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ANIMALS USED IN EXPERIMENTAL PHARMACOLOGY AND 3 RS

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ABSTRACT

Drugs, vital weapons in healthcare, are only used in therapeutics after being tested in the lab. In both animals and humans, the roles of cells and organs are essentially the same. Many of the functions of animal cells are similar to those of human cells. Humans are classified as animals biologically. Animals are used to study how diseases impact living tissue, develop and test interventions, including animal medications, and educate future scientists and health-care practitioners. Animals have also played an important role in the advancement of research; several new drug discoveries have been made possible thanks to animal testing. Animal experimentation has its drawbacks, which include animal procurement, the complexity of conducting animal experiments, stringent rules, ethical considerations, and, more often than not, the interests of students. That is why, in today's world, other approaches and emerging technology, such as computer simulations, are increasingly replacing animal use. The article reveals animals used in experimental pharmacology. Alternatives for using animals in pharmacology education, on the other hand, have been suggested.

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Introduction

Animal experimentation [1, 2] is a term used to describe the use of animals in study, training, and education. Cutting into or dissecting a live animal is known as vivisection [3, 4], and it is a concept preferred by those who reject the use of animals in research. It has been reported that animals are exposed to stressful educational and training practices that are unnecessary. Concerns have been posed on how animals are killed in these "irrelevant tests" due to the extensive use of animals in toxicity research and the testing of dermatological preparations." CPCSEA aims to ensure that animals are not subjected to unnecessary suffering or pains during, before, or after the experiments on them. In India, a large number of animals are used in various experiments and studies [5]. Animals have been used and are still approved for drug testing, bioassay screening, and preclinical testing, including general and detailed studies of toxicity [6-8]. This preclinical safety and efficacy data must be submitted to the drug regulatory authorities before permission to conduct further human trials can be granted. [9-11].
In pure research, a greater variety and a larger number of animals are used than in applied research [12]. Examples include embryogenesis, developmental genetics, behavior, and breeding experiments in fruit flies, nematodes, mice, and rats. In the pharmaceutical industry or universities, applied research aimed at addressing specific questions is normally carried out. The University Grants Commission (UGC), CPCSEA, and Medical Council of India (MCI) all recommend three Rs [13] in animal experiments: replacement, reduction, and refinement, with a fourth R, rehabilitation, added as an added step for their treatment [14-18]. The creation of alternatives is a necessity in today's changing scenario. In several medical colleges across India, using live animal experiments [19] is reducing. These are increasingly being replaced by those alternatives that are available with demonstrated educational effectiveness and at reasonably low cost [20, 21].

Different Species are used in Experimental Study

Rat

Albino rats are one of the commonest laboratory animals because of their small size and greater sensitivity to most drugs. There is a wide head, rough fur, and long ears on the albino rat. The length of the tail is often less than the length of the body. It has a long cylindrical body, a long thin tail, and legs that are very short.

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The head has 2 silt-like nostrils with a pointed snout, a narrow mouth with a split upper lip and short lower jaw, 2 tiny beady eyes, set so that they can look forward and sideways diagonally, and they have several long whiskers. It has a short neck. The trunk is slightly wider than the head. Rats do not vomit because they lack a vomiting center. They do not have a gall bladder.

Experimental Use: Analgesic and anticonvulsant studies, bioassays of various hormones such as insulin, oxytocin, and vasopressin, chronic blood pressure studies, gastric acid secretion studies, acute and chronic toxicity studies The rats are ideal for determining the teratogenicity and carcinogenicity of drugs.

Tissues of Rat: Tissues of rats used for various drug actions.

Blood Collection: By snipping the tip of the tail, small blood samples can be obtained from a tail vein, large amounts of blood can be collected from anesthetized rats by heart puncture or by orbital sinus.

GUINEA PIG

For decades, the guinea pig has been utilized as a laboratory animal in human experimental subjects, and it is a docile animal. The guinea pig is a short, tail-free rodent, with small ears, the head is seen in the profile is rectangular and blunt. The thick short, neck of the trunk emerges. The length of the limbs is unequal, while the forelegs are shorter than the hind legs. In many respects, the guinea pig differs from other laboratory rodents. It needs Vitamin C in the diet and is very susceptible to anaphylactic shock and tuberculosis. They are highly sensitive to histamine. Guinea pig is usually utilized as a metaphor for a scientific experimentation subject. They have been used to separate various bacterial strains in the past, but they have been replaced by mice and rats in modem laboratories, which replicate faster.

