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Beyond the petri dish: Model organisms in non-clinical exploration

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Abstract

Animal models have been integral to scientific research for decades, offering invaluable insights into complex biological processes, advancing medical knowledge, and aiding the development of new treatments. This review explores the significance of animal models in various research fields, emphasizing their biological similarity to humans as a key factor. Animals, ranging from mice and rats to primates, fruit flies, and worms, share fundamental physiological, anatomical, and genetic characteristics with humans, making them crucial proxies for studying diseases and biological pathways. This review underscores the essential role of animal models in preclinical research, particularly in evaluating drug candidates for safety, efficacy, and potential side effects before human trials. The controlled experimental environment provided by animal models allows researchers to manipulate variables, isolate factors, and establish cause-and-effect relationships, enhancing the understanding of complex genetic disorders.

Keywords: Animal model; Anatomical; Genetic; Physiological and pre-clinical studies

1. Introduction

Animal models have played a crucial role in scientific research for decades, serving as valuable tools for understanding complex biological processes, developing new treatments, and advancing medical knowledge.[1] These models, which encompass a wide range of organisms such as mice, rats, Primates and even simpler organisms like fruit flies and worms, provide researchers with invaluable insights into human biology and disease.[2]. While it is essential to recognize the ethical considerations and strive for alternatives, animal .models continue to be indispensable in numerous fields of research, including medicine, pharmacology, toxicology, genetics, and neuroscience.[3] One of the key reasons for the significance of animal models in research is their biological similarity to humans.[4] Animals share many fundamental physiological, anatomical, and genetic characteristics with humans, making them suitable proxies to study human diseases and biological processes. By utilizing animal models, scientists can investigate the underlying mechanisms of diseases, test potential therapies, and unravel intricate pathways that would be otherwise challenging to explore in humans directly.[2]. This knowledge forms the foundation for medical advancements and the development of novel treatments.

Animal models are particularly crucial in preclinical research, where they help identify promising drug candidates before they are tested on human subjects.[2]. These models allow researchers to evaluate the safety, efficacy, and potential side effects of new drugs, ultimately guiding decisions about their viability for human clinical trials. Moreover, animal models contribute to understanding drug metabolism, pharmacokinetics, and dosage optimization, ensuring better treatment outcomes, and minimizing risks for patients.

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Another vital aspect of animal models is their suitability for studying complex genetic disorders and elucidating the genetic basis of diseases. By selectively breeding animals with specific genetic modifications or mutations, researchers can mimic human genetic conditions and investigate the resulting phenotypes. These studies enable scientists to unravel the intricate interplay between genes, environment, and disease progression, providing valuable insights for the development of targeted therapies and personalized medicine.[3]

Furthermore, animal models offer researchers a controlled experimental environment, allowing them to manipulate variables and isolate specific factors for investigation. This level of control is often not feasible or ethical in human studies, where numerous confounding variables may be present. Animal models provide a way to understand cause-and-effect relationships, evaluate hypotheses, and acquire detailed data that can inform subsequent human studies.

Despite their essential role, it is important to note that ethical considerations are crucial when using animal models in research. Ethical guidelines and regulations are in place to ensure the humane treatment of animals and minimize unnecessary suffering.[3] Furthermore, scientists are continuously exploring alternative methods such as in vitro models, computer simulations, and tissue engineering to reduce reliance on animal models whenever possible. The goal is to strike a balance between scientific progress and the ethical responsibility towards animal welfare.

In this review we have elaborately discussed the use of some conventional animal models in various kind of preclinical research and advantages of one model over the another.

2. Rabbit

For many years, rabbits (*Oryctolagus cuniculus*) have been employed in scientific investigations because they have numerous physiological and anatomical similarities with humans, such as circulatory system, respiratory system, digestive tract which renders them a perfect model for researching many diseases and ailments.

Notably, the initial studies in rabbit model were to study the usefulness of lung reduction surgery as a therapy for emphysema and chronic obstructive pulmonary disorder (COPD) that resulted in successful human trials, and this approach is now routinely used clinically. Different delivery of drugs mechanisms into the airways play a significant role in the effective treatment of asthma and other airway diseases. According to one study, utilizing the rabbit model to examine drug delivery strategies can be a valuable tool in evaluating optimum administration of inhaled medications in new-borns.[4]

Due to patient's aesthetic mindset, surgeon hesitate to employ novel materials for surgery since smidgen of complication could cause catastrophic deformity. Rhinoplasty is one of the surgical procedures for which there is no good animal experimental model.[5] Using rabbit nose research, several experimental techniques such as nasal packing, maxillary sinus augmentation, acute and chronic sinusitis, and alterations in mucosal bacterial ecology owing to nasal intubation have been characterized. [6] Experiment models have also been used to study and report on septoplasty, submucous resection, saturation, septal cartilage regeneration, and facial structure development. [7,8]

Human lipoprotein metabolism and that of rabbits are very similar. The metabolism of lipoproteins differs in mice and humans. ApoB editing enzyme is found in the gut and the liver in mice and rats, but exclusively in the intestine in humans and rabbits. While apoB100 is a prominent apolipoprotein of VLDL, IDL, and LDL, which are endogenous lipoproteins generated from liver, apoB-48 is a crucial apolipoprotein of chylomicron and chylomicron remnants in humans and rabbits, which carry exogenous lipids received from meals.[9] In Watanabe Heritable Hyperlipidemic rabbit (WHHL), competitive inhibitors of rate limiting cholesterol production have been shown to be beneficial in treating hypocholesterolemia and heart attacks, but not in mice or rats. Studies have shown that statins are beneficial for treating hypocholesterolemia in people. These results emphasize how crucial it is to choose suitable animals for translational research. [10,11]

