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CONTRIBUTION OF ANIMAL MODELS IN PHARMACOLOGY AND IN VARIOUS DISEASES: A BRIEF HISTORY

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ABSTRACT
Animals have been used in drug development since the era of Aristotle. All the drugs developed till now have been used in animals to test their safety and efficacy before applying to humans. Even for establishing the safety of surgical procedures, first, they have been applied to animals since 12th century by Moorish Spain. Due to opposition by animal lover activists and other organisations ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines were established in 2010. Animal research has a very important role in drug development for various diseases, even many scientists have been awarded Nobel prizes for drug development with the use of animals. Total elimination of animal testing will significantly set back the development of drugs and treatment, So we should use animals in Biomedical research by following rules and regulations, and if possible we should use computer-based models and mathematical models for real-time monitoring of drug effects.

KEYWORDS: Animal model, Drug discovery, Nobel prize, Biomedical research.

INTRODUCTION

Animals have been used repeatedly throughout the history of biomedical research. Early Greek physician-scientists, such as Aristotle (384-322 BC) and Erasistratus (304-258 BC), performed experiments on living animals, and Galen (129-199)/217 AD, a Greek physician who practiced in Rome and was a giant in the history of medicine conducted animal experiments to advance the understanding of anatomy, physiology, pathology and pharmacology.[1] Ibnzuhr (Avenzoar) an Arab physician in the 12th century Moorish Spain, introduced animal testing as an experimental method for testing surgical procedures before applying them to human patients.[2]

In recent years, the practice of using animals for biomedical research has come under immense criticism by animal supporters and animal rights groups. Laws have been passed in several countries to make the practice more 'humane'.

Issues such as 'cruelty' to animals and the inhumane treatment of animals are a valid concern and hence the use of animals in experimentation is greatly regulated. This has led to the 3'R's campaign:

(1) Replacement of animals with non-living models.
(2) Reduction in the use of animals.
(3) Refinement of animal use practices.

However, the total elimination of animal testing will significantly set back the development of essential medical devices, medicines & treatments. Moreover, the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines, established in 2010 and recently, in 2020, provides practical and detailed instructions on the design of a research project. These guidelines are applied to all areas of research involving living animals from fruit-fly to mammalian species.[3] Animal testing and research have advanced the human health by playing important role in the development of drug, trial of drug, and understanding and modifications of etiology of diseases.

CONTRIBUTION OF ANIMAL MODELS TO THE FOLLOWING DISEASES[4]

(1) Aging – Experiments on mice showed that Metformin improves the life and health span of the mice, leading researchers to further investigate the drug's potential protective effect against diseases that become more common with age, like cancer, heart disease, and Alzheimer's disease.

Our body's natural ability to repair our DNA declines with age, but experiments on mice showed that mice who received an NAD+ booster improved cells repairability significantly and treated old mice cells were indistinguishable from those of young mice. Human

trials started in late 2017, and if they succeed the drug could be available in coming years.

(2) Alzheimer's disease – Monkeys have many similarities with human being so, by studying brain function in healthy monkey brains, researchers can pinpoint where human brains suffering from Alzheimer's, dementia, and other neurological disorders are failing. Mouse models are also being used to pioneer immunotherapy treatments for Alzheimer's that are currently in clinical trials.

(3) Artificial blood - Rats and mice have been used to understand the components of human blood for decades. Stem cell-based blood substitutes can be used to create artificial blood. Our knowledge of stem cells improves and continues to progress due to animal research.

(4) Cancer - Many of the cancer-fighting drugs today would not be possible without the use of mice. Chemotherapy was first developed by using modified mustard gas to reduce tumors in mice. Herceptin, a drug for improving long-term survival in breast cancer patients, was developed with mice. Continuing to play a role in improving current therapies, mice will remain a vital part of newly developing therapies. Hemangiosarcoma (HSA) in dogs, and angiosarcoma in humans, both are extremely aggressive with very poor prognosis. Researchers have developed a drug, eBAT, to target the tumor with minimal damage to the immune system. eBAT with traditional chemotherapy extended the life spans of the dogs tested and researchers are expecting it to be beneficial in humans also.

(5) Cholesterol - The science of cholesterol-lowering drugs is based on animal research; Commonly used Cholesterol lowering agents (Rosuvastatin, Ezetimibe) were tested in mice, dogs, and rats, and Ezetimibe was also developed using rabbit and monkey models. Mice, rat and rabbit models led to the development of statins, a staple in lowering cholesterol and preserving cardiovascular health. James Black developed the first beta-blockers after studying guinea pig heart muscles and monitoring the cardiovascular functions of anesthetized cats. Antihypertensive drugs began with research on mice, eventually proving safe and effective for mice, dogs, and cats and they are safely used in humans to reduce blood pressure.

(6) Cystic- fibrosis (CF) - Recently, researchers in Japan have genetically engineered mice that can now closely reproduce the symptoms found in humans, as opposed to the previous mice models which lacked the infections of humans. This helps researchers understand and develop treatments for CF, and other pulmonary diseases. Ferret animal models are also helping in improving CF treatments, as Ferrets experience similar resulting ailments such as liver and pancreatic disease. Due to similarities in lung cell biology, the Ferret model has been vital to our continued progress in combatting lung infections.

(7) Diabetes – Discovery of Insulin would not have been possible without research with dogs. Beginning in 1893, dogs were crucial to identify the role of the pancreas and the eventual isolation of insulin and successful injection in humans in 1922. First, by monitoring blood sugar levels in rabbits, James Collins successfully used insulin in dogs and then in humans.

Recently researchers have been developing an insulin shot that would last an entire month. The shots have proved effective in controlling the glucose levels in rhesus monkeys for over 14 days. Monkeys and mice have faster metabolism than humans making the shot likely to work as effective glucose control for a month or longer in humans.

(8) Ebola – Ebola has evolved from primate viruses, so animal research is essential for the study of disease. Rhesus and cynomolgus macaques and marmosets exhibit a disease course like that of humans and are necessary to understand the full progression of the disease. Vaccines and antivirals that are the result of this non-human primate research have promising results, and the eventual eradication of the disease is possible with continued animal research.

(9) Epilepsy - Mice are helping to pioneer new therapies and even possible cures for Epilepsy. The identification of several gene mutations in mice has allowed a better understanding of the cause of epilepsy. The mice used in tests for cell therapy are especially important as they closely resemble drug-resistant human epilepsy

(10) Eyesight - Research using cats gave an important initiation in understanding of the visual system. Scientist David Hubel studied the visual system of cats and find that all mammals have partially developed visual systems at birth. Studies on the nervous system of kittens, helped Hubel to discover that stimulation of the visual neurons by light is necessary for the proper development of the eyes, optic nerve and visual centres of the brain. Developments like the implantation of retinal stem cells in mice are showing positive results to restore vision. Worldwide, 80% of all visual impairment can be prevented or cured, which would not have been possible without animal research.

(11) Flu - Researchers are working on developing a universal flu vaccine with the help of animal models. Historically, ferrets, mice, and guinea pigs have been important animal models in influenza research. In 2015 universal vaccine was proven successful in protecting mice from eight different flu strains. The vaccine proved effective for six months in mice. The research team wants to move to ferrets, to prove their safety and efficacy before moving to humans. The development of a

universal vaccine would be a huge step in reducing the impact of influenza.

(12) Hepatitis B - Chimpanzees were instrumental in the development of diagnostic tests for hepatitis A and B because the infection patterns in chimpanzees closely resembled those in humans. The HBV vaccine was developed in 1976, and became commercially available in 1982, after proving safe and effective on chimpanzees.

