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Zebrafish in Drug Research: Decoding Biological Mechanisms and Finding Novel Therapeutic Targets

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Abstract

The zebrafish, scientifically known as Danio rerio, is a powerful model organism in biological research because of its favorable traits, which include rapid development, genetic tractability, and transparent embryos. Zebrafish were first identified for their use in studies of vertebrate development, but they have since spread to a variety of disciplines, such as pharmacology, clinical research as a disease model, and most notably, drug development. Zebrafish have emerged as a cost-effective and practical alternative to traditional mammalian models in numerous biomedical research areas. Their utility spans a wide range of applications such as lead compound screening, identification of molecular targets, morpholino oligonucleotide-based gene function studies, assay development, and the evaluation of drug toxicity. Due to their genetic similarity to humans, transparent embryos, and rapid development, zebrafish serve as an ideal in vivo model for understanding complex biological processes. This review highlights the key characteristics that contribute to the zebrafish's growing significance in research, especially in the field of pharmacology. It emphasizes their vital role in accelerating drug discovery and development, offering a simplified yet reliable system for studying disease mechanisms, therapeutic responses, and adverse drug reactions, making them an invaluable asset in pharmaceutical research.

KEYWORDS: Toxicity, zebrafish, pharmacology, drug development, embryos, genetic

INTRODUCTION

Originating in South Asia, the zebrafish has become well-known in several biological research domains such as toxicology, neurology, genetics, developmental biology, and pharmacology. Certain characteristics make it an important model organism [1, 2]. Finding chemical entities with potential for medical use is known as drug discovery. The objective is to find new compounds that address unmet medical needs, with an emphasis on serious illnesses for which there are no effective treatments. Current drugs primarily focus on enzymes and target ion channels, nuclear receptors, and G-protein coupled receptors [3].

Due to things, like unknown variables during lead discovery, compounds may encounter difficulties in development even with promising leads. Failures can be caused by problems such as unacceptable toxicity, inadequate biopharmaceutical qualities, and inadequate in vivo efficacy. Complexity of the synthetic process, unclear toxicological results, and low potency can also impede development [2–4].

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Since the 1980s, the zebrafish (Danio rerio), a commonly used freshwater teleost, has been a popular model in studies. The discovery of millions of zebrafish mutant models with conserved disease genes broadened its applications, which were initially concentrated on developmental biology. With the help of their external embryo development, zebrafish's relevance to inherited and acquired human disorders increased the potential for translational research.

The zebrafish's role in translational research has grown since its 2013 genome sequencing revealed

significant genomic homology with humans. It developed into an essential tool for drug research and development over the course of 20 years [4]. Zebrafish have become more popular in the industry because of their robustness and ease of maintenance, which make them suitable for medium-to-high-throughput screening in pharmacology investigations.

Zebrafish, reaching about 5 cm in adulthood and 5 mm in the larval stage, are ideally suited for systems-level studies aimed at exploring the complex interactions between genes, biological processes, and chemical environments. Large stocks are made possible by their high fecundity, and non-invasive observation of the morphology and function of internal organs is made possible by their optical transparency [3]. They are superior to other methods for high-throughput phenotyping because they prioritize phenotype over genetics or experimental tools. The versatility of the model is demonstrated by recent applications, such as the study of zebrafish neuron regeneration, using fluorescence-labeled mauthner cells [1, 4].

ADVANTAGES OF ZEBRAFISH AS A MODEL ORGANISM

Zebrafish stand out as a flexible model for modern research because of their astounding 80% genetic overlap with humans. Their highly characterized genes are cost-effective for product development because they can be precisely manipulated. Zebrafish and mammals have similar genomic characteristics, which emphasizes their applicability in drug development processes. Figure 1 illustrates the fundamental idea of zebrafish models, their advantages over humans, and a comparison [5–7].