Furthermore, the rabbit hypercholesterolemia model, which was initially established for the study of atherosclerosis, has several benefits that make it one of the top models for AD research as well. Rabbits that were given high-cholesterol diets exhibit many of the neuro pathologies which are seen in Alzheimer's disease patients. Such rabbits' brains have higher levels of cholesterol and Amyloid [A] and lower levels of acetylcholine, as well as increased A, tau, and ApoE immunoreactivity. For studying human myocardial ischemia WHHL rabbit and the myocardial infarction-prone (WHHLMI) rabbit are used frequently. [12–14]. The heart of a rabbit and that of human are electro physiologically similar; it may be beneficial to monitor the ECG for cardiac function. The human ECG waveforms were discovered to resemble rabbits more than mice or other rodents.[15]

In comparison with people, rabbits are born at an earlier stage of development. There have been some very interesting findings from studies carried out on the development of the bladder in rabbits. The contractile response to adrenergic agonists as well as the density of adrenergic receptors is almost non-existent at birth, but grows rapidly throughout the first weeks of development. The response to muscarinic stimulation, as well as the existence of muscarinic receptors, are present from birth. Since the typical laboratory rabbit is consistently susceptible to ovulation triggered by sexual intercourse (coitus-induced ovulation), it is an excellent illustration for studying the effect of sex hormone modification. Castration and estrogen delivery have been demonstrated to have significant impacts on adrenergic and cholinergic receptor density, as well as autonomic stimulation. In a recent investigation on the effect of pregnancy on rabbit bladder function, along with muscarinic receptor density, the sensitivity to bethanechol were shown to be considerably lower in pregnant rabbit bladders than in age-matched virgin controls [16,17]. Rabbits are easily infected with *T. pallidum*, a causative agent of syphilis which may be connected to their natural vulnerability to a very closely related bacteria, *Treponema paraluiscuniculi,* which is transmitted sexually. The clinical, histological, and immunological parallels between rabbit and human syphilis infection are significant. [18]

Rabbit immune sera can be utilized in a variety of assays, including ELISA, western blots, and testing for functional antibody responses such as neutralizing antibodies.[20] The rabbit model was originally utilized to evaluate polyclonal antibody responses, and the immunogenicity of DNA vaccination as a novel immunization approach was demonstrated

The rabbit model has further aided in elucidating the pharmacologic features of traditional and novel anti-tubercular drugs, displaying similar drug distribution and pharmacokinetic/pharmacodynamic qualities as in human lung resection surgical investigations. Vaccine development, antiviral therapy, papillomavirus biology, and latent viral infections have all been extensively researched utilizing two rabbit papillomavirus models. [19] Also, rabbit immune systems can prevent infection and displaying a latent, condition comparable to humans, and in rare circumstances can manage TB infection so efficiently that the bacilli seem to be entirely eradicated. While human reactivation from a dormant state occurs spontaneously, rabbit reactivation involves immunosuppression. Thus, these characteristics make rabbit a valuable model for studying human latent TB. [21]

Rabbits are also good for ophthalmic research because they are easy to handle also are cost-effective compared to larger breed models. With major benefit of hundreds of years of gathered data on the anatomy and physiology of the rabbit eye, as well as its similarities to the human eye they are the most ideal model for studies related to the eye. Young rabbits are employed as they exhibit stronger post operative inflammatory response, like that of children making them the most suitable model.

3. Rodents and Pig

Rodents, belong to the order Rodentia, primarily mice (*Mus musculus*) and rats (*Rattus norvegicus*), but also guinea pigs (*Cavia porcellus*) and others, are often employed in animal research study. Some common examples of mice & rats used are, Sprague-Dawley rats, Nude Mouse (Athymic Mouse). Mice and rats have long been the preferred species due to their physical, physiological, and genetic similarities to humans. Rats, mice, and humans exhibit a 95% similarity in their genes. Consequently, rodents have several advantages, such as their portability, low care requirements, relatively short life spans, and rich genetic diversity.

Mice and pigs (*Sus domesticus*) have both played critical roles in biomedical research in modelling human illnesses and explaining their underlying processes. Pig models have been established for diseases including as cystic fibrosis muscular dystrophy, and cardiovascular abnormalities, offering information on disease development and potential therapies. [22,23] Meanwhile, mice have played a crucial role in the study of cancer, Alzheimer's disease, and the modelling of autoimmune disorders. CRISPR/Cas9 gene editing technology has brought a paradigm change to the use of mice and pigs in scientific research. Scientists may now precisely alter genes in these animals to examine gene function and analyse the impact of certain genetic mutations on disease development using this innovative technique.[24]

Due to a shortage of human organ donors, xenotransplantation using pig organs has been researched.[25] To lessen the likelihood of immunological rejection and cross-species infections, significant progress has been achieved in genetically engineering pigs. Mice and pigs continue to play an essential role in preclinical drug research by allowing the assessment of prospective medications' safety and efficacy prior to human trials. Treatments for cancer, viral diseases, and autoimmune disorders have all benefited greatly from the insights provided by these animal models. Mice have played an important role in the study of learning, memory, neurodegenerative disease, and mental health issues, all of which are vital to the field of neurobiology. Understanding brain networks and functions in both mice and pigs has been

boosted using innovative imaging methods and optogenetics. Mice and pigs have been crucial to the research of infectious diseases and developing of vaccines. [26]

Understanding the human immune system and how it reacts to microbial exposures is greatly aided by mouse models. Immune responses & potential therapeutics for both short and long-term can be learned. Many types of immune cells work together to fight off infections, and CD8 T cells are only one of them. Short-lived effector cells (SLECs) which is a distinct type of CD8 T cell, clear pathogens and provide immediate protection on the other hand memory precursor effector cells (MPECs), develop into long-lived central memory T cells, which are essential for long-term immunity and faster, stronger responses to recurring pathogens. Thus, it was observed that when the initial CD8 T cell response is biassed towards SLECs, the immunological response is strengthened in the short term, but the number of MPECs is decreased, potentially weakening the immune system in the long run. [27]

Researchers frequently choose to artificially induce preterm birth in mice and rats by inoculating them with infectious agents, administering progesterone blockers, or treating them with lipopolysaccharide. However, guinea pigs are the go-to model for researchers studying dysfunctional labor because they undergo preterm labor and uterine dystocia at rates that are comparable to humans. [28] Also, the study used rats to examine how cross-reactive B and T lymphocytes defend against dengue virus infection. The rat model was essential for understanding the dengue immune response and paving the way for new treatments and vaccinations [29]

Mice are also utilized to assess the effectiveness and safety of drugs before human trials. Researchers conducted in vivo experiments on mice to study the acute toxicity, pain-relieving, sedative, and fever-reducing properties of *Monotheca buxifolia* and *Bosea amherstiana*. The results indicated that both plants reduced abdominal constrictions in mice in a dose-dependent manner [30]. Another example, researchers used mice to test a new drug i.e., Orally Bioavailable Azaindole Inhibitor (VX-787) for Influenza.

The screening of metabolites in mice helped to evaluate the effects of benzene exposure on mice and to find possible biomarkers relevant to human health or early identification of benzene-related disorders. The amount of peripheral blood cells, especially white blood cells (WBC), were shown to be reduced in the benzene-exposed mice. After analyzing the mice exhaled air, the researchers identified many chemicals that may act as biomarkers for benzene exposure such as phenol, hydroquinone/catechol, benzenetriol, and trans, trans-Muconic acid (t, t-MA). They also found additional chemicals in exhaled breath that might be used as indicators of benzene-induced adverse effects. Some of these biomarkers were glutamate, cysteine, malondialdehyde [MDA], and -carboxylic fatty acids (C5H10O3 and C6H12O). [31]

Insulin insufficiency and insulin resistance (type II diabetes) were studied in detail using pigs with INS gene mutations and deletions. Humanized islet amyloid polypeptide (IAPP) and a dominant-negative glucose-dependent insulinotropic polypeptide (GIP) receptor have been expressed in pigs, providing a useful model for investigating insulin secretion, glucose homeostasis, and the development of islet amyloid deposits. IAPP plays a role in amyloid deposits production causing type II diabetes.

Humans with cystic fibrosis (CF), a genetic disorder that affects the lungs, digestive system, pancreas, liver, genitourinary system, and sweat glands, have a defective CFTR gene. To study human CF, pig models are genetically modified with CFTR allele deletion and the most common mutation [F508] were created via recombinant adeno-associated virus (RAAV) delivery resulted in reduced pancreatic and biliary production. [32]

A pig model with a deletion of the tumor suppressor gene RUNX3 was developed, opening exciting possibilities for study into stomach cancer. The BRCA1 gene, which is linked to breast cancer, was knocked out in a pig model.[33] This genetic engineering resulted to a rise in perinatal mortality, or deaths in the first few weeks after a piglet was born. Pigs with adenomatous polyposis coli (APC) gene mutations developed extensive bowel lesions and adenomas like those seen in humans with familial adenomatous polyposis. Cloned pigs with rhodopsin gene mutations have diminished light sensitivity, comparable to humans with hereditary retinal degeneration. This model helps us understand how eye degeneration happens.[34]

4. Zebrafish

The use of zebrafish (*Danio rerio*) as a test animal hinges heavily on important characteristics including its ease of maintenance in experimental settings and similarity in genotype and phenotype to humans. Because zebrafish are vertebrates, they contain all human organs. They also share 80% of the genetic characteristics of genes linked to human diseases. Zebrafish are, therefore, a popular choice for biomedical research and an excellent experimental animal. The zebrafish model is a potent, well-researched platform for evaluating the actions of novel pharmacological compounds

and the physiological responses to them. It also serves as a well-liked platform for research on the effectiveness of existing antibiotics and new anti-staphylococcal medicines in vivo as it is even susceptible to pathogens like Staphylococcus aureus that are typically not thought of as natural pathogens of fish. The degree of resistance to the development of a *S. aureus* infection in zebrafish larvae is greatly influenced by the injection site selection, as [35] have previously demonstrated. Since almost every dose eventually results in 100% mortality of zebrafish larvae, the yolk body is the injection site that is most vulnerable to staphylococcal infection. To assess the host's innate immune responses after being infected with strains of *S. aureus* that are both susceptible and resistant to daptomycin, used a zebrafish infection paradigm that provided a significant in vivo understanding of the pathogenicity of daptomycin-resistant *S. aureus* strains. The model is already utilized to examine bacterial virulence, disease pathophysiology, host-pathogen interactions, and host immune responses. In conclusion, zebrafish larvae are becoming a crucial in vivo model in *S. aureus* infection research [36] The antibacterial activity of Kalafungin, a compound discovered from a marine sponge-derived *Streptomyces sp*., was studied in a recent publication by zhou *et.al.* [37] To generate an infection model, the procedure involved microinjecting GFP-tagged *S. aureus* into the caudal vein of zebrafish larvae. Treatment given at 12 hpi (hours post infection) with 1- and 2-fold MIC showed how successful Kalafungin was in eliminating infection caused by bath immersion (minimum inhibitory concentration).

