IJBPAS, November, 2023, 12(11): 4956-4965

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

# ZEBRAFISH (DANIO RERIO) AS BIOMEDICAL MODELS IN RESEARCH

**RUTUJA SONAWANE*[1,2] AND KARTHICKEYA KRISHNAN[3]**

**1:** Research Scholar, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies, VISTAS, Pallavaram, Chennai, Tamil Nadu

**2:** Department of Pharmaceutical Chemistry, SCES's Indira College of Pharmacy, Tathawade, Pune - 411033

**3:** Professor and Head, Ph.D. Guide, Department of Pharmacy Practice, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu

***Corresponding Author: Prof. Rutuja Sonawane: E Mail: kamblerv23@gmail.com**

**Received 19th Sept. 2022; Revised 16th Oct. 2022; Accepted 21st Feb. 2023; Available online 1st Nov. 2023**

https://doi.org/10.31032/IJBPAS/2023/12.11.7526

## ABSTRACT

In comparison to other vertebrate models used to simulate human diseases, zebrafish have several advantages. This is especially true for large-scale genetic mutant and therapeutic chemical screenings, as well as other biomedical research applications. Disease modeling in zebrafish is advancing our understanding of the molecular pathways behind human genetic illnesses because of significant advancements in sequencing technology. Because they offer fresh diagnostic and treatment options, these initiatives are crucial for the development of precision medicine. This article focuses on development in Disease modeling in zebrafish.

**Keywords: Zebra Fish, Disease Modeling, Therapeutic screening, Biomedical research**

## INTRODUCTION

Zebrafish (Danio rerio) may be a tiny freshwater fish that's associated with extensively studied vertebrate model organisms. It may breed speedily in massive numbers, is amenable to biology and genetic studies, and encompasses a clear embryo, creating it engaging to review vertebrate development, physiology, and sickness. Zebrafish are also superior to rodent models for the study of vertebrate development and illness. Recent research found that medications that were beneficial in treating

metabolic disorders in people also worked well in a zebrafish model. Interestingly, the publication of the first paper on zebrafish development investigated the effect of different toxins, alcohol, and different levels of carbohydrate or fat diets on zebrafish embryos, larvae, and adult developmental stages **[1]**.

**Common Fish Species Used as Model Species**

Goldfish (Carassius auratus), the first model species, has been utilized by scientists for more than 200 years. Most of the applied research on aquatic toxicology utilized goldfish. Additional fish species have also been used, including zebrafish (Danio rerio), goldfish (Carassius auratus), medaka (Oryzias latipes), roach (Rutilus rutilus), three-spined stickleback (Gasterosteus aculeatus), pufferfish (Takifugu rubripes), and the swordtail (Xiphophorus hellerii) **[2]**. Each fish species has specific benefits and drawbacks. For instance, research on growth, stress, immunology, and reproduction has been done using goldfish. The most common species of fish utilized to research genetics, reproduction, and development was the medaka fish. Due to its characteristics being appropriate for numerous research fields, zebrafish models have become more and more popular in recent years.

**General Features of Zebrafish**

Danio rerio the Latin name for zebrafish formerly called Brachydanio rerio is a small tropical freshwater fish originating in the Ganges River and its tributaries in northern India **[3]**. Zebrafish are typically found near the bottom of the ocean in their natural habitat to reduce attack by predators. Male and female zebrafish morphology is depicted in 1. Zebrafish are currently thought to be an appropriate model to study development, genetics, immunology, behavior, physiology, and nutrition.


