Review Paper

Moral Values and Ethical Aspects to be Adhered in Preclinical Experiments with Laboratory Animals: an updated review

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ABSTRACT

Throughout the last century, seminal advances in pharmacology and medicine have been made and helped find medication for various life-threatening ailments. However, all drugs tested in preclinical studies do not reach meaningful outcome and this gives context for people's moral concerns about using animals in research for human benefit. Both animal and human studies raise serious ethical issues. This is principally because the lack of demonstrable advantages, insufficiency in rehabilitation process and possibility of injury to the animals involved in the research, present several ethical concerns. This review addresses the moral and ethical issues raised in animal testing and how to find a fine balance where both research and ethics needs to be pursued for the betterment of both humans and laboratory animals.

Keywords: Institutional Animal Ethics Committee, animal studies, animal experiments, and animal research

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Introduction

Medical research is an ever-growing need and animal research is at the forefront in drug development. The use of laboratory animals in biomedical and behavioural research have yielded significant benefits for drug development. Laboratory animals are an absolute necessity for research. But, of course, their use should be warranted and carried out in an ethical manner. The ethical principles do not get compromise in the animal kingdom. Hence, animal for either in vitro or in vivo experiments need to be conducted according to the approved ethical norms. The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) is the regulatory point of conduct for animal research. The lower forms and higher-level forms of

animals have need for ethical research. It is the institutional animal ethics committee that ensures ethical research, locally.
The use of laboratory animals is an important aspect of the search for a cure for human illness and well-being. In this objective it is especially very important that in testing of novel drug/formulation formulations and surgical treatments and procedures animals need to be used to test the efficacy of the modality in test. In lieu of this, animal experiments and models are critical for the discovery of new medications and the assessment of the safety of novel surgical interventions.
From a bioethics perspective, utilitarianism promotes using laboratory animals in research despite the inherent danger of injury, arguing that the benefits to humanity exceed the risks involved in the experimentation. The outcomes may promote human well-being in ways that existing technologies cannot. However, use of surgical procedures and novel drugs can be extremely painful for the animals involved, increase morbidity, and may significantly reduce their quality of life. Furthermore, there is no evidence of any prospective human advantages of all interventions tested.

The widely held belief that animal pain perception is comparable to that of humans presents ethical concerns concerning animal cruelty methods. Researchers acknowledge this and agree on the most humane way to perform research. According to the US Department of Agriculture, animals only suffer mild discomfort in around 62% of tests [1]. After getting an anaesthetic or analgesic, 32% displayed no discomfort. This raises the concern that using animals in studies could generate serious moral issues and if it is wrong to subject animals to suffering. However, this data does not justify the continued use of animals in scientific studies for the simple fact that ethically it is wrong to subject laboratory animals to undue stress and pain [1].

Animal Research Ethical Problems
From a societal perspective, the morality of animal testing continues to pique the public's curiosity. There are two schools of thinking emerging: deontological (intrinsic) and consequentialist (utilitarian) [2]. The animal rights movement is frequently attributed to utilitarian ethicist Peter Singer. According to his classification of animal rights within the context of human rights, animals' best interests should be considered regardless of whether they are cute, endangered, valuable to people, or whether any humans care about them or not. He argues in his ground-breaking 1975 book Animal Liberation that humans have no right to be treated differently than other animals. Even if animals and people have some rights, he emphasizes that there are times when those rights must be compromised for the greater good. Jeremy Bentham expressed similar views, questioning the use of animal research. Questions were raised concerning their communication, mental clarity, and pain perception. Despite his opinions on the subject, he was not opposed to the use of animals in research if it could promote medical sciences and assist humanity. This, however, necessitates an honest assessment of the costs and rewards of using laboratory animals. Tom Reagan, a deontological ethicist, disagrees with this utilitarian viewpoint, arguing that using animals in scientific research is always unethical due to the species' inherent worth.

Due to the shared evolutionary lineage between humans and other primates, certain diseases, including AIDS, have been investigated using primate models in research endeavours. The animal result translation to humans was problematic because primates have higher immune system than humans, allowing them to fight sickness and aid the body in recovering from illness when treated. However, when tested on humans, the drug was shown to be ineffective, hastening the spread of the disease and eventually leading to death. What was distressing was that it took a considerable amount of time for this knowledge to be known and substantial numbers of primates were used for the study. The comparative observations brought in to fore the disparities in physiological, immunological, and biochemical functioning between the two species and questioned the need to use primates as predecessor for translational experiments in clinics.

