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Exploring Drug Discovery: Advances in Alternatives to Animal Testing Methods

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ABSTRACT

Animals including aquatic organisms are widely used in a variety of drug discovery processes, from the traditional "trial-and-error" method to the contemporary rational drug synthesis, to evaluate the effectiveness and safety of the compounds. Each year, millions of animals are sacrificed as a result of this practice. Laws to safeguard animal rights have been passed in response to concerns regarding animal cruelty on a worldwide scale. Drug discovery experts started looking for alternatives to animal trials in response to these rules protecting animals. Recently non-animal methods are employed in toxicity testing and biomedical research. These methods include improving data sharing and analysis before further studies, as well as physicochemical evaluation and computerized modeling using structure-activity relationships and expert systems. Additionally, minimal sentient animals from lower taxonomic orders, early vertebrate developmental stages, and higher plants and microbes may be used. Tissue cultures, like immortalized cell lines, embryonic and adult stem cells, and organotypic cultures, are also employed for animal testing. To effectively address the needs of high-throughput chemical testing, emerging testing standards, and the ongoing advancement of human therapeutic treatments, an in-depth focus is required on establishing and implementing methodologies to replace, reduce and refine animal use in experiments.

1. INTRODUCTION

Recent developments in pharmacological and medicinal research have created the need for using a huge number of animals in order to test the efficacy, purity, and safety of newly developed drugs. At least 192.1 million animals are reported to be used for experimental research every year, worldwide (Taylor et al., 2015). Like two sides of a coin, animal testing has led to various drug discoveries and, at the same time also created controversy due to ethical reasons. Animal usage added immense knowledge to research advances made in the past several decades, but the scenario is rapidly changing. Increased use of animals in toxicity testing has raised controversy and criticism due to ethical concerns such as the pain, stress, trauma, and even death experienced by these animals during research. Following a global debate on the “cruelty of animals”, a number of legislative initiatives have been proposed from time to time to limit animal research or ensure proper treatment of animals. The new European Chemicals Legislation of REACH policy favored the usage of validated and appropriate alternative methods instead of conventional in vivo testing (Lilienblum et al., 2008). However, in the last three decades, there has been an inclination towards using fewer animals and performing new methods to serve as new biological sciences options (Ajmera *et al.*, 2017). Non-animal models rely on investigating the physicochemical characteristics of drugs outside of living organisms. After validation with in-silico or in-vitro methods, these drugs are administered to animals, reducing the number of animals used for testing and maintaining the well-being of animals. However, there is a requirement for more focus on their research and implementation in order to effectively address the needs of high-throughput chemical testing programs, significant emerging testing needs, and the ongoing advancement of human clinical interventions (Ajmera *et al.*, 2017).

2. Definition of alternative to animal testing

Animals are used in scientific and medical research to assess the efficacy, safety, and possible risk or toxicity of various drugs, products, and treatments (Mukherjee et al., 2022). This approach is known as animal testing, animal experimentation, or vivisection. The creation and use of test methodologies without the use of live animals are alternatives to animal testing. "Alternatives" or "Substitutes" are any procedures that replace the use of laboratory

animals (complete) or reduce the number of animals needed (partial), minimize the amount of stress they experience, cut down on the number of animals needed, or improve an existing treatment.

3. Regulations for animal experiments

To regulate the usage of animals in scientific research, various acts and laws have been passed (Fig 1). The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) was established under the Prevention of Cruelty to Animals Act, 1960, providing guidelines for conducting animal experiments. In 2006, Institutional Animal Ethics Committees (IAECs) were established for Education and Research purposes involving small animals. To ensure adherence to regulations, IAEC will review, examine and approve proposals for conducting experiments on small animals, and ensure that experiments are not performed in a routine manner.