(13) Hepatitis C - Chimpanzees enable the discovery of hepatitis C and have been a valuable tool in the preclinical analysis of developing antivirals. Sovaldi (sofosbuvir) tablets were approved in 2013 for the treatment of chronic hepatitis, and, in most patients, cure the disease in a few months. Before approval Sovaldi proved safe and effective in preclinical rodent and non-human primate models. With continued animal research a vaccine is insight for Hepatitis C and will be a major step in greatly reducing the rate of infection.

(14) HIV - HIV research has been grounded in animal research from the start. The first antiretroviral was developed in 1986 through research with monkeys and mice. Staggering progress in treatment and prevention has already been made with animal research, but continued research with animals is necessary for an effective and safe HIV vaccine.

(15) Joint replacement - Researchers are experimenting with a cementless fixation, a biological implant that allows the bone to grow onto the implant. This is being researched with dogs and rabbits to pioneer implants that would be reabsorbed by the host tissue. With tissue engineering, researchers have successfully created a moving joint in rabbits.

(16) Kidney disease - The invention of dialysis would not have been possible without dogs. Dogs and rabbits were the first animals, dialysis was tested with, then testing moved to monkeys before it was used on humans to save the lives of the millions who have suffered from kidney failure. Currently, other treatments are being pioneered with the help of mice.

(17) Leukaemia - Research using mice has made crucial steps in understanding and tackling leukemia. In the 1970s mice were used to determine that all malignant cells must be destroyed and that the earlier treatment begins the higher likelihood of eliminating cancer. In the 1960's aggressive chemotherapy regimen increased the remission rate from 25% to 60% by research on mice.

(18) Malaria - Chloroquine was developed with rodent models and is continually tested alongside new drugs in rodent models. Rodent models have become especially prominent in severe malaria to ensure treatments, by tracking the progression of the parasite in rodents. Researchers are working to develop a malaria vaccine with the help of rodents and non-human primates.

(19) Mental health - Abilify, is the fourteenth most prescribed drug in America. It was tested on rats, dogs, monkeys, and rabbits to ensure its safety and efficiency in managing the symptoms of schizophrenia and bipolar disorder. Neuroscientists in California are researching the ways the lithium can be incorporated into bipolar treatments by injecting lithium into mutant mice.

(20) Organ transplant - Joseph E. Murray, recipient of the Noble Prize for his work on organ transplants, and Thomas E. Starzl, known as "the father of modern transplant", both acknowledge the importance of dogs in the study of immunosuppressive therapies for transplanted organs to be successfully accepted. As part of dialysis research, dogs were the obvious choice to better understand kidney transplants. Dr. Christiaan Barnard performed the first successful heart transplant in 1967, after years of research in which Barnard and his colleagues performed nearly 50 dog heart transplants.

(21) Paralysis - Rats are integral to the study of spinal cord injuries, offering promising new therapies, and hope for a cure for paralysis. Researchers at Johns Hopkins University, through the injection of embryonic stem cells, allowed paralyzed rats to move their limbs. The facial nerve of cats and humans are extremely similar so, cats have helped to study acute facial paralysis or Bell's palsy.

(22) Parkinson's disease - Monkeys, mice and rabbits are the most popular animal models for the study of the disease. Nobel Prize-winner Avid Carlsson discovered the role of dopamine in the brain's ability to control movement through tests with rabbits whose movement was restored with injections of the drug L-dopa.Deep Brain Stimulation improved the movements of monkeys with Parkinson's before it was trialled on humans and approved by the FDA in 2002.

(23) Polio - In 1908 scientists Karl Landsteiner and Edward Popper proved the specific cause of polio through the infection of monkeys. After that polio vaccine could be developed with the help of monkeys, mice, and cows.In 1960, Polish-American scientist Albert Sabin created an oral polio vaccine with the help of "approximately 9,000 monkeys, 150 chimpanzees, and 133 human volunteers.

(24) Smallpox - Research and development in the 19th and 20th centuries involved cowpox material being extracted from calves and then purified to produce the vaccine. This vaccine and its mass distribution would not have been possible without the use of calves to harvest cowpox material. Without animal research, smallpox would still be a disease affecting masses of people.