Despite heated disputes about the best way to treat animals, utilitarianism remains popular, albeit with certain adjustments, since we continue to believe that "*the end justifies the means*" or "*the greatest*

good of the greatest number" is more important than any single life. From a moral dimension there are two key points of dispute in animal studies. The first is whether utilizing animals in research will yield useful results that would otherwise be difficult to get to treat humans. The second pertains to the ethics of potentially harmful animal use for these experiments primarily if it causes unnecessary pain to the animals. The benefits of animal research to humans are not always clear and add to the conundrum and question the use for experiments that may turn to be futile.
The animals' inherent worth must be recognized, and their well-being must always take precedence. Animal protection is unequally distributed due to its hierarchical character, with only cats, dogs, horses, and primates (among others) obtaining legal protection and when choosing on a study topic and approach, researchers must consider the demands of each laboratory animal [3].

William Russel and Rex Burch defined the three R's in their 1959 book The Principles of Humane Experimental Technique. The initial three R's have given way to the current six R's [1]: refinement, reduction, replacement, rejection to employ animals in favour of other approaches, and responsibility. Following these guidelines is the same as using humane methods while doing animal research.
All worldwide legislation governing the use of animals for scientific purposes, as well as all organizations committed to the development of alternatives to animal research, have allowed the use of these alternatives. It also prohibits the use of animals in research that cause severe, unwarranted, or prolonged pain. By enforcing the 3 R's and considering the rewards of the inquiry, the experimental design should reduce expenses. Each academic institution should have an established Animal Ethics Committee comprised of qualified individuals who can advise researchers interested in using animals in their study and can keep track of how well the three guidelines are being followed [1].
They have the right to reject proposed study protocols for improved animal welfare if they believe that continuing to use animals in experimentation is not justified [3]. Each study proposals should be critically evaluated and considering prospective costs and benefits thoroughly. The study's importance, objectives, and likelihood of yielding decisive results must all be made obvious and possible. The use of genetically modified (GM) mice presents extra ethical concerns. The insertion of a foreign gene into the genome of an organism results in phenotypic and physiological functions that are not found in the wild-type species and both the deontological theory, and the modified utilitarian theory disagree on their use. In the absence of a negative phenotype, however, there is no difference in animal wellbeing between wild-type and mutant animals since the animal is ignorant that its genome has been altered.

Replacement
The principle of replacement can be categorized into two distinct types: absolute replacement and relative replacement. According to Russell and Burch, animal replacement refers to the utilization of scientific methodologies that involve the substitution of non-sentient substances for conscious vertebrates in the context of animal experimentation. This observation suggests that clever higher creatures have been supplanted by unconscious matter. This statement suggests that if there is an alternative scientific approach available that can effectively achieve the desired outcome without involving animals, then studies on animals of that species should be avoided. When there is a need for animal cells, tissues, or organs, in vitro research is often undertaken using perfused organs, tissue slices, tissue cultures, and cellular and subcellular fractions thereby reducing pain/sacrifice of animals.

Reduction
In the past, a variety of animals were employed to fulfil the necessary quality control regulations for vaccines. The utilization of animals has experienced a significant decrease after the implementation of the 3Rs. Adhering to the principles of the 3Rs (Replacement, Reduction, and Refinement) in vaccination management has the potential to result in the complete elimination of animal utilization. The researchers bear the responsibility of ascertaining if a reduced number of animals may be employed in the proposed experiment, while ensuring that the scientific rigor of

the techniques and the significance of the results are upheld. This statement suggests that it is imperative for researchers to engage in literature reviews, evaluate various experimental designs, and undertake design calculations prior to commencing their investigations and work towards mitigating the effective number of animals for the study.

Refinement

The concept of refinement underscores the objective of reducing the frequency or intensity of harsh methodologies employed in animal experimentation. Any alteration made to a methodology that reduces the level of distress experienced by animals is considered ethically acceptable. This encompasses the topics of humane endpoints, non-invasive monitoring, and implanted monitoring technology. Deliberate attempts should be made to reduce or eliminate pain and misery to the treated animals using anaesthetics and analgesics, appropriate animal care, habitat enrichment, and humane euthanasia. There is mounting evidence that emotional distress can have profound effects on an animal's psychology, biology, and immunity and that these can act as a compounding factor and give inconsistent experimental outcomes, undermining the validity and reproducibility of research. Considering this all facets of animal experimentation, from animal housing and care to the actual scientific techniques, needs to be focused and improved upon. More importantly ensuring that the animals are given with housing that allows the expression of species-specific characteristics is vital and examples of refinement.

