time, a Researcher can study four to five products with alternative methods while only one product can be studied by using animal testing.

Alternative methods are cost-effective.

The alternative method means no animal involved, which means there is no need for animal purchasing, housing, feeding, and care. As alternative methods are faster, they help to bring the product to the market faster which helps in early earning.

Alternative methods are more environmentally friendly.

Animal waste is classified as hazardous or pathogenic waste. Alternative methods are less harmful and create less waste. These methods reduce or eliminate the use of test products on animals.

CONCLUSION

It is incontrovertible that animal testing has contributed considerably to scientific progress. As important as human well-being is animal ethics. To effectively execute the 3Rs while using animals in laboratories, significant effort must be made. Varioussubstitutions to using animals are suggested, but they must be coerced in a decent way. The incorporation of multiple computational models necessitates in vitro cell cultivation, enzymatic assays, bioinformatics resources, including biological models. Reliable findings will be provided by the application of modern analytical methods, information gathering, and applied mathematical procedures that store search outcomes of other protocols. These coordinated approaches would reduce the need for using animals in scientific processes.

Non-animal options could likewise produce outcomes almost immediately.Additional benefits revolve around the possibility for tremendous human and economic resource reductions, the significant reduction of usage of animals, and the need for just minuscule amounts of test compounds.A significant shift in focus far from the extremely time- and resource-consuming the rat bioassay is required to reduce the immense expenses related to cancer to community.It is vitally essential and important that more effort be put towards the creation and use of non-animal alternate tests.

AKNOWLEDGEMENT

The authors thank Oriental college of Pharmacy for constantly supporting and encouragement for the research work.

CONFLICT OF INTEREST

The creators announced that there are no irreconcilable circumstances with respect to the production of this paper.

ABBREVIATIONS

VS: Virtual screening.
HTS: High throughput screening.
DNA: Deoxyribonucleic acid.
RNA: Ribonucleic acid.
OECD: Organisation for Economic Co-operation and Development.
ASCs: Adult somatic stem cells.
ICH: The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.
CPCSEA: Committee for Control and Supervision of Experiments on Animals
SAR: Structure Activity Relationship.
QSAR: Quantitative structure-activity relationships.

REFERENCES

1. Wallig MA, Haschek WM, Rousseaux CG, Bolon B, editors. Chapter 22 - Special Senses. In: Fundamentals of Toxicologic Pathology (Third Edition) [Internet]. Academic Press; 2018. p. 673– 747. Available from: https://www.sciencedirect.com/science/article/pii/B9780128098417000228
2. Teixeira L, Dubielzig RR. Chapter 53 - Eye. In: Haschek WM, Rousseaux CG, Wallig MA, editors. Haschek and Rousseaux' s Handbook of Toxicologic Pathology (Third Edition) [Internet]. Boston: Academic Press; 2013. p. 2095– 185. Available from: https://www.sciencedirect.com/science/article/pii/B9780124157590000534
3. Russell WMS, Burch RL. The principles of humane experimental technique. Methuen; 1959.
4. Gipson IK. Cell biology of the corneal epithelium. Principles and practices of ophthalmology. 1994;3– 16.
5. Doke SK, Dhawale SC. Alternatives to animal testing: A review. Saudi Pharmaceutical Journal [Internet]. 2015;23(3):223– 9. Available from: https://www.sciencedirect.com/science/article/pii/S1319016413001096
6. Knight A, Bailey J, Balcombe J. Animal carcinogenicity studies: 3. Alternatives to the bioassay. Alternatives to Laboratory Animals. 2006;34(1):39– 48.
7. Sousa SF, Ribeiro AJM, Coimbra JTS, Neves RPP, Martins SA, Moorthy N, et al. Protein-ligand docking in the new millennium– a retrospective of 10 years in the field. Curr Med Chem. 2013;20(18):2296– 314.
8. Test No. 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method [Internet]. OECD; 2021 [cited 2023 May 19]. (OECD Guidelines for the Testing of Chemicals, Section 4). Available from: https://www.oecd-ilibrary.org/environment/test-no-439-in-vitro-skin-irritation-reconstructed-human-epidermis-test-method_9789264242845-en
9. Björnstad K, Helander A, Hultén P, Beck O. Bioanalytical investigation of asarone in connection with Acorus calamus oil intoxications. J Anal Toxicol. 2009;33(9):604– 9.
10. Moody JD, Zhang D, Heinze TM, Cerniglia CE. Transformation of amoxapine by Cunninghamella elegans. Appl Environ Microbiol. 2000;66(8):3646– 9.
11. Jaworski A, Sedlaczek L, Długoński J, Zajaczkowska E. Inducible nature of the steroid 11-hydroxylases in spores of Cunninghamella elegans (Lendner). J Basic Microbiol. 1985;25(7):423–7.
12. Hezari M, Davis PJ. Microbial models of mammalian metabolism. Furosemide glucoside formation using the fungus Cunninghamella elegans. Drug metabolism and disposition. 1993;21(2):259– 67.

13. Hannah Simmons. Drosophila as a Model Organism. [cited 2023 May 20]; Available from: https://www.news-medical.net/life-sciences/Drosophila-as-a-Model-Organism.aspx
14. Hoeksma J, Misset T, Wever C, Kemmink J, Kruijtzer J, Versluis K, et al. A new perspective on fungal metabolites: identification of bioactive compounds from fungi using zebrafish embryogenesis as read-out. Sci Rep. 2019;9(1):17546.
15. Arora T, Mehta AK, Joshi V, Mehta KD, Rathor N, Mediratta PK, et al. Substitute of animals in drug research: an approach towards fulfillment of 4Rs. Indian J Pharm Sci. 2011;73(1):1.
16. Dr Tobias Pusterla. High-throughput screening (HTS) [Internet]. BMG LABTECH. [cited 2023 May 20]. Available from: https://www.bmglabtech.com/en/blog/high-throughput-screening/
17. Rester U. From virtuality to reality-Virtual screening in lead discovery and lead optimization: a medicinal chemistry perspective. CurrOpin Drug DiscovDevel. 2008;11(4):559– 68.
18. Rollinger JM, Stuppner H, Langer T. Virtual screening for the discovery of bioactive natural products. Natural compounds as drugs Volume I. 2008;211– 49.
19. Pottel J, Moitessier N. Customizable generation of synthetically accessible, local chemical subspaces. J Chem Inf Model. 2017;57(3):454– 67.
20. Animal Testing Statistics [Internet]. [cited 2023 May 20]. Available from: https://spots.com/animal-testing-statistics/
21. Limitations of Animal Tests [Internet]. [cited 2023 May 20]. Available from: https://www.hsi.org/news-resources/limitations-of-animal-tests/
22. Ethical Guidelines for the Use of Animals in Research, Published: 8/7/2019. [Internet]. [cited 2023 May 20]. Available from: https://www.forskningsetikk.no/en/guidelines/science-and-technology/ethical-guidelines-for-the-use-of-animals-in-research/

5487847845**1230512**