Zebrafish have also been used as an experimental model to study neurodegenerative disorders such as Parkinson's, Alzheimer's, and Huntington's diseases. Researchers examined the effects of an ethanol-based fruit extract from the Alpinia oxyphylla plant using zebrafish as a model.[38] The extract has a neuroprotective effect and has long been utilized in traditional Chinese medicine. The findings showed that the extract prevented and reversed the neurodegeneration caused by 6-hydroxydopamine. A zebrafish model was created by [39] to find new targets and potential medications for autism spectrum disorder (ASD), a range of diverse neurodevelopmental diseases that appear throughout childhood and are brought on by both hereditary and environmental causes. The use of zebrafish cntnap2 mutant's behavioral profiling as a platform for pharmacological screening led to the discovery of novel ASD-related pathways and phenotypic suppressors. It was discovered that the medication phytoestrogen biochanin A, which was screened from zebrafish, had the ability to selectively reverse the mutant behavioral phenotype. In addition, zebrafish has fulfilled several promises as an in vivo animal model for screening nanomaterials.[40]

The aim of zebrafish genomic research is to identify novel therapeutic targets. Using a zebrafish model, newly emerging targeted genome editing technologies like TALEN, CRISPR/Cas9 and ZFN, have been used to replicate pathological situations that are similar to those in humans and to study the in vivo effects. Duchenne muscular dystrophy, spinal muscular atrophy, severe congenital anemia, etc. are a few human disorders that have been researched in zebrafish. Zebrafish embryos exposed to ethanol at various concentrations and for various amounts of time allowed researchers to study the effects of fetal alcohol syndrome on zebrafish. They caused developmental flaws that are directly comparable to flaws in human birth.[41] Zebrafish suffer from pericardial edema and circulatory disturbance brought on by cardiotoxic medications. Using 5-fluorouracil, mitoxantrone, doxorubicin, and cyclophosphamide as well as terfenadine as an antihistamine, tested for cardiotoxicity in zebrafish. Overall, the zebrafish model is frequently utilized as a model for heart issues and seems to be helpful in preclinical research. Zebrafish have also gained popularity as an experimental animal for investigations on human cancer. To enable the dynamic visualization of individual cells after engrafting several kinds of human malignancies,[42] created an optically clear prkdc−/−, il2rga−/− zebrafish mutant that is devoid of adaptive and natural killer immune cells. They also uncovered the preclinical efficacy of a combination therapy for rhabdomyosarcoma employing the DNA-damaging drug temozolomide and the Olaparib PARP inhibitor. Phase I trials have now been conducted for the same combination therapy used to treat Ewing sarcoma and rhabdomyosarcoma (ClinicalTrials.gov with the number of NCT01858168).

Antibiotic residues in the environment have sparked worries about the possible harm they could do to ecosystems and human health. Zebrafish have lately become a potential animal model to explore the toxic effects of medicines and their therapeutic efficacy. Recent research has focused on both single as well as combined effects of different representative types of antibiotics, such as aminoglycosides, macrolides, tetracyclines, sulfonamides, quinolones, etc. on zebrafish. Following both acute and chronic exposure to antibiotics, oxidative stress, developmental toxicity, neurotoxicity, nephrotoxicity, oculotoxicity and cardiotoxicity have been noted. As an example, the antibiotic netilmicin, which is typically used to treat a variety of infections [100], caused cardiotoxicity in 6 hpf [hours post-fertilization] zebrafish embryos but not in 3 dpf (days post-fertilization) zebrafish, indicating a toxic effect during embryonic development [43]. Studies on the toxicity of another aminoglycoside, gentamicin, on zebrafish revealed that its administration can result in oculotoxicity, nephrotoxicity and ototoxicity as well as having an impact on the animals' locomotor behavior [44]. Additionally, zebrafish embryos exposed to binary mixtures of sulfonamides displayed increased developmental toxicity and a greater impact on the detoxification pathway.[45] Thus, to test the developmental toxicity of potential medications and chemicals, zebrafish embryos are being employed. In studies investigating the effects of the microbiome, intestinal inflammation, congenital abnormalities, enteric nervous system/motility disorders and

intestinal cancer, zebrafish have been utilized as intestinal disorder models. Recent advancements in visualizing gut motility and assessing gut transit time in zebrafish have increased sensitivity and throughput for toxicology. Drug-induced liver damage (DILI) is a significant issue for the pharmaceutical business. The zebrafish liver performs the same tasks as the human liver, being completely functional 5 days after fertilization. Thus, zebrafish larvae can be used to test xenobiotics for hepatotoxicity in an in vitro format, providing data from multiwell plates on the whole organ/whole animal, making zebrafish a compelling model. To view and quantify cell-specific compound effects or gross changes in the zebrafish liver, techniques utilizing fluorescent markers for different cell types or lipid stains, like oil-red-O, are used, respectively.[46]