Responsibility

This includes reducing the risk of animal suffering and improving animal welfare, preserving biological diversity, including when intervening in a habitat, openness and sharing of data and materials, the need for animal knowledge, and the obligation to treat animals with respect. Similar to other nations, India continues to undertake animal experiments and has a thriving research industry. We are a long way from being morally oriented toward the humane treatment of animals, despite the existence of ancient religions such as Hinduism and Buddhism that advocate a tolerant attitude and messages to preserve animals and treat them with respect, compassion, and dignity. In contrast, article 51A(g) of the Indian Constitution emphasizes that it is everyone's fundamental duty to preserve the nation's natural resources, which includes compassion for all other living things. In 1960, the Indian Parliament enacted the prevention of cruelty to animals' act. According to Section 15 of this Act, the Central Government of India establishes the Committee for the Control and Supervision of Animal Experiments (CPCSEA) [4].

Animal Experimentation -The changing facade

Many researchers breach ethical principles, neglect them, or give them little weight when doing study. Some brief examples of actual animal studies demonstrating ethical issuesIn a 2002 experiment on awake lambs, third-degree burns and significant lung injury were also seen. The researchers aimed to demonstrate that there are alternatives to the current treatments for respiratory failure [5]. For this 5-day blinded trial, 14 lambs were divided into two groups: those getting volume-controlled mechanical breathing and those receiving par corporeal prosthetic lungs. After a tracheostomy, thoracotomy, and venous cut-down, the animals were sedated. They were then burned along the flanks with propane, resulting in cutaneous burns covering more than 40% of their body surface area. To force smoke into their lungs, a modified bee smoker was utilized, which burnt as it entered. After then, the experimental lambs were woken while still getting artificial ventilation. ARDS, or acute respiratory distress syndrome, was diagnosed in the animals. It was standard practice to kill animals that were thought to be in pain. The Institutional Animal Care and Use Committee (IACUC) approved the research, and the researchers informed everyone that the animals were treated humanely in accordance with the Guide for the Care and Use of Laboratory Animals. The creatures' pain and suffering were beyond comprehension. The magnitude of the suffering was unimaginable, regardless of any potential benefits to human health from the study [6].

Challenges to Animal Experimentation and Research at an Ethical Level

Humans are endowed with intelligence and judgment capacity and are constantly confronted with decisions that put his moral compass to the test. Beecher correctly predicted that the number of animals used in trials would increase over time and according to estimates, more than 500 million animals are used in studies in the United States each year [7]. Using animals in experiments involves difficult moral quandaries and maintaining Brambell's five freedoms is a serious challenge. In animal care, the ability to eat and drink without limitation comes first. To preserve their health and vitality, all animals require simple access to clean water and healthy food. The absence of pain is a close second. It is critical to have a comfortable and secure environment. The third benefit is that you won't have to worry about things like discomfort, injury, or preventable diseases. The fourth fundamental right is the freedom to behave in a socially sanctioned manner. It necessitates proper habitat, adequate facilities, and the company of other creatures of the same kind. The goal is to be free of all concerns and pain.

Conditions and therapies are offered to lower the likelihood of mental anguish. When the five freedoms of Brambell are considered objectively, it is evident that many at times laboratory animals' rights and conditions have been infringed and unethically treated. Even though water can be given freely as needed, it is common for examinations to need the animals to have fasted for several hours. Even though the trials were approved by the Institutional Animal Ethics Committee (IAEC), the treated animals suffered greatly. Consensus (or lack thereof) is an ongoing problem that must be addressed.