**Figure 1: - An illustration of a male zebrafish (A) and female zebrafish (B) [4]**

**Why Do Zebrafish Make Such Good Animal Models?**

The criteria to select animal models for biomedical research are directly related to the final goal of the research. The development of two significant genetic mutations, one by Nobel Prize winner Christiane Nusslein-Volhard in Tubingen, Germany, and the other by Wolfgang Driever and Mark Fishman in Boston, USA, in the 1990s, gave rise to the use of zebrafish as a model organism. One of the most critical methods used in the study of numerous aspects of biology is the identification of mutants. The use of zebrafish as a biomedical model was suggested by George Streisinger and colleagues at the University of Oregon, who launched the modern era for zebrafish in the field of biomedical research **[5]**. Due to their many benefits over other species, zebrafish are a favorite among scientists who study animal models. The fully sequenced genome, ease of genome manipulation, high fecundity, short generation period (approximately 3 mo.), quick embryonic development (24 hr.), and external fertilization of zebrafish are its most favorable traits. The translucent zebrafish embryo makes it possible to observe the various developmental phases, starting with the embryonic stage. In addition, zebrafish embryos form complete organ systems, including heart, intestine and blood vessels within 48 hr. after fertilization. More than 10,000 mutants in protein-coding genes have been generated **[6]** and several transgenic lines of zebrafish have been made to study human diseases. Another key benefit of this species is the availability of several zebrafish strains. Additionally, keeping a large number of zebrafish in a condensed amount of lab space is quite economical. Even though zebrafish are generally simple to manage, special care must be taken to guarantee a balanced food and sufficient water quality to promote fish health and growth. Specific genes have been knocked out or knocked in to create mutant zebrafish. New biomedical models are produced by these modifications. For instance, different mutations in zebrafish genes related to metabolism can be produced, and changes in gene expression can subsequently be seen using various molecular techniques if the patient has an illness related to metabolism. It is challenging to create stable transgenic adults or homozygous mutant embryos in zebrafish due to their short generation time, which typically takes 4 months.

**Table 1: Developments of Zebra Fish Models**

| Zebrafish model | Year | References |
|---|---|---|
| As a cancer model | 2008 | [7] |
| | 2002 | [8] |
| As a Developmental Model Organism for Pediatric Research | 2008 | [9] |
| In developmental biology | 1989 | [10] |
| In Angiogenesis | 2006 | [11] |
| As a Model Animal for Diet-induced Obesity | 2010 | [12] |
| | 2018 | [13] |
| | 2017 | [14] |
| As Model for Glucose Metabolism and Type 2 Diabetes Mellitus | 2014 | [15] |
| | 2017 | [16] |
| | 2016 | [17] |
| | 2007 | [18] |
| | 2017 | [16] |
| | 2018 | [19] |
| | 2010 | [20] |
| | 2014 | [15] |
| | 2016 | [21] |
| | 2015 | [22] |
| | 2012 | [23] |
| As Model for Dyslipidemia and Atherosclerosis Diseases | 2012 | [24] |
| | 2014 | [25] |
| | 2010 | [12] |
| As a Model for Nonalcoholic Fatty Liver Disease and Other Liver Disorders | 2018 | [26] |
| | 1937 | [27] |
| | 2018 | [28] |
| | 2019 | [29] |
| | 2009 | [30] |
| | 2015 | [31] |
| | 2018 | [32] |
| As a Model for the Study of Intestinal Diseases and Host–Microbe Interactions | 2016 | [33] |
| | 2018 | [34] |
| | 2018 | [35] |
| | 2011 | [36] |
| | 2018 | [37] |
| | 2012 | [38] |
| | 2018 | [39] |
| | 2007 | [40] |

## Toxicology and drug discovery

As discussed previously, the zebrafish has recently become a well-known model for toxicological investigations and drug development due to various advantages. The length and shape of the zebrafish body as well as the morphology of internal organs including the brain, liver, circulatory system, pancreas, intestine, kidney, notochord, etc. allow one to visually study how medications affect growth and development. The zebrafish model also has been used to know the organ function assays and assessment of drug effects **[41]** Zebrafish embryos are used as a predictive model to assess the toxicity in mammals. The lethal concentration (LC50) of different chemicals has been determined in embryos of zebrafish and has been compared with the mammalian LC50, and it has been found that the median lethal dose of zebrafish is lower than mammals **[42]**. The effects of drugs on specific organs have also been studied, and it has been found that organ toxicity is similar in both zebrafish and mammals. The drugs that were used to evaluate the organ toxicity were gentamicin, cisplatin, vinblastine, quinine, neomycin,

doxorubicin, dexamethasone, cyclosporin A, caffeine, camptothecin, MPA, fluorouracil, etc. **[43-48]**.

**Zebrafish and drug discovery**

The zebrafish model has been used potentially in drug discovery and to know the effects of neurotoxic, ototoxic, and neuroprotectant drugs. The process of drug discovery is divided into four main components: screening of lead compounds, target identification, target validation, and assay development **[49]**. The process of target identification involves the recognition of the target gene or protein which when modulated by a drug can have positive effects on the progression of disease. After identification of possible target, the validation process of target begins through determination of protein function and assessment of the druggability of the target **[42, 50, 51, 52]**. Zebrafish has great role in each of these areas of drug discovery.

**Limitations**

Zebrafish have certain limitations, including the dissimilarity of some organs like the respiratory system and the reproductive system, despite their significance as a biological model. Therefore, it is challenging to use zebrafish as a model for human breathing or reproduction. Another restriction is the inability to screen some water-soluble medicines in zebrafish due of their watery habitat.

**CONCLUSION**

The zebrafish is a crucial biological model in all areas of biology. Zebrafish have a number of characteristics that make them excellent for genetic, physiological, and developmental research, such as external fertilization and translucent embryos. Zebrafish are an appealing model for a number of human illnesses and the development of potential treatments for humans due to the high degree of functional conservation of morphology, genetics, and physiology between zebrafish and humans. Zebrafish are used to examine several human diseases because to advancements in nanotechnologies and molecular approaches. In this review, we focused on a few biomedical fields where the use of zebrafish as a model is common to study the mechanisms and processes underlying metabolic diseases, such as diet-induced obesity, type 2 diabetes mellitus, dyslipidemia and atherosclerosis, liver-related illnesses, and intestinal illnesses. Zebrafish have also been utilized by researchers to create novel treatments and vaccines for these significant human diseases.

**Acknowledgment**

The author is thankful for the guidance and support of Dr. Karthickeyan Krishnan, Professor and Head, Ph.D. Guide, Department of Pharmacy Practice, School of Pharmaceutical Sciences, Vels Institute of

Science, Technology and Advanced Studies (VISTAS). Also, Principal Dr. Anagha Joshi & Vice-Principal Dr.D.M. Kannur for providing all necessary Facilities at SCES's Indira College of Pharmacy, Pune.

**REFERENCES**

**[1]** Roosen-Runge EC. An observation on differentiation without cleavage in an egg of the zebra fish, Brachydanio rerio. The Anatomical Record ,1939; 74:349–353.

**[2]** Ribas L, Piferrer F. The zebrafish (Danio rerio) as a model organism, with emphasis on applications for finfish aquaculture research. Reviews in Aquaculture. 2013; 6:209–240.

**[3]** Tavares B, Santos Lopes S. The Importance of Zebrafish in Biomedical Research. Acta Médica Portuguesa,2013; 26:583.

**[4]** Avdesh A, Chen M, Martin-Iverson MT, Mondal A, Ong D, Rainey-Smith S, Taddei K, Lardelli M, Groth DM, Verdile G, *et al*. Regular Care and Maintenance of a Zebrafish Laboratory: An Introduction. Journal of Visualized Experiments. 2012.

**[5]** Clark KJ, Boczek NJ, Ekker SC. Stressing zebrafish for behavioral genetics. Revneuro, 2011; 22:49–62.

**[6]** Butler D. Wellcome Trust funds bid to unravel zebrafish genome. Nature, 2000; 408:503–503.

**[7]** Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, *et al*. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Research, 2008; 18:997–1006.

**[8]** Amatruda JF, Shepard JL, Stern HM, Zon LI. Zebrafish as a cancer model system. Cancer Cell, 2002; 1:229–231

**[9]** Veldman MB, Lin S. Zebrafish as a Developmental Model Organism for Pediatric Research. Pediatric Research, 2008; 64:470–476.

**[10]** Kimmel CB. Genetics and early development of zebrafish. Trends in Genetics. 1989; 5:283–288.

**[11]** Pandya NM, Dhalla NS, Santani DD. Angiogenesis—a new target for future therapy. Vascular Pharmacology. 2006; 44:265–274.

**[12]** Oka T, Nishimura Y, Zang L, Hirano M, Shimada Y, Wang Z, Umemoto N, Kuroyanagi J, Nishimura N, Tanaka T. Diet-induced obesity in zebrafish shares common pathophysiological pathways with mammalian obesity. BMC Physiolog. 2010;10.

**[13]** Chen B, Zheng YM, Zhang JP. Comparative Study of Different Diets-Induced NAFLD Models of Zebrafish. Frontiers in Endocrinology 2018; 9.

**[14]** Landgraf K, Schuster S, Meusel A, Garten A, Riemer T, Schleinitz D, Kiess W, Körner A. Short-term overfeeding of zebrafish with normal or high-fat diet as a model for the development of metabolically healthy versus unhealthy obesity. BMC Physiology 2017;17.

**[15]** Capiotti KM, Antonioli R, Kist LW, Bogo MR, Bonan CD, Da Silva RS. Persistent impaired glucose metabolism in a zebrafish hyperglycemia model. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 2014; 171:58–65.

**[16]** Zang L, Shimada Y, Nishimura N. Development of a Novel Zebrafish Model for Type 2 Diabetes Mellitus. Scientific Reports. 2017;7.

**[17]** Connaughton VP, Baker C, Fonde L, Gerardi E, Slack C. Alternate Immersion in an External Glucose Solution Differentially Affects Blood Sugar Values in Older Versus Younger Zebrafish Adults. Zebrafish. 2016; 13:87–94.

**[18]** Gleeson M, Connaughton V, Arneson LS. Induction of hyperglycaemia in zebrafish (Danio rerio) leads to morphological changes in the retina. Acta Diabetologica. 2007; 44:157–163.

**[19]** Yang Q, Yan C, Wang X, Gong Z. Leptin induces muscle wasting in kras-driven hepatocellular carcinoma (HCC) model in zebrafish. Disease Models & Mechanisms. 2019.

**[20]** Eames SC, Philipson LH, Prince VE, Kinkel MD. Blood Sugar Measurement in Zebrafish Reveals Dynamics of Glucose Homeostasis. Zebrafish. 2010; 7:205–213

**[21]** Michel M, Page-McCaw PS, Chen W, Cone RD. Leptin signaling regulates glucose homeostasis, but not adipostasis, in the zebrafish. Proceedings of the National Academy of Sciences. 2016; 113:3084–3089.

**[22]** Kimmel RA, Dobler S, Schmitner N, Walsen T, Freudenblum J, Meyer D. Diabetic pdx1-mutant zebrafish show conserved responses to nutrient overload and

anti-glycemic treatment. Scientific Reports. 2015;5

**[23]** Olsen AS, Sarras MP, Leontovich A, Intine RV. Heritable Transmission of Diabetic Metabolic Memory in Zebrafish Correlates with DNA Hypomethylation and Aberrant Gene Expression. Diabetes. 2012; 61:485–491.

**[24]** Fang L, Miller YI. Emerging applications for zebrafish as a model organism to study oxidative mechanisms and their roles in inflammation and vascular accumulation of oxidized lipids. Free Radical Biology and Medicine. 2012; 53:1411–1420

**[25]** Miyares RL, de Rezende VB, Farber SA. Zebrafish yolk lipid processing: a tractable tool for the study of vertebrate lipid transport and metabolism. Disease Models & Mechanisms. 2014.

**[26]** Ferrari JT, Ayres R, Hammes TO, Silveira TRD, Uribe-Cruz C. Experimental model of hepatic steatosis by fructose in adult zebrafish: A pilot study. Clinical & Biomedical Research. 2018; 38:151–154.

**[27]** Roosen-runge ec. On the early development—bipolar differentiation and cleavage—of the zebra fish, brachydanio rerio. The Biological Bulletin. 1938; 75:119–133

**[28]** Ferrari JT, Ayres R, Hammes TO, Silveira TRD, Uribe-Cruz C. Experimental model of hepatic steatosis by fructose in adult zebrafish: A pilot study. Clinical & Biomedical Research. 2018; 38:151–154.

**[29]** Yang Q, Yan C, Wang X, Gong Z. Leptin induces muscle wasting in kras-driven hepatocellular carcinoma (HCC) model in zebrafish. Disease Models & Mechanisms. 2019.

**[30]** Passeri MJ, Cinaroglu A, Gao C, Sadler KC. Hepatic steatosis in response to acute alcohol exposure in zebrafish requires sterol regulatory element binding protein activation. Hepatology. 2008; 49:443–452.

**[31]** Shimada Y, Kuninaga S, Ariyoshi M, Zhang B, Shiina Y, Takahashi Y, Umemoto N, Nishimura Y, Enari H, Tanaka T. E2F8 promotes hepatic steatosis through FABP3 expression in diet-induced obesity in zebrafish. Nutrition & Metabolism. 2015;12.

**[32]** Imran M, Sergent O, Tête A, Gallais I, Chevanne M, Lagadic-Gossmann D, Podechard N.

Membrane Remodeling as a Key Player of the Hepatotoxicity Induced by Co-Exposure to Benzo[a]pyrene and Ethanol of Obese Zebrafish Larvae. Biomolecules. 2018; 8:26.

**[33]** Brugman S. The zebrafish as a model to study intestinal inflammation. Developmental & Comparative Immunology. 2016; 64:82–92. doi: 10.1016/j.dci.2016.02.020.

**[34]** Ji J, Thwaite R, Roher N. Oral Intubation of Adult Zebrafish: A Model for Evaluating Intestinal Uptake of Bioactive Compounds. Journal of Visualized Experiments. 2018.

**[35]** Arias-Jayo N, Abecia L, Alonso-Sáez L, Ramirez-Garcia A, Rodriguez A, Pardo MA. High-Fat Diet Consumption Induces Microbiota Dysbiosis and Intestinal Inflammation in Zebrafish. Microbial Ecology. 2018; 76:1089–1101

**[36]** Oehlers SH, Flores MV, Okuda KS, Hall CJ, Crosier KE, Crosier PS. A chemical enterocolitis model in zebrafish larvae that is dependent on microbiota and responsive to pharmacological agents. Developmental Dynamics. 2010; 240:288–298.

**[37]** Koch BEV, Yang S, Lamers G, Stougaard J, Spaink HP. Intestinal microbiome adjusts the innate immune setpoint during colonization through negative regulation of MyD88. Nature Communications. 2018;9.

**[38]** Semova I, Carten J, Stombaugh J, Mackey L, Knight R, Farber S, Rawls J. Microbiota Regulate Intestinal Absorption and Metabolism of Fatty Acids in the Zebrafish. Cell Host & Microbe. 2012; 12:277–288.

**[39]** Valenzuela MJ, Caruffo M, Herrera Y, Medina DA, Coronado M, Feijóo CG, Muñoz S, Garrido D, Troncoso M, Figueroa G, *et al*. Evaluating the Capacity of Human Gut Microorganisms to Colonize the Zebrafish Larvae (Danio rerio). Frontiers in Microbiology. 2018;9.

**[40]** Bates JM, Akerlund J, Mittge E, Guillemin K. Intestinal Alkaline Phosphatase Detoxifies Lipopolysaccharide and Prevents Inflammation in Zebrafish in Response to the Gut Microbiota. Cell Host & Microbe. 2007; 2:371–382.

**[41]** Kari G, Rodeck U, Dicker AP. Zebrafish: An Emerging Model System for Human Disease and Drug Discovery. Clinical

Pharmacology & Therapeutics. 2007; 82:70–80.

**[42]** Kari G, Rodeck U, Dicker AP. Zebrafish: An Emerging Model System for Human Disease and Drug Discovery. Clinical Pharmacology & Therapeutics. 2007; 82:70–80.

**[43]** Zhang C, Willett C, Fremgen T. Zebrafish: An Animal Model for Toxicological Studies. Current Protocols in Toxicology. 2003;17.

**[44]** Daroczi B, Kari G, McAleer MF, Wolf JC, Rodeck U, Dicker AP. In vivo Radioprotection by the Fullerene Nanoparticle DF-1 as Assessed in a Zebrafish Model. Clinical Cancer Research. 2006; 12:7086–7091.

**[45]** Langheinrich U, Hennen E, Stott G, Vacun G. Zebrafish as a Model Organism for the Identification and Characterization of Drugs and Genes Affecting p53 Signaling. Current Biology. 2002; 12:2023–2028.

**[46]** Dicker A, McAleer M, Santana E, Farber S, Rodeck U. Novel use of zebrafish as a vertebrate model to screen radiation protectors and sensitizers. International Journal of Radiation Oncology* Biology* Physics. 2004; 60: S348–S349.

**[47]** Ton C, Parng C. The use of zebrafish for assessing ototoxic and otoprotective agents. Hearing Research. 2005; 208:79–88.

**[48]** Wu X, Zhong H, Song J, Damoiseaux R, Yang Z, Lin S. Mycophenolic Acid Is a Potent Inhibitor of Angiogenesis. Arteriosclerosis, Thrombosis, and Vascular Biology. 2006; 26:2414–2416.

**[49]** Handen JS. The industrialization of drug discovery. Drug Discovery Today. 2002; 7:83–85.

**[50]** Fillit H, Refolo L. Introduction: Advancing Drug Discovery for Alzheimers Disease. Current Alzheimer Research. 2005; 2:105–107.

**[51]** Lindsay MA. Target discovery. Nature Reviews Drug Discovery. 2003; 2:831–838.

**[52]** SAMSDODD F. Target-based drug discovery: is something wrong? Drug Discovery Today. 2005; 10:139–147.