(25) Stem cells - Researchers have been using animals to understand stem cells since 1961, beginning with the identification of nerve cell replication in rats. In 1981 embryonic stem cells were derived from mouse embryos,

which then led to the ability to derive stem cells from human embryos in 1988. Scientists inject human embryonic stem cells into mice with suppressed immune systems to study the differentiation process as well as to identify whether the cells are pluripotent, meaning that a single cell can become specialized in any tissue of an organism.

(26) Surgery- Functions of the heart were first pioneered with calf hearts. Echocardiography is still being improved with the use of rodents, dogs, and pigs to improve the detection of heart problems. The first dog to undergo open-heart surgery was a Doberman- German shepherd in 2015 for rare congenital heart disease. Lifesaving procedures such as open-heart surgery, coronary artery bypass surgery, etc are now common with lab animals.

ANIMAL MODELS USED IN TESTING OF SAFETY PROFILE AND TRIAL OF DRUGS

Animals are behind, in almost every prescription of medicine available today. Animal testing is necessary for understanding the safety and proper dosages of new medicines and treatments. So, animal research and testing are important first steps. Contribution of lab animals to most common drugs that are part of most of the prescriptions.

Table1; Animal models used in drug trials.[4]

Sr.no	DRUG	DRUG USAGE	SPECIES
1	Synthroid (Levothyroxine sodium)	Enlarged thyroid, hypothyroidism	Rats, dogs, mice
2	Crestor (Rosuvastatin)	High cholesterol	Rats, beagle dogs, mice, cats, cynomolgus monkeys, rabbits
3	Nexium (Esomeprazole)	Heartburn, acid reflux	Rats, beagle dogs, rabbits, mice
4	Ventolin HFA (Albuterol sulfate inhalation aerosol)	Bronchospasm	Rats, beagle dogs, New Zealand white rabbits
5	Advair disks (Fluticasone propionate / Salmeterol)	Asthma, COPD flare-ups	Rats, dogs, mice, rabbits, guinea pigs, monkeys, hamsters
6	Diovan (Valsartan)	High blood pressure	Rats, mice, rabbits, marmosets
7	Lantus Solostar (Insulin glargine)	Diabetes mellitus	Rats, beagle dogs, rabbits, guinea pigs
8	Cymbalta (Duloxetine)	Fibromyalgia, major depression, general anxiety disorder	Mice, rabbits, rats, dogs
9	Vyvanse (Lisdexamfetamine)	ADHD, Severe binge eating	Rats, guinea pigs, beagle dogs
10	Lyrica (Pregabalin)	Fibromyalgia, epilepsy	Mice, rats, monkeys, rabbits
11	Spiriva handihaler (Tiotropium bromide)	COPD (inducing bronchitis & emphysema)	Mice, rats, rabbits, dogs
12	Lantus (Insulin glargine)	Type 2 Diabetes	Mice, rats, dogs, guinea pigs
13	Celebrex (Celecoxib)	Arthritis, ankylosing spondylitis	Rats, mice, dogs, guinea pigs
14	Abilify (Aripiprazole)	Schizophrenia, bipolar disorder	Rats, dogs, monkeys, rabbits
15	Januvia (Sitagliptin)	Type 2 Diabetes	Rats, rabbits, dogs, monkeys
16	Namenda (Memantine)	Moderate to severe dementia	Rats, mice, beagle dogs
17	Viagra (Sildenafil)	Erectile dysfunction	Rats, Dogs, Mice, Rabbits
18	Cialis (Tadalafil)	Erectile dysfunction	Mice, Wistan rats, Beagle dogs
19	Zetia (Ezetimibe)	High Cholesterol	Rats, Dogs, Mice,
20	Nasonex (Mometasone furoate)	Seasonal/year- round allergy	Rats, Dogs, Rabbits
21	Suboxone (Buprenorphine)	Narcotic Addiction	Rats, Dogs, Mice, Baboons, Rabbits
22	Symbicort (Budesonide and Formeterol fumarate)	Asthma	Rats, Dogs, Mice,
23	Bystolic (Nebivolol)	Hypertension	Rats, Dogs, Mice,
24	Flovant hfa (Fluticasone propionate)	Prevent Asthma Affects	Rats, Dogs, Rabbits
25	Oxycontin (Oxycodone hydrochloride)	Long lasting, Moderate to severe pain	Rats, Rabbits

Adapted from foundation for Biomedical research[5]

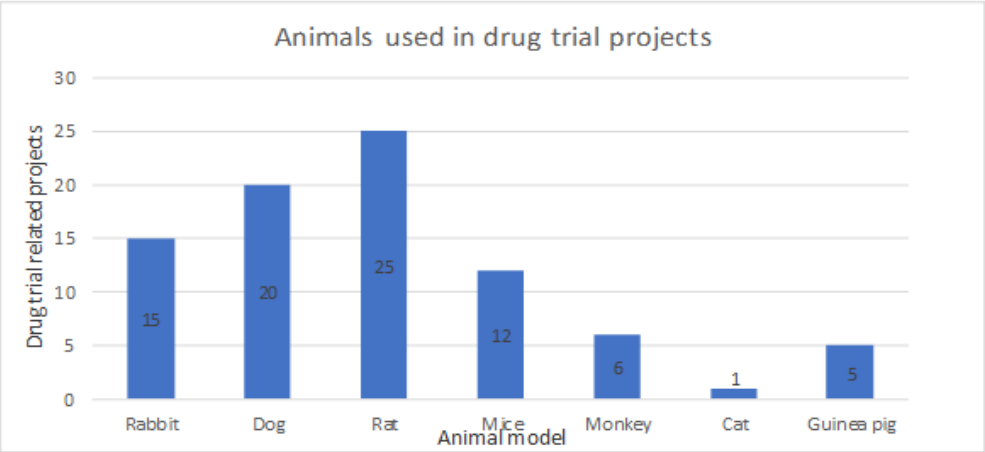

Figure 1: shows the number of animal models used in drug trial projects.

CONTRIBUTION OF ANIMAL MODELS IN NOBEL PRIZE AWARDED PROJECTS RELATED TO PHARMACOLOGY

Lab animals have made important contributions to nearly every Nobel prize in medicine happens practically every year. In fact, out of 224 award recipients in the Physiology or Medicine category, 188 used animal models in their research. The first prize in 1901 was won by German scientists for developing the diphtheria vaccine through research with horses. Afterward, each year Nobel prizes were awarded for major discoveries, most of the time for research involving animals. In 2021 winners David Julius and Adam Patapoutian won for their work with mice for discoveries of receptors for temperature and touch. Out of 188 Nobel prizes that used animal models, 22 Nobel prizes were awarded to projects related to Pharmacology.

Table 2: Nobel Prize awarded Projects related to Pharmacology from 1901-2021[4,5]

S.No	Year	Animals Used	Subject	Researcher	Ref
1.	1901	Guinea pig, horse, and rabbit	Development of diphtheria antiserum	Emil Adolf von Behring	[6]
2.	1906	Dog and horse	Characterization of the central nervous system	Camillo Golgi and Santiago Ramón y Cajal	[7]
3.	1913	Dog and rabbit	Mechanisms of anaphylaxis	Charles Robert Richet	[8]
4.	1923	Dog, rabbit, and fish	Insulin and mechanism of diabetes	Frederick Grant Banting And John James Richard Macleod	[9]
5.	1929	Chicken	Antineuritic and growth-stimulating vitamins	Christiaan Eijkman;	[10]
6.	1929	Chicken	Antineuritic and growth-stimulating vitamins	Sir Frederick Gowland Hopkins	[11]
7.	1934	Dog	Liver therapy for anemia	George Hoyt Whipple, George Richards Minot, and William Parry Murphy	[12]
8.	1939	Mouse and rabbit	Antibacterial effects of prontosil	Gerhard Domagk	[13]
9.	1943	Rat, dog, chick, and mouse	Function of vitamin K	Edward Adelbert Doisy	[14]
10.	1945	Mouse	Penicillin and its curative effect in various infectious diseases	Sir Alexander Fleming, Ernst Boris Chain and Sir Howard Walter Florey	[15]
11.	1951	Monkey and mouse	Yellow fever vaccine	Max Theiler	[16]
12.	1952	Guinea pig	Streptomycin, the 1st effective antibiotic against tuberculosis	Selman Abraham Waksman	[17]
13.	1960	Rabbit	Acquired immunological tolerance	Peter Brian Medawar	[18]
14.	1961	Guinea pig	Physical mechanism of stimulation within the cochlea	Georg von Békésy	[19]