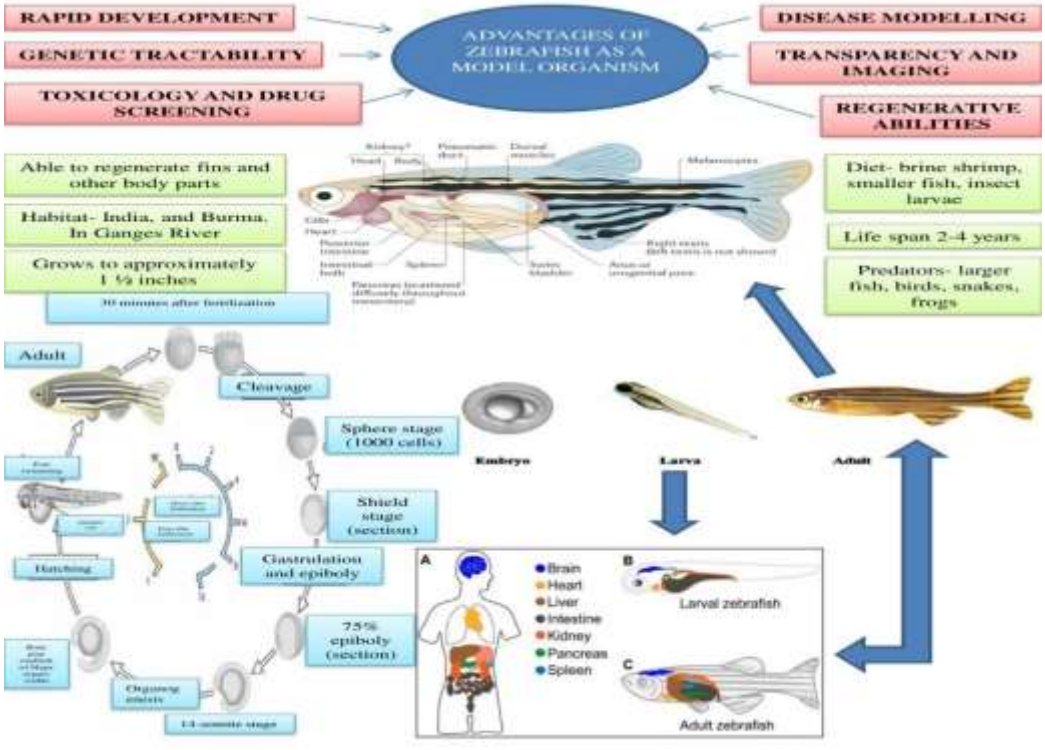

Figure 1. Fundamental overview of Zebrafish models, their comparison with humans, and associated advantages [1, 8].

Transparency and Imaging

Real-time imaging of drug interactions, tissue development, and disease progression is made possible by the transparency of zebrafish embryos and larvae. This special characteristic offers important information about the cellular and subcellular pharmacological effects [9].

Genetic Tractability

The genome of zebrafish is well-characterized, and researchers can make precise genetic changes using genetic manipulation methods like CRISPR/Cas9. This makes it easier to research pharmacological targets and pathways [10].

Rapid Development

Zebrafish embryos develop rapidly, with their organs and systems forming in just a few days. This fast-paced development enables scientists to assess drug effects and toxicity across multiple stages, offering a comprehensive view of how drugs interact with biological systems [11].

Regenerative Abilities

Zebrafish possess remarkable regenerative abilities, particularly in restoring fins, heart tissue, and the central nervous system. This capacity makes them a valuable model for studying tissue regeneration and repair mechanisms [12].

Developmental Biology

Zebrafish embryos experience fast embryonic development, offering valuable insights into vertebrate growth processes. Their transparency allows clear observation of organ formation, making them an ideal model for exploring developmental biology.

Disease Modelling

Zebrafish are effectively used to model a wide range of human diseases, including neurological conditions, heart disorders, and cancer. Their physiological resemblance to humans, along with ease of genetic modification, enables researchers to investigate disease processes and evaluate potential treatments.

Toxicology and Drug Screening

Zebrafish models serve as a vital tool in toxicology and drug screening research. They play a key role in evaluating the toxicity of both environmental contaminants and pharmaceutical agents, greatly supporting safety assessment efforts.

ZEBRAFISH MODELS IN DISEASE RESEARCH

Zebrafish serve as an excellent model organism for disease and translational research, witnessing a surge in utilization over the last five years. Table 1 shows Different types of zebrafish models and organ used in disease and activity research.

Table 1. Different types of zebrafish models and organs are used in disease and activity research.