Experimental Use: It is mainly used for the evaluation of the bronchodilator drugs against experimentally induced asthma (histamine or acetylcholine aerosols). It is also used in the field of immunology particularly in the area of delayed hypersensitivity by using various antigens such as egg albumin, horse serum, etc. They have widely used in the studies of the local anesthetics as well as the bioassay of digitalis and suitable for hearing experiments as they have sensitive cochlea and experiments on oxygen consumption. It resembles a man in that it also needs an exogenous source of vitamin C and so it is useful in the study of its metabolism. Being an appropriate host for Mycobacterium, it is also suitable for tuberculosis studies.

Various Isolated Tissues of Guinea Pig: Isolated organ preparations such as guinea pig lungs and intestines are widely used. These organ and tissue preparations were considerable in the discovery and early development of medicines to treat stomach ulcers and also beta-blockers to treat high blood pressure. The terminal ileum is most sensitive for the preliminary screening of the spasmodic and antispasmodic compounds and suitable for the detection and assay of histamine and related compounds.

Blood Collection: Small blood samples (less than 0.25 ml) can be collected by simple venesection of the marginal ear vein; large amounts of an anesthetized guinea pig by cardiac puncture or by metatarsal veins; repeated samples of small quantities can be collected from the orbital sinus.

MOUSE (Mus Musculus)

Albino mice are the smallest laboratory animals, which can be bred uniformly. They have a smooth hair coat and a slim body with a long-pointed snout, prominent round ears, and long flat front teeth. They are cheap and easy to handle.

Experimental Use: Mice are widely useful in acute toxicity studies. They are also used in the assay of insulin and analgesics and also for the general screening of the chemotherapeutic agents specially bred mice arc mainly useful in the study of problems in genetics and cancer. They are most frequently used for testing drugs due to teratogenicity. The Nude mice which lack the thymus gland are mainly useful in the study of tissue immunity and transplantation research.

Tissues of Mice: Tissues of mice are used in the vas deferens and the ileum is the only tissue used in mice for the experiments because it is small and delicate.

RABBIT

Rabbits are docile animals. The rabbit's body, except in a few areas (the tip of the nose, a small part of the scrotum, and the inguinal spaces), is tightly coated with smooth hair or fur. With readily visualized musculature, the rabbit has an erect and large pinna (external ears).

An undivided lower lip and a cleated upper lip are connected by the tiny external opening of the mouth. The external nostrils are ovoid and in the upper lip are attached to the cleft. Around the nose, over each eye, and one or two on each cheek, prominent whiskers are present. The rabbit has wide eyes that are more laterally focused (pink in albino rabbit) than most mammals. The hind legs are longer than the forelimbs, muscular and strong.

It has a huge caecum and a long appendix. In rabbits, the gene for atropinesterase is linked with the color of the fur. The enzyme atropine esterase is present in rabbit plasma and liver, so it can tolerate a large dose of belladonna.

Experimental Use: Rabbits are mainly used for pyrogen testing in intravenous fluids. The agents affecting capillary permeability are primarily examined by intracutaneous injection of the substances and accompanied by intravenous dye

injection, such as Evans blue. Studies of miotics and mydriatics, Insulin, and other antidiabetic drugs, curare, and sex hormones are tested in the rabbit. It is used in serological work and for the screening of embryo toxic agents. It is mainly used for the research on reproduction as ovulation is nonspontaneous and its semen is also collected easily. The convenience of injecting into and withdrawing blood from the ear marginal vein is helpful in bioavailability studies.

Used Tissues of Rabbit: Isolated heart, Jejunum, and ileum are some of the preparations routinely used for the testing of the drugs.

Blood Collection: The marginal ear vein is the chosen blood collection site; blood may also be withdrawn from the jugular vein, orbital sinus, for significant volumes (20 ml) of blood, an anesthetized rabbit cardiac puncture is preferred. The details of some common species such as mice, rats, guinea pigs, and rabbits used in the laboratory were shown in **Table 1.**

HAMSTER

Mesocricetus auratus is also known as the Syrian or Golden hamster. They belong to the family *Cricetidae*, to the subfamily *Cricetinae*, to the genus Mesocricetus, and the species *Mesocricetus auratus*. When they burrow, play, chew, and dig, the Syrian hamsters are mostly active at nighttime. Their nocturnal nature means that circadian rhythms are perfect for learning.

The Syrian hamster is intensely protective, and other hamsters introduced in their environment are known to fight, bite, and even kill. Aggressive territorial behavior starts to evolve at about 8-10 weeks of age in the Syrian or Golden hamster.

Experimental Use: Among other research fields, including oncology, immunology, and physiology, the hamster is widely used in IVF research. However, their peculiar reproductive system involves a continuous 4-day estrous cycle (all non-higher primate mammals' reproductive cycle) and a very short 16-day pregnancy period.

CAT

The cat (*Felis catus*) belongs to the Felidae (feline) family and is found on Earth in most regions except Australia and Antarctica. Usually growing to 28 inches (71 cm), they are the smallest member of the feline family. Cats are carnivores, mostly animal flesh is their diet, and they have a stomach capable of digesting raw meat. However, their diet, with the occasional hunting activities of the cat to support their diet, is typically dependent on their human owners. Though cats are not widely used in research, many diseases are similarly encountered by cats to humans. Cats also suffer from diseases such as leukemia, Alzheimer's disease, heart disease, infection, and immunodeficiency, and thus, they are excellent animal models for the mentioned diseases. Their relatively long lifespan of 20 years also makes them ideal models for illnesses and disorders that are age-related and slowly evolving.

The cat has a highly developed nictitating membrane, which is contracted by stimulation of the cervical sympathetic trunk and also by several drugs like adrenaline, histamine, etc. Contraction of the nictating membrane is recorded for the investigation of ganglionic blocking agents. In cats, morphine produces excitation of the CNS.

Experimental Use: Cats are employed in acute experiments for the study of drugs affecting blood pressure. Both anesthetized and spinal preparations are used, the latter being and particularly for the assay of catecholamines. Contractions of the nictitating membrane are recorded for the investigation of ganglionic blocking action of the drugs.

Cats are essential in the study of the nerve centers in the brain because they can produce methemoglobinemia, cats are most suitable for the toxicity of compounds like acetanilide. Cats are also the model of choice for neurological research, as are studies on movement, balance, hearing, and motor neuron studies related to spinal cord injuries. They have been used for mapping trials due to anatomical similarities in brain structure. They can be also used as models for viral disease syndromes.

FROG

Frog belongs to the class of amphibians. It has been used since 200 years ago. The amphibian animal is safe to handle. It cannot breed in the lab. In India, it is the biggest frog. Rana Tigrigna's size ranges from 5 to 17 cm. They come in a variety of colors, from yellow to olive green to grey, and have dark irregular markings. Their snout is long and pointed, and their hind limbs are long and muscular. They have an average-sized head. Their toes are almost fully webbed. The eardrum is very large. The nose is slightly closer to the end of the mouth than the eye; the inter-orbital area is smaller than the upper eyelid; and the tympanum is distinct, measuring around two-thirds the size of the eye. Males have two lateral vocal sacs, which are visible externally as skin folds on the sides of the throat. Males are darker in color and have breeding pads on the first finger, but females are larger.

They are most commonly used in physiology, pharmacology, and toxicology. The isolated preparation of frogs need not be maintained at 37℃ and the experiments are performed at room temperature. In frogs, adrenaline is the neurotransmitter for the sympathetic nervous system.

Experimental Use: Study of a drug on the CNS, the study of isolated tissue such as rectus abdominus muscle, heart preparation, drugs acting on CNS, and drugs acting on the neuromuscular junction, as well as to determine the retinal toxicity of the drug. For several years, the African frog *Xenopus laevis* has been used as a biological assay to determine human pregnancy.

Experimental Use: Human diseases, vertebrate embryology and growth, basic cell and molecular biology, genomics, neurobiology, and toxicology are all studied and modeled using *Xenopus Laevis*. Xenopus eggs and embryos have several characteristics that make them an excellent tool for biomedical research.

DOG

Dogs (*Canis familiaris*) belong to the Canidae family and are considered to be one of the first domesticated animals. For more than a century, they have been used in research.

Dogs are carnivores but can survive in the domestic situation on a well-designed, suitably processed omnivorous diet. Dogs are useful among large laboratory animals because they can be tamed trained without much difficulty. For many reasons, dogs have been very useful research models for such a long time. One of the reasons being that dogs are physiologically quite similar to humans, they also have roughly the same number of genes as humans, and their genome has been sequenced. This makes dogs particularly useful in genetic studies. Dogs are also known to suffer from diseases such as diabetes, epilepsy, autoimmune diseases, cancers, and eye diseases that are similar to human diseases.

Experimental Uses: Acute experiment for medication affecting blood pressure and intestinal movement, gastric acid secretion research, pharmacokinetic study, antidiabetic agent study, and pharmacokinetic study.

Used Tissues of Dog: Chronically prepared gastric fistula and pouches by earlier operations are also employed for the study of gastric secretion in the dog.

MONKEY

Monkeys and apes belong to the primates, the highest order of Mammals, which includes man. Most research primates are macaques or marmosets. Both structurally and functionally monkeys and apes closely resemble man. They are used in relatively limited numbers, but they've played a role in a variety of big medical breakthroughs, including the polio vaccine, premature baby life support systems, and deep brain stimulation for Parkinsonism.

Experimental Uses: Primates are used in the field of virology, parasitology, immunology, nutrition, reproduction, etc. Primate research is currently focused on infectious diseases, such as developing vaccines and therapies for HIV/AIDS. They are also used in modern drugs and vaccines for safety research. The details of some common species such as monkeys, hamsters, dogs, cats, and frogs used in the laboratory were shown in **Table 2.**

Table 1. Details of Some Common Animals Used in Laboratory

	Mice	**Rat**	**Guinea Pig**	**Rabbit**
Scientific name	*Mus musculus*	*Rattus norvegius*	*Cavia porcellus*	*Oryctolagus cuniculus*
Order	Rodentia	Rodentia	Rodentia	Lagomorpha
Body Temperature	37.4ºC	37.5-39ºC	37.6-38.9ºC	38.7-39.1ºC
Respiration Rate	90-230/minute	70-180/minute	40-110/minute	38-55/minute
Heart Rate	300-750/ minute	260-500/ minute	240-400/minute	135-300/minute
Blood pressure	120/75	130/90	75-52	130-90
Blood volume (ml/kg)	7-9/ 55-80	6-7/ 64 (50-70)	6-12/ 75 (67-92)	4-8/ 56 (44-70)
Food consumption	15gm/100gm/day	10gm/100gm/day	6gm/100gm/day	5gm/100gm/day
Water consumption (body weight /day)	15ml/100gm/day	10-12ml/100gm/day	10ml/100gm/day	5-10ml/100gm/day
Life span	1-3 years	2-3.5 years	4-5 years	4-5 upto 15 years
Prefer Humidity	60-70%	44-60%	45%	40-50%
Room Temperature	20-27 ºC	18.5-27 ºC	18.5-27 ºC	15.5-18.5 ºC
Mating Age	6-8 weeks	70-84 days	12-20 weeks	5-6 months
Esterous cycle	4-5 days	4 to 5 days	15-19 days	There is no regular estrous cycle. Receptivity periods last between 5 and 14 days.
Gestation Periods	19-21 days	21-23 days	59-72 days	31 days
Body Weight	25-40 gm	250-500gm	200-1000gm	2-6 kg

Table 2. Details of Some Common Animals Used in Laboratory

	Monkey	**Hamster**	**Dog**	**Frog**	**Cat**
Scientific name	*Macaca mullata*	*Mesocricetus auratus*	*Canis familiaris*	*Rana tigrana*	*Felis catus*
Order	primates	Rodentia	Carnivora	*Anura*	Carnivora
Body Temperature	37-39 ºC	36.2-37.5 ºC	37.7 ºC	26-8° C.	38.06°c.-39.17°*c*.
Respiration Rate	76-90	74/minute	14-28/minute	66-104/minute	24 - 42 / minute
Heart Rate	Upto 150/minute	280-412/ minute	77-138/minute	64 times per minute	140 - 220 / minute
Blood pressure (mmHg)	130/100	94 99/67	140/80	25-35 systolic to 18-28 diastolic	140-90

Blood volume	54 ml/kg	78 ml/kg	86 (79-90) ml/kg	15,93,600 in females & 10,29,700 per mm^3 in male	60 ml/kg
Food consumption	1-2.4 kg/day	12gm/day	3-4 meals/day	five crickets per meal. (0.2 to 0.8 grams/cricket)	40 gms /kg of body weight 10 or more meals/day
Water consumption	18.5 ml /kg/day	20 ml/day	20-70 ml/kg /day	———	45 ml/kg/day
Life span	30 years	2-3 years	10-13 years	10-12 years	2-16 years
Room Temperature	37-40 ºC	37 ºC	24-27 ºC	18-25 ºC	21.111°C
Mating Age	4-5 years	6-8 weeks	90 days	4 years	6 months-female 8 months-male
Esterous cycle	26-28 days	4 days	180 days	40 days	21 days
Gestation Periods	165 days	15-18 days	62 days	33 days	58-67 days
Body Weight	About 5000-6000 gm	110-140 gm	1.5-75kg	25-500 gms	4.1-5.4kg

Alternatives to Animal Experiment

In biomedical research and experimentation, alternatives or substitutes for live animals are defined as the replacement of live animals in any form, from complete to partial. [22, 23]. To replace animal experiments, several alternative methods have been accepted around the world.

The Zebrafish, [24, 25] a recent vertebrate model, is a very effective model for measuring toxicity. Zebrafish are fast-growing, small, and easy to keep in large numbers. Ninety percent of chemicals tested in Zebrafish triggered specific tissue, organ, and behavioral toxicity, according to reports [26]. Chemicals may be injected directly into fish water or in small quantities by microinjections. PS1 and PS2 orthologues found in Zebrafish assist in research on Alzheimer's disease [27] studies. It seems likely that intact animal models in pharmacology education will eventually replace these alternative methods and models, either partially or entirely.

In several nations, computer-based alternatives are being used. Two versions, i.e. Expharm and Xcology, are presently available in India as free modules and advanced paying models, [28, 29] For years, both have been well tested and used. All countries have used computer-based alternatives to some degree. They tested these software systems and discovered that the solutions are feasible and minimize the expense and time spent on animal experiments. The students enjoyed the alternatives and considered them more effective in learning the drug action process. Although updated versions of previous software programs are available, no comprehensive revolutionary software has been released to date. International regulatory authorities have developed, validated, and/or accepted approximately 50 alternative methods and testing strategies. The three Rs are the guiding principles for using animals in scientific research in a humane manner. Any researcher who plans to use animals in their study must first show why there is no other way and what steps will be taken to reduce the number of animals used and the suffering they cause, i.e.

- Replacement: Methods of replacement are available. Absolute alternatives, such as in silico computer modelling [30] and *in vitro* methodologies are possible, as are relative substitutes that exclude or substitute the use of 'protected' animals. Examples include known animal cell lines, animal cells, tissues, and organs acquired from human-sacrificed animals, slaughter materials, invertebrates such as Drosophila and nematode worms, larval amphibians and fish, bacteria, fungi, and other microorganisms.
- Reduction: The use of a few animals as possible to obtain statistically meaningful outcomes and to find ways to reduce the number of animals used by each species. Only a few examples include improved experimental design and statistical analysis, new imaging methods, avoiding test repetitions to prove tested hypotheses, and data and resource sharing.
- Refinement [31]: Refers to reduced invasiveness, enhanced instrumentation, improved pain management that minimizes real or possible pain, discomfort, and distress [32-34]. For example, non-invasive strategies involve the use of adequate pain relief anesthetic and analgesic regimes.
- The 4^{th} R i.e., Rehabilitation of the animals after their use, is also emphasized.

Conclusion

Animals are used by humans for several reasons, including science. It is important to consider the health of animals used in research. Removing animals entirely from the laboratory, on the other hand, would obstruct our knowledge of health and illness, as well as the creation of innovative and critical therapies. Both countries used computer-based alternatives to some degree. They examined these software systems and discovered that the alternatives are feasible to incorporate and reduce the expense and time spent on animal testing. The 'three Rs' are a set of guidelines that researchers must follow to help minimize the harm that animals may suffer while being studied in the lab. There are the following: The word "alternative" is used to

describe any change in an animal test that results in one or more of the "three Rs," which are eliminating animals, decreasing the number of animals, refining the mechanism for alleviating or minimizing potential animal suffering, and emphasizing the fourth R, which is animal rehabilitation after their use.

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