5. Primates

In the intricate tapestry of pre-clinical research, where the quest for medical breakthroughs unfolds, one group of beings stands out with striking familiarity - primates. Far beyond the conventional laboratory mice or zebrafish, these intelligent and empathetic creatures bring a unique humanized perspective to the world of scientific exploration. Non-human primates are divided into the strepsirrhines, which include the lemurs, galagos, and lorisids, and the haplorhines, which include the tarsiers and the simians, monkeys, including apes. [47]

At the heart of the primate model organism narrative lies the remarkable proximity to humans on the evolutionary scale. With genetic similarities and shared physiological traits, primates provide an unparalleled platform to study the intricate mechanisms that govern our bodies. The subtle nuances in their biology offer a more accurate reflection of human responses to various stimuli, making them indispensable allies in the pursuit of medical knowledge.

One of the standout features of primates in pre-clinical research is their advanced cognitive abilities. Their complex social structures, problem-solving skills, and emotional depth echo shades of our own human experiences. This cognitive complexity enables researchers to investigate not only the physiological aspects of diseases but also the behavioral and psychological dimensions, offering a holistic understanding of the impact of interventions and treatments.

Primates serve as invaluable models for a range of diseases that afflict humans, including neurodegenerative disorders, infectious diseases, and cardiovascular conditions. The ability to closely mimic the progression and manifestation of these diseases in primates provides researchers with a real-world context, allowing for more accurate predictions of how potential therapies might translate from the laboratory to clinical settings.[48]

In the realm of drug development, primates offer a critical bridge between initial laboratory studies and human trials. Their physiological similarities facilitate the assessment of drug safety, efficacy, and potential side effects in a manner that is more predictive of human responses. This humanized approach not only enhances the success rate of drug development but also ensures a safer and more effective transition from the pre-clinical to clinical phases.

While the use of primates in research raises ethical concerns, strides have been made to ensure their welfare and minimize their use whenever possible. Stringent regulations and ethical guidelines govern their involvement in studies, emphasizing the importance of humane treatment and the pursuit of alternatives whenever feasible [49]

In the ever-evolving landscape of pre-clinical research, primates stand as unparalleled collaborators, offering a humanized lens through which we gain profound insights into the intricacies of human health. As we navigate the ethical considerations surrounding their use, it is undeniable that the contribution of primates as model organisms has been, and continues to be, instrumental in advancing medical knowledge and fostering a deeper understanding of our own biological tapestry.

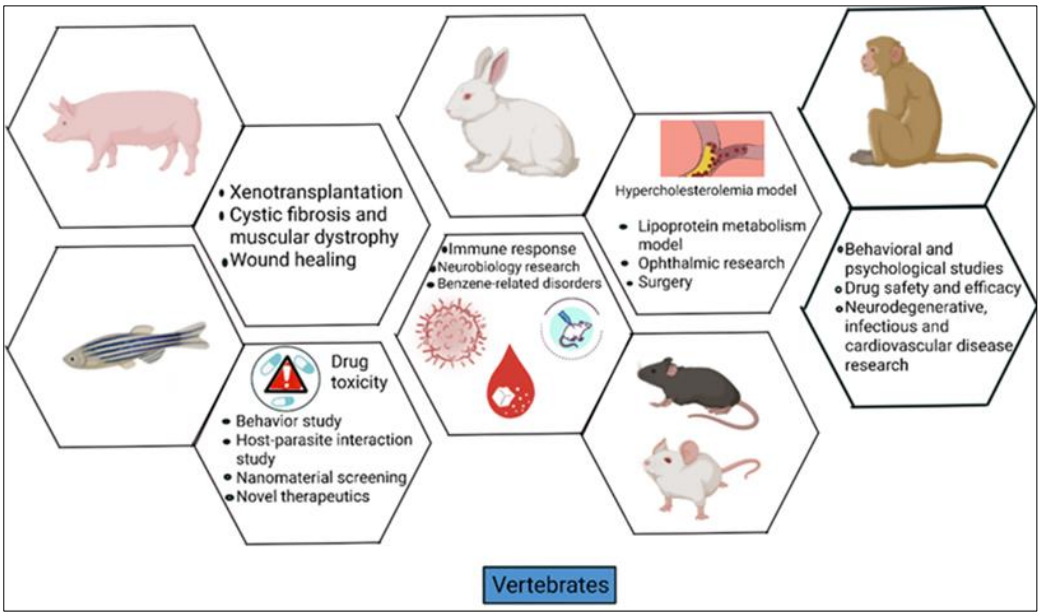

Figure 1 Vertebrates: key research applications across various species

6. Round worm

The well-known nematode *Caenorhabditis elegans* [*C. elegans*], has been used in numerous studies on ageing and aggregation, as an animal model. While 60–80% of nematode genes have human equivalents, it shares 35% of its sequence with the human genome [50]. Since C. elegans is simple to genetically modify to produce knock-out, knock-down and/or transgenic nematodes, it is perfect for studying crucial pathways and associated genes [51]. In terms of mechanistic and high-throughput screening methodologies, the ability of worms to self-fertilize and produce vast numbers of offspring, along with the presence of complex tissue systems, makes them excellent for a variety of biological studies. Modern methods such as high-throughput screening (HTS) are employed to evaluate the biological effects of different compounds, medications, and nanoparticles [52]. In 2006, the first HTS method utilizing C. elegans was carried out. Kwok and colleagues screened over 14,100 smaller compounds using worm transfer units of a Complex Object Parametric Analyzer and Sorter (COPASTM BIOSORT, Union Biometrica) and semi-automated image recovery. [51] By examining morphological defects in wild-type animals, such as sluggish growth, mortality, inappropriate mobility, and other traits, molecules were assessed for various bioactivities. Such assays are provided to the pharmaceutical industry by several businesses, including Celescreen, Sunny biotech, and Nagi Biosciences, to identify novel candidate compounds and validate pharmacological targets. The Caenorhabditis elegans model has also become popular in toxicological research. According to a recent study, continuous exposure to the pesticide paraquat might cause reproductive decrease in afflicted populations, which may have adverse, long-term ecological effects. Although C. elegans has only recently been used as a platform for drug screening, certain substances have been found in this nematode, and after being verified in vertebrate models, are currently being tested in human patients. For instance, zebrafish and mice were used to validate results from a medication screening carried out in a model of amyotrophic lateral sclerosis (ALS) in C. elegans. A brief randomized controlled study of individuals with sporadic ALS revealed the effectiveness of one of these successes, the neuroleptic Pimozide, a Ca^{2+} channel blocker that stabilizes neuromuscular transmission in C. elegans. Another study combined investigations on C. elegans with a clinical trial to test pearl powder, a Chinese medication. The greater antioxidant capacity discovered in blood samples taken from patients who used pearl powder therapy has been linked by the scientists to the lifetime extension observed in C. elegans. [53] Plant extracts high in polyphenols have also been examined for their impact on C. elegans lifespan as lifespan extension caused by their administration to nematodes was directly correlated with the polyphenol concentration of the examined extracts. The polyphenolic substances have been studied in relation to ageing and aggregation-related disorders and have been found to lengthen life expectancy and/or slow the progression of diseases [51].

C. elegans use in nano-biotechnology has also been expanded to include its viability as an in vivo model. Nanoparticles are frequently ingested by C. elegans either through the digestive tract while the organism is feeding or they can diffuse directly through the excretory pores, vulva, anus, and cuticle. [54] Because of the presence of glycoproteins, the cuticle's top layer has a negative charge that can interact electrostatically with a nanoparticle and affect how well it enters C. elegans. By way of the worm's epithelial surface, superparamagnetic iron oxide nanoparticles (SPIONs), for instance,

can be taken up. [55] Through its conjugation to the surface of Au nanoparticles (CNMA-GNPs), cinnamaldehyde (CNMA) was evaluated for its effectiveness as a broad-spectrum antimicrobial agent. Interesting findings revealed that this antimicrobial nanodrug delivery system (CNMA-GNPs) greatly lessens pathogenic infections by successfully reducing antibiotic-resistant strains and pathogenic biofilms. Similar to how it decreased C. elegans mortality, it also increased worm survivability when *Staphylococcus aureus* infection was present, with a reported twofold increase. [56]

It has been established that the worm C. elegans is a potent model for studying the genetics of fatty acid production and the control of fat accumulation in the context of obesity and other metabolic diseases. This nematode is a useful in vivo model for dietary ingredient screening, identifying bioactive compounds with beneficial qualities [such the capacity to regulate fat storage] and characterizing the molecular process behind this action. This is since C. elegans and mammals have extremely conserved energy balance regulatory mechanisms. [54] Previous findings imply that vanillic acid, along with the phenolic substances resveratrol and apigenin, may be helpful in preventing the excessive fat buildup associated with obesity. Additionally, the various mechanisms by which each phenolic compound works to reduce fat point to the possibility of a future assessment of their combination as a potential therapeutic agent in diseases associated with obesity. C. elegans has also grown to be the most often used model in geroprotective medication screening. This is seen by the difference between the percentage of medications studied in C. elegans [nearly 70%] and mice [only 10%] in the DrugAge database (https://genomics.senescence.info/drugs/stats.php). Surprisingly few geroprotective pharmacological hits discovered in C. elegans have been confirmed in further model organisms, nevertheless. Yu Liu studied the effects of para-hydroxybenzyl alcohol (HBA) on ageing and neurodegenerative illnesses in C. elegans models. [57] HBA is a byproduct of the traditional Chinese medicine *Gastrodia elata*, also known as "Tianma" in Chinese. Their findings showed that HBA significantly slowed the progression of neurodegenerative illnesses like Alzheimer's disease, Parkinson's disease, and Huntington's disease. Additionally, HBA could significantly enhance the physiological processes associated with ageing in worms and extend the average lifespan of wild-type N2 worms by more than 25%. Additionally, HBA increased the likelihood that worms would survive under conditions of oxidation, heat, and pathogenic bacteria. Overall, the results indicate that HBA is a prospective candidate for future development of antiaging pharmaceutical use since it increases stress resistance and protects against ageing and aging-related disorders by activating several cellular protective pathways.

7. Fruit fly

As the human and *Drosophila melanogaster* genome sequences were finished, it became clear that while many genes are shared by the two species, only around 60% of human disease-related genes have fly homologs, and Drosophila has fewer members of conserved gene families [58]. Because fewer genes need to be altered to create a drug-screening condition, Drosophila's smaller and simpler gene families may be advantageous for drug discovery. One of the most important aspects of the Drosophila model is its capacity to control the expression of genes using potent molecular tools, such as the GAL4/upstream activation sequence (UAS) system for targeted gene expression studies, the FRT/FLP recombination method and the RNAi-mediated gene knockdown in particular cells and tissues.

Drosophila models have also developed into a crucial tool for the development of new treatments for neurological illnesses. Recent research has identified numerous pathogenic factors that contribute to Parkinson's disease (PD), including mitochondrial failure, α-syn aggregation, defective autophagy, disruption of calcium (Ca^{2+}) homeostasis, microglial activation, endoplasmic reticulum (ER) stress and metabolic changes. ROS levels rise because of mitochondrial malfunction, which is a major factor in Parkinson's disease. As potential treatments for the condition, several antioxidant substances, including tocopherol, curcumin, and resveratrol, have been tested in Drosophila models of PD, which revealed that these substances are able to suppress other abnormalities in model flies, such as locomotor deficits and shortened life spans, in addition to lowering ROS levels. [58] Despite divergent evolutionary histories, the dopaminergic systems of mammals and flies both functions similarly in modulating locomotion, sexual function, and the response to drugs. As a result, drugs that target these pathogenic pathways are currently being evaluated to create new treatments for Parkinson's disease (PD) and have been tested in fly models of the condition. The physiological and biochemical effects of Cabergoline, a powerful ergot dopamine agonist that was administered in the form of cabergoline alginate nanocomposite (CANC), have been studied in Parkinson's disease (PD) model flies. The findings indicate that CANC is effective in postponing and easing PD symptoms. [59] The Drosophila dopamine receptor and well-known mammalian dopamine 2 like receptors share a significant amount of homology. As a result, this invertebrate serves as a great model organism for studying how dopamine works in higher creatures. Several drugs that block α-syn aggregation have been explored in Drosophila PD models as α-syn aggregation causes the death of dopaminergic (DA) neurons. For example, a Drosophila model expressing the human pathogenic A30P α-syn protein showed that the antidepressants nortriptyline or rifampicin, successfully decreased neurotoxicity as well as α-syn aggregation [60]. In Drosophila, neuronal malfunction can be fatal at any of the four stages of the organism's development, i.e., embryonic, larval, pupal, and adult. The literature has numerous cases demonstrating that Drosophila models of human diseases

frequently experience developmental block, typically passing away at any point prior to adult eclosion. This has been seen in a transgenic Drosophila model of Huntington's disease that expresses the human Huntingtin (Htt) protein's 588 amino acid N-terminal segment with a pathogenic polyglutamine tract that has 138 repeats (HttQ138). In a different Drosophila model of myotonic dystrophy 1 (DM1), female-specific semilethal pupal phenotypes were produced by the targeted production of a 480 interrupted CUG repeat RNA in Drosophila mushroom bodies, which are brain regions involved in sleep, learning and memory. This characteristic was utilized to find medications that could boost fly viability by screening pharmacological suppressors of neuronal toxicity brought on by CUG RNA.[61]

Drosophila models have been developed for a variety of complicated illness characteristics, some of which were employed in drug screening. For instance, mutations in at least two genes may cause Drosophila larvae to develop epithelial tumours. Common tumour models combine one oncogenic mutation and homozygous loss of one tumour suppressor, such Scribble, a homolog of the human tumour suppressor hScrib, in the same cell. In the Drosophila neuroepithelium, scrib+/+ and RasG12V-expressing clones develop into metastatic tumours. When Ras and Scrib mutations are present in the same cell, this may occur cell-autonomously, while it may not if the mutations are present in nearby cells. Medullary thyroid carcinoma (MTC) is one type of human cancer that has been linked to mutations in the proto-oncogene that encodes RET kinase. Inhibitors of RET signalling, such as ZD6474 (Vandetanib), which is currently licensed for the treatment of medullary thyroid carcinoma, have been studied using Drosophila RET models [62]. Partial lethality occurs between the embryo and pupa stages because of tissue-specific expression of a cancer-relevant mutant version of Drosophila Ret, dRetMEN2B, in a subset of epithelial cells of larva. Because these models are fatal, it is possible to test for medications that block RET signaling by utilizing the recovery from lethality as a read-out.

It is possible that more than 40% of the human and Drosophila genes that code for pharmacological targets are functionally conserved. Various multi-hit Drosophila models are being tested in an ongoing study involving patients with colorectal and Medullary Thyroid cancer. In these models, genomic information from patients is used to create Drosophila "avatars" with multiple genomic alterations that mimic the complex genotype of the patient's tumour. Then, both separately and in combination, the avatars are employed to thoroughly test FDA-approved medications. A potential medicine becomes a strong clinical candidate when it possesses a variety of qualities beyond effectiveness. One of these characteristics is toxicity, which is considered in the design of several Drosophila screens. For instance, p53 and Chk1 mutations were used in a screening process to find radiation modulators, while wild type was used as a control. Finding substances that were more effective against cancer-related mutations than wild type was the aim. 1,000 FDA-approved medicines were tested to see which ones decreased transgene-induced larval mortality. Trametinib, an inhibitor of MEK1/2 kinases, and fluvastatin, a statin medicine used to treat cardiovascular disease, were the two hits from the screen. When given simultaneously, the two medications were found to synergize to increase each other's efficacy in Drosophila and in human lung cancer cells. It is interesting to note that high doses of trametinib lowered the survival of non-tumor larvae and were toxic, while fluvastatin co administration decreased this toxicity.

Fruit fly offers a very useful substitute for the current mammalian models used in SARS-CoV-2 research, allowing for the investigation of virus protein function as well as the development of targeted treatments. A recent study examined 12 SARS-CoV-2 proteins in flies and ranked them according to how likely they were to cause pathogenic host interactions. Research on these proteins mostly examined the trachea, the fly equivalent of the lung, and muscle, two tissues affected by COVID-19. Notably, the SARS-CoV-2 genes that were found to be the most pathogenic in the in vitro human cell culture (HEK 293 T) model were likewise the most detrimental in these in vivo transgenic fly systems (Orf6, Nsp6, and Orf7a) [63]. Fruit fly is a suitable drug screening model due to its conserved pathways, genetic tools, target identification using mutants that are no longer susceptible to a drug, and the capacity to address mechanisms of drug response/resistance. Despite numerous benefits, limitations in automation technology prevent Drosophila screens from being used more widely. Making Drosophila the go-to model for drug-screening would be worthwhile if we concentrated our efforts on automating the current screenings.

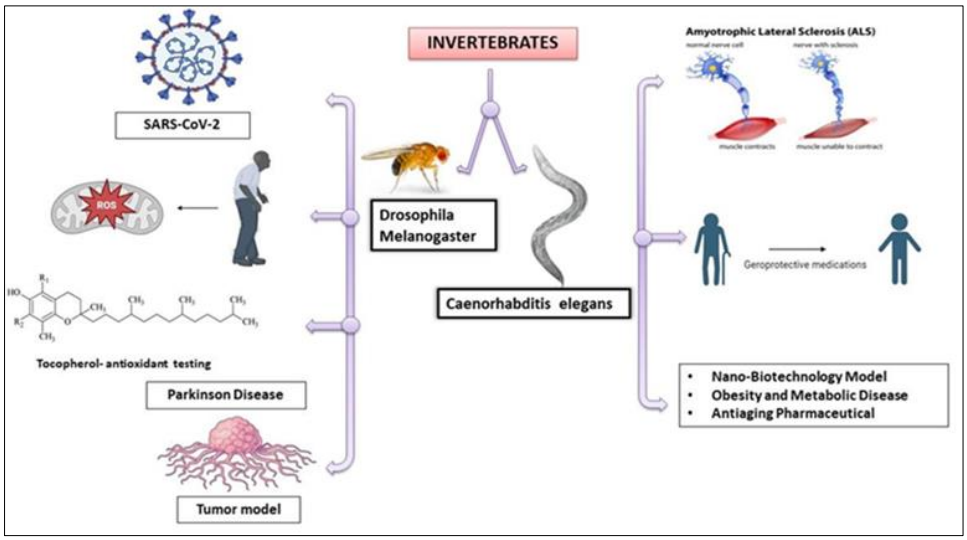

Figure 2 Invertebrates: key research applications across various species

Table 1 Comparisons between different animal models

Model	Advantages	Disadvantages	Source
Rabbit	Very docile Short puberty and gestation period Comes under small animals hence require ethical clearance only from institutional body and not from a central body	Not very easily available. Not many operative and post-operative medicines available specific to rabbit Scarcity of literature	[64]
Rodents	Small in size Easily maintained Lifecycle is short Ethical clearance only from institute	Capture only single aspect of disease. In case of tumours, genetics are more often not representative in humans	[65]
Pigs	Genome thrice closer to humans than mouse model Docile to human Good model for osteoporosis as knee thickness is like humans	Special facilities required for housing Different gastrointestinal system than humans Ethical clearance is more stringent	[23]
Primates	High degree of genetic similarity Capability of genetic control Experimental manipulations can be carried out	Maintenance is highly expensive. Gene map is still in early stage Stringent ethical clearance	[66]
Zebrafish	Embryos develop outside the uterus Development is rapid Easy to get ethical clearance Easy and cheap maintenance	Genes are present in more than one copy [paralogs]	[67]
Fruit Fly	Cheap Genetic cross breeding is easy since very short life cycle Produce large number of eggs	Most pathogenetic factors are vertebrate specific. Anatomically very different from human.	[68]
Round worms	Grow large in number. Exhibits distinct phases of life cycle which can be observed physiologically and genetically.	Lacks mammalian organs. Far from humans in terms of evolution	[69]

8. Conclusion

In the vast canvas of non-clinical research, our exploration into model organisms has provided us with profound insights into the intricate workings of biological systems. While our focus in this review cantered on the remarkable contributions of model organisms such as mice, primates, and others, it is crucial to acknowledge the diverse array of creatures contributing to pre-clinical studies. The likes *of Caridea, artemia, Canines, Felis spp*., and many more have each played unique roles in unraveling the complexities of biology and disease.

As we conclude this exploration, it is evident that the collaborative efforts of scientists, whether with the close companionship of primates or the microscopic wonders of *Caridea* and *artemia*, continue to expand the frontiers of knowledge. The journey of non-clinical research is a testament to the ingenuity of researchers who, with ethical considerations in mind, meticulously select and study these diverse organisms to enhance our understanding of health and disease.

While this review primarily delved into the nuanced world of certain model organisms, it is important to recognize the ongoing exploration into alternative species and their potential roles in shaping the future of pre-clinical studies. As we move forward, the world of non-clinical research promises to weave in new chapters, each revealing the extraordinary contributions of various model organisms, both traditional and unconventional, to advance our collective pursuit of scientific understanding and medical progress.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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