S. N.	Activity or Disease Activity	Organ or Models of Zebrafish
1.	Antibacterial Activity	Embryos and Tissue [13].
2.	CNS Effect of Antimicrobial Drugs	Brain [14].
3.	Seizures Induced (Behavioral Characterization)	Adult Zebra fish [15].
4.	Epigenetics in renal disease	Kidney [16].
5.	Neurotoxic Effects and Neurodegeneration (Behavioral Assessment)	Brain [17].
6.	Senescence Independent Anti-Inflammatory activity	Zebra fish Larvae [18].
7.	Inflammatory Bowel Disease	Intestine [19].
8.	Lipid-Lowering drug Induced Myopathies	Muscle [20].
9.	Cancer therapy Induced Cardiovascular Toxicity	Heart [21].
10.	Glioblastoma Cellular and Molecular Mechanisms	Glial Cells [22].
11.	Toxicity	Embryo [23].
12.	Drug-Induced Liver Injury	Liver [24].
13.	Neuropsychopharmacology & CNS Drug Discovery	Brain [25].
14.	Locomotor Activity	Brain [26].
15.	Optical Mapping of Neuronal Activity During Seizures	Brain [27].

16.	Alzheimer's Disease	Brain [28].
17.	Neutrophilic Inflammation	Larvae Neutrophils [29].
18.	Embryo Toxicity	Zebrafish Blood [30].
19.	Autism Spectrum Disorder (ASD)	Brain [31].
20.	Analysis of the Retina	Eye (Retina) [32].
21.	Thyroid Gland Development and Function	Thyroid gland [33].
22.	Dual Oxidase – Anti-Bacterial Properties	Larvae [34].
23.	A Model of Superinfection of Increased Susceptibility to Bacteria Associated with Neutrophil Death.	Virus-Infected Zebrafish Larvae [35].
24.	Antibiotic Chlortetracycline Causes Transgenerational Immunosuppressant Ianf-Kb:	Larvae [36].
25.	Anticarcinogenic And Antioxidant Action of An Edible Aquatic Flora Jussiaea Repens L.	Larvae [37].
26.	Mucosal Inflammation at The Repository Interface	Zebrafish gills [38].
27.	Quantitative analysis of Protein Turnover and Tissue Regeneration	Tissues [39].
28.	Screening Antimycobacterial Compounds	Naturally Infected Zebrafish Larvae Model [40].
29.	Antifungal Activity Of 2′-Hydroxychalcone Loaded in Nanoemulsion Against Paracoccidioides Spp.	Whole Zebrafish [41].
30.	Influenza A And Exacerbate Disease –Duchenne Muscular Dystrophy	Virus Infection Damage Zebrafish Skeletal Muscle [42].
31.	Spermatogonial Stem Cell Niche Spermatogonial Stem Cell Transplantation	Spermatogonial Stem Cells (SSCS) [43].
32.	Estrogen Signaling Influences Nephron Segmentation	Zebrafish Embryonic Kidney [44].
33.	Exocrine Pancreas Development	Pancreas [45].
34.	Ocular Tuberculosis.	Eyes [46].
35.	Bacterial Meningitis in Streptococcus Agalactiae Infection.	Adult Zebrafish [47].
36.	Visual Of Von-Hippel Lindau Disease	Eye [48].
37.	Attention Deficit Hyperactivity Disorder (ADHD).	Zebrafish [49].

Nutritional Abnormalities in Zebrafish

Zebrafish, whose major organs closely resemble those involved in human metabolic regulation, serve as a valuable model for researching obesity and metabolic disorders. By experimentally altering their blood glucose levels to mimic diabetes, researchers have gained important insights into complications like retinal inflammation. Additionally, studies on osmoregulation have highlighted links to glucose absorption, providing a distinctive approach to exploring diabetic retinopathy.

Frameworks of Inflammatory Pathology in Zebrafish

Zebrafish models are instrumental in exploring inflammation and tissue regeneration, particularly through tail injury-induced acute inflammation. They provide a platform to investigate glucocorticoid activity, anti-inflammatory effects, and the discovery of novel therapeutic agents. Pro-resolution pathways, such as those involving lipoxins, play a key role in resolving inflammation. Moreover, the presence of mast cells in zebrafish, which are comparable to those in mammals, offers valuable insights into inflammatory processes.