15.	1966	Rat, rabbit, and hen	Hormonal treatment of prostatic cancer	Charles Brenton Huggins	[20]
16.	1982	Rat, rabbit, and guinea pig	Prostaglandins	Sune K. Bergström, Bengt I. Samuelsson, and John R. Vane	[21]
17.	1988	Guinea pig, cat, dog, and rat	Important principles for drug treatment	Sir James W. Black	[22]
18.	1988	Mouse, dog, rabbit, and monkey	Important principles for drug treatment	Gertrude B. Elion	[23]
19.	1998	Rabbit	Regulation of blood pressure with nitric oxide	Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad	[24]
20.	2015	Mouse, dog, sheep, cattle, chicken, and monkey	Therapy against infections caused by roundworm parasites	William C. Campbell and Satoshi Ōmura	[25]
21.	2015	Mouse, dogs, sheep, cattle, chicken, and monkey	Therapy against Malaria	Youyou Tu	[26]
22.	2018	Mouse	Cancer therapy via inhibition of negative immune regulation	Dr. Tasuku Honjo and James P. Allison	[27]

Adapted from foundation for Biomedical research, Nobel prize on project related to Pharmacology[4,5]

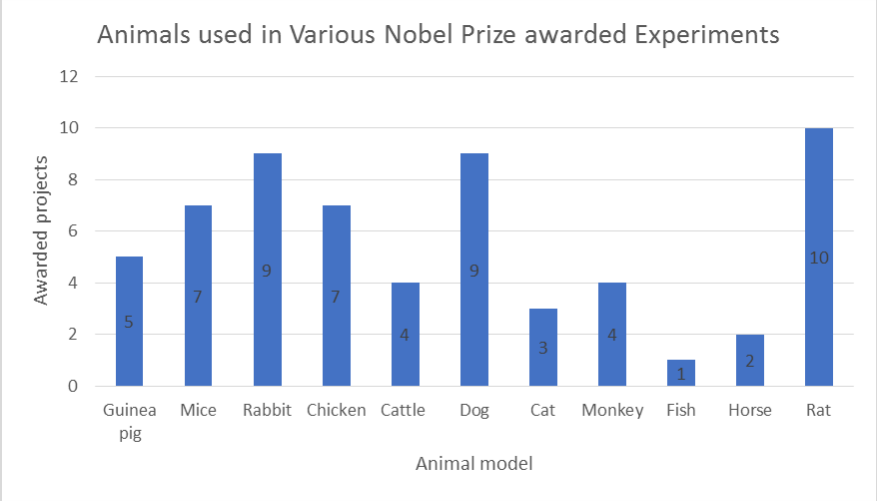

Figure 2: Shows number of animal models used in Nobel prize awarded experiments in Pharmacology

CONCLUSION

The development of any medicine/ vaccine will not be possible without use of animals. Without animal research, many diseases would still be health problems affecting population.

We concluded that animal models are essential for the advances in Medicine, Biology and in the field of Pharmacology like immunotherapy, new drug development, drug trial, toxicology and vaccine. However, before using animals we must ensure and follow new rules and regulations of animal use, so animal models become so 'humane'.

In future, we should concentrate more on the use of computer-based models, and mathematical models for real-time monitoring of drug effects, so less quantity of animals are used.

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