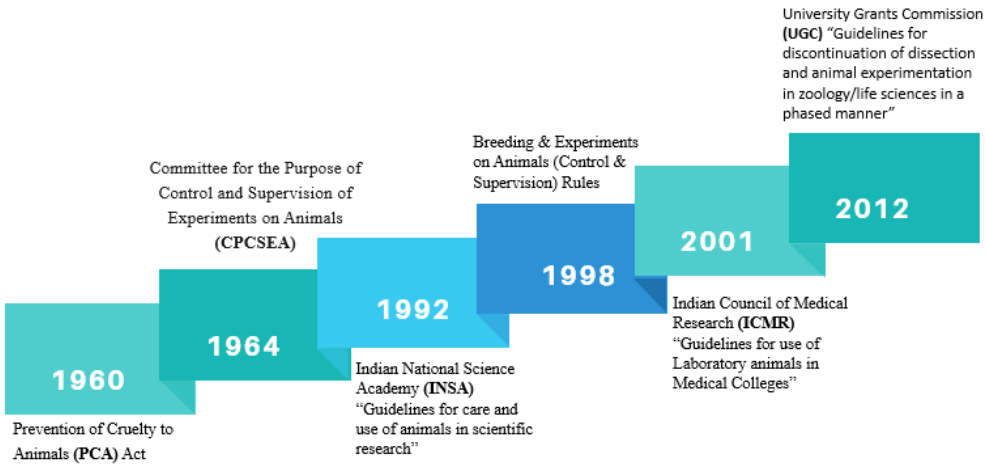

Fig 1. Regulations/ Guidelines for the use of experimental animals

4. CONCEPT OF 3 R

The principle of 3R introduced by Charles Hume and William Russell at the Universities Federation of Animal Welfare includes Reduction, Refinement, and Replacement is becoming a popular alternative (Russell & Burch, 1959) and it gained widespread interest in the scientific community after the special edition of *The Principles of Humane Experimental Technique.*

A Special Edition containing the original text was reissued in 1992 after its ideas had gained widespread interest in the scientific community. Later, 4th R i.e., Responsibility is also added (Banks, 1995).

i. **Reduction:** Decrease the number of laboratory animals required for a test method. Achieved by good experimental design and controlled environment

ii. **Replacement:** Replace by non-animal systems or phylogenetically lower species of animals. Achieved by the use of an in-vitro system and computer simulation

iii. **Refinement:** Reduces animal suffering with procedures that bring less distress to animals. Achieved by less invasive technologies, pain relievers & proper environment

iv. **Responsibility:** Humans are responsible for the safety and prevention of cruelty to animals. It also indicates that animal life is precious for biomedical advancement.

5. ALTERNATIVE METHODS

Diverse techniques and alternative organisms must be used to accomplish the 3R approach effectively. Researchers have also started using non-animal methods to overcome the limitations imposed by various acts and laws. Various non-animal testing methods are given below.

Fig 2. Methods of Non-Animal Testing

5.1. MINIMALLY SENTIENT ORGANISMS

Utilizing minimally-sentient animals from lower phylogenetic orders or early developmental vertebral stages, as well as microorganisms and higher plants, has the potential to enhance compliance with animal use regulations (Ajmera et al., 2017). Additionally, it's acknowledged that many fundamental processes are common among a broad spectrum of organisms, including invertebrates (Susan et al., 2011). Numerous lower organisms have demonstrated a higher degree of genetic similarity to mammals, particularly humans. These alternative organisms include lower vertebrates, invertebrates, and microorganisms such as *Drosophila melanogaster*, the nematode, *Caenorhabditis elegans*, the brewing yeast Saccharomyces cerevisiae, the Branchiopods *Daphnia magna*, the fungi *Cunninghamella elegans*. The advantage of employing minimally-sentient animals lies in their short life cycle, compact size, and reduced requirements for maintenance and housing (Khabib et al., 2022). Organisms from lower phylogenetic orders offer extensive applications in investigating conditions such as cancer, heart diseases, neurological disorders, behavioral patterns, and the examination of mutations and developmental issues in organs resulting from exposure to test compounds.

5.2. IN-SILICO METHODS

Without any animal dissection, computer-generated simulations can be used to predict the various possible biological effect and hazard potential of chemicals. *In silico* models are based on basic principles of biology. Specially designed computer models and computer software help to design new drugs. *In-silico* methods such as Quantitative Structure-Activity Relationships (QSAR), Computer Assisted Learning (CAL), and Computer-aided Drug Design (CADD) are widely used to avoid the use of chemicals which does not have any biological activity (Kapetanovic, 2008). Computer models predict possible binding sites of drugs and their receptors, thus reducing or decreasing the number of animals used. For *in vivo* experimentation only the most promising molecules obtained from primary screening are used. For example, to know the receptor binding site of a drug, *in vivo* experimentation is necessary. *In-silico* methods can effectively employed in drug designing, development, and screening and these computer models are more accurate, inexpensive, and give us quick results (Li et al., 2007).