Cancer Research in Zebrafish

Zebrafish are highly useful in cancer research, exhibiting spontaneous tumor formation and supporting techniques like genetic engineering, transgenic modelling, allografts, xenografts, and high-throughput genetic screening. Their transparent embryos enable real-time observation of tumor development, offering a distinct advantage for studying cancer biology. These models effectively mimic a range of human cancers and are instrumental in identifying and testing potential therapeutic agents.

CARDIOVASCULAR STUDY USING ZEBRAFISH AS A GENETIC MODEL

Zebrafish present key advantages for investigating cardiovascular genetics, with their transparent embryos allowing for detailed phenotypic screening. The clearly visible embryonic heart facilitates

direct observation of heart development. Genetic screens help identify heart-related abnormalities, enhancing our understanding of circulatory disorders. These models also replicate human responses to cardiac drugs, making them valuable tools for drug development. Additionally, high-throughput assays support rapid and efficient testing of cardiovascular impacts.

Zebrafish in Toxicology and Safety Assessment

Zebrafish are a valuable model for evaluating developmental and reproductive toxicity during drug development, enabling early detection of potential risks. Assessing organ-specific toxicity, especially in the heart and liver, is a key aspect of preclinical testing. As vertebrate organisms, zebrafish play a significant role in in vivo toxicity screening, as well as in evaluating drug efficacy and overall safety. Safety pharmacology focuses on identifying adverse effects on the respiratory, neurological, and cardiovascular systems, which can vary depending on the compound's class and mechanism of action.

Embryotoxicity and Teratogenicity

Zebrafish models are instrumental in investigating embryotoxicity and teratogenicity, offering insights into compound-induced structural abnormalities and disease mechanisms. Their fully sequenced genome supports the study of signaling pathways associated with teratogenic effects. The impact of drugs on development and reproduction is thoroughly assessed, following protocols refined since the discovery of thalidomide's harmful effects. The rapid development of zebrafish embryos enables efficient evaluation of viability, morphological defects, and emerging endpoints, moving beyond basic survival analysis.

Cardiotoxicity and Hepatotoxicity

Zebrafish embryos are highly effective in toxicological research, particularly for examining the effects of environmental pollutants and developmental toxins on the cardiovascular system. Investigations involving cigarette smoke and chronic alcohol exposure underscore their value in assessing cardiovascular toxicity. Zebrafish models also play a significant role in evaluating the cardiotoxic effects of human medications, reinforcing their importance in drug safety studies. In hepatotoxicity research, both in vitro and in vivo approaches are employed, with zebrafish embryos exhibiting gene expression changes comparable to those seen with human liver toxins. The distinctive tubular structure of the zebrafish liver allows for real-time observation of specific cellular responses, aiding in the study of liver damage caused by drugs and environmental agents.

CHALLENGES AND LIMITATIONS

Zebrafish model presents several challenges. While it allows to produce large numbers of embryos at a low cost, issues persist, such as the difficulty in correlating toxic water dosing levels with mammalian plasma concentrations, along with the need for more research on absorption, distribution, metabolism, and excretion (ADME) data. Other limitations include partial genome duplication, which leads to gene redundancy, and a relatively moderate generation time. While zebrafish are convenient for laboratory studies, these challenges, particularly in toxicological research, raise concerns about their applicability to human models.

Future Direction

As a preclinical model, the zebrafish requires further research to pinpoint the molecular targets of tested chemicals, particularly those affecting cardiac-related genes and proteins. A thorough analysis of how these chemicals influence relevant pathways and disrupt associated traits is essential. Investigations should prioritize established molecular targets and signaling pathways, while also remaining open to discovering new ones if needed. Addressing these factors is key to advancing zebrafish research and improving its utility in drug safety evaluation [49].

CONCLUSIONS

The zebrafish, known for its genetic strengths, swift developmental cycle, and impressive regenerative abilities, serves as a vital model in a wide range of biological research fields. It is integral to exploring basic biological functions, modelling diseases, and assessing drug efficacy. Despite its indispensable role, there is

a continuing need to advance welfare practices, refine disease models, and enhance drug screening methods. Sustained efforts in these areas will further the study of *Danio rerio*, offering deeper insights into its biomedical research potential.

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