In practice, there is either no assessment or an inadequate assessment of the animal injury. The possibility of zoonotic infection transmission in both directions cannot be ruled out and guidelines must be followed appropriately. When there are no benefits for the animals, it is unequally distributed, and attempts should be towards reducing this. In most cases, we cannot extrapolate observations from animal disease studies to human health. This is because factors beyond the laboratory's control may have a substantial impact on the study outcomes. Also, there could be significant differences between diseases modelled in animals and those modelled in people and the variations in the physiology and genetics between the two species can affect the study outcome [8]. Solving these difficulties necessitates persistent faith and moral reflection. There must be evidence to support undertaking this type of animal study. There must be a compelling rationale why this study is necessary, how it will benefit people or other species, and how the findings will improve our understanding of the disease and the therapeutic benefit. The researcher should be aware with the relevant literature and realistic alternatives to animal experimentation before proposing a study. The animals employed in the study must be the finest possible representations of the research's goals along with concern for the animals in accordance with moral values.

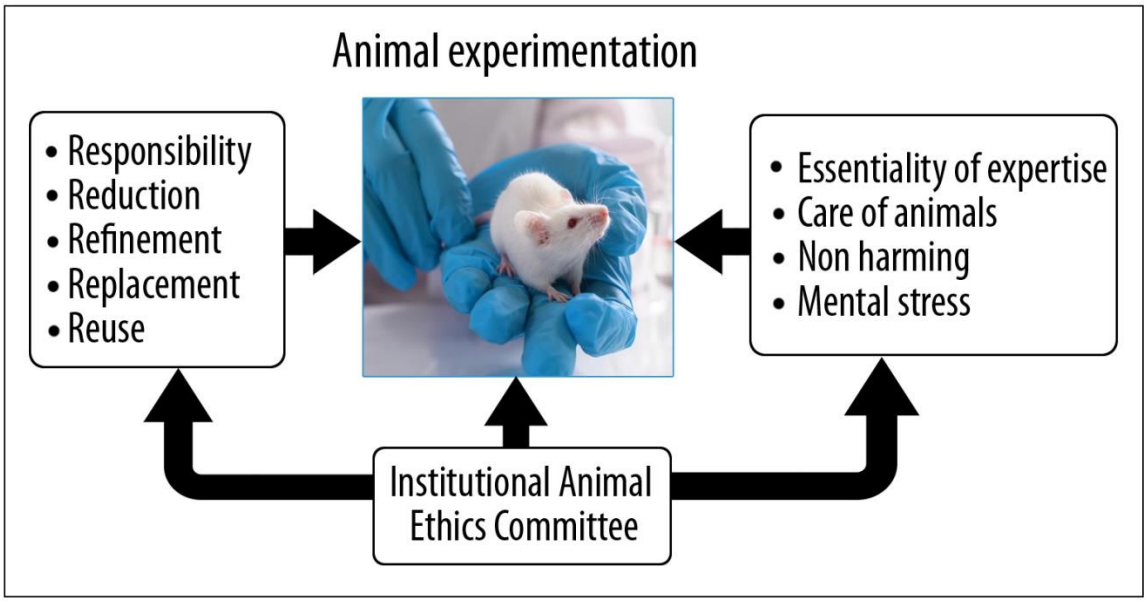
Before undertaking any study involving experimental animals, the Institutional Ethics Committee (IEC) and the Institutional Animal Ethics Committee (IAEC) must extensively review the procedure, need, and ethics of the study. When conducting the tests, it is critical to utilize humane techniques and adhere to the 3Rs principles. Providing an explanation for this would validate the use of animals in the study considering how the animal will feel and how much pain, suffering, and harm it will likely undergo. When an animal is used in a study, its behaviour must be closely monitored and subtle signs like the animal's cries, the presence or lack of protests, and resistance should be used to determine agreement or acquiescence. The investigator's primary objective should always be the welfare of the animals. There is a chance that animals will experience pain during therapies that humans would experience pain throughout. Everyone involved in the project should be conversant with animal welfare standards, have received proper training and any changes in behaviour that are unusual for their species could be signals of concern and analysed [9].

Conclusion

Animal researchers must raise their standards of conduct and assume responsibility for their actions if they are to prevent unfavourable outcomes. To establish and maintain ethical standards

in animal research, it is necessary to acknowledge the current unsatisfactory state of affairs and address the regulatory gaps that exist. By strictly adhering to norms and standards, it is possible to implement empathy and compassion for animals effectively. It is essential to enact laws that modify the existing rules, and it is also essential to punish those who violate these regulations. The imperative for the exploration of alternatives to animal experimentation must be emphasized. This strategy will uphold the right of animals to a decent standard of living and provide fair treatment.

Figure 1: Schematic representation of the ethical aspects, responsibilities and essentialities needed while using animals for research.



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