5.3. IN- VITRO METHOD

In-vitro methods comprise every type of experiment in the life sciences, which makes use of living material but does not involve animals or humans except as donors of tissue or cells. The development of *in vitro* methods based on biological materials that will be suitable for reliably verifying the safety and compatibility of product ingredients, and the development of testing and evaluation strategies that efficiently combine and use information from different sources is vital in the current scenario. The different *in vitro* techniques include primary cell cultures, established cell lines, stem cells, tissue slices, and organ cultures. To assess the toxicity of eye and skin corrosion, as well as carcinogenic potential, researchers use *in vitro* tests that utilize bacterial, yeast, human cell cultures, and mammalian cells. The benefit lies in the fact that certain assays have undergone scientific validation, while others are currently undergoing rapid development (Khademhosseini et al., 2006). Some examples include perfused cultures, human hepatocytes, and green-screen genotoxicity assays. *In vitro* assays are having applications in various fields such as model systems, cancer research, virology, vaccine production, genetic engineering, gene therapy, and drug screening and development.

5.4. ORGAN ON CHIPS

The term "Microfluidics" is the combination of two words: "micro," refers to small, and "fluidics," refers to the intricate movement of liquids and gases. In recent decades, remarkable progress has transformed the field of microfluidics, with a prominent focus on "organ-on-chips" (Li et al., 2023). These miniature chips 2 cm in width, house an array of minute chambers, each delicately cradling tissue samples sourced from distinct body regions (Haeberle

and Zengerle 2007). A blood substitute flows through the channels interconnecting the compartments, and the test drugs are added to the blood substitute as it circulates around the device. The chip's sensors provide information for computer analysis. This can be used to research drug metabolism and the progression of disease (Ingber, 2022). In order to enable the growth of human cells in physiologically realistic environments, tissue engineering and micro-engineering are combined to allow the influence of growing environments on both human cell cultures and animal models. The chips have been demonstrated to replicate human physiology, diseases, and drug responses more closely than basic animal trials, making them an alternative to the use of animals in disease research, drug testing, and toxicity testing.

6. APPLICATIONS

 Usage of protozoa over rodents for bacterial infection assays: This is due to the shared protective mechanism of the bacterium *Pseudomonas aeruginosa* in both unicellular amoebas and multicellular mammalian cells (Cosson *et. al.*, 2007)

 Usage of fruit flies as multiple model organisms: Approximately 75% of genes exhibit functions homologous to human genes (Wilson, 2011). The embryo stage is utilized for exploring organogenesis, cell fate determination, and axon pathfinding. The larval stage investigates physiological and growth processes like foraging. The adult stage focuses on structural studies of organs such as the heart, gut, kidney, and lungs. Each stage contributes to research, making the fruit fly a versatile model organism (Susan *et al.*, 2011).

 Brewing yeast as a model organism: *Saccharomyces cerevisiae* can be employed for investigation of apoptosis, and cellular progression of various diseases like Alzheimer's, Parkinson's, and Huntington's diseases (Madeo *et al.*, 2002).

 Ames test: Microorganisms are commonly employed in toxicology and carcinogenesis studies. The Ames test which uses several strains of the bacterium *Salmonella typhimurium* can identify 80-90% of carcinogenic chemicals studied, primarily serving as a screening system. It requires validation through animal studies. This test relies on a chemical's ability to induce mutagenic changes in a cell's DNA, which holds the cell's genetic information.

 ***Invitro* models:** Several liver-based in vitro model systems, including liver tissue slices, isolated microsomes, perfused liver, and immortalized cell lines, are efficient for early-stage screening of highly toxic compounds (Ajmera *et al.*, 2017).

 ***In-vitro* permeation studies:** Chitosan films, serving as alternatives to animal and human epidermal sheets, are employed for *in vitro* permeation studies of both polar and nonpolar drugs (Rana *et al.*, 2004).

7. CONCLUSION

The complete replacement of animal models with alternative methods for assessing drug effects in biomedical research is currently unfeasible. Nevertheless, by integrating these approaches, it becomes possible to gain valuable insights while minimizing animal usage in scientific experiments without compromising research quality.

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