Review Article

# Zebrafish as a Fascinating Animal Model: A Robust Platform for *in vivo* Screening for Biomedical Research

**Manish Kumar Gautam[1], Pritish Kumar Panda[2], Anubhav Dubey[3,4], Mamta Kumari[4], Niladry Sekhar Ghosh[5,*]**

[1]Department of Pharmacology, Faculty of Pharmaceutical Science, Assam Down Town University, Guwahati, Assam, INDIA.
[2]Department of Pharmaceutics, Faculty of Pharmaceutical Science, Assam Down Town University, Guwahati, Assam, INDIA.
[3]Department of Pharmacology, Adarsh Vijendra Institute of Pharmaceutical Sciences, Shobhit University Gangoh, Saharanpur, Uttar Pradesh, INDIA.
[4]Department of Pharmacology, Maharana Pratap College of Pharmacy, Kanpur, Uttar Pradesh, INDIA.
[5]Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Science, Assam Down Town University, Guwahati, Assam, INDIA.

## ABSTRACT

**Background:** Zebrafish have emerged as an invaluable asset in biomedical research, particularly in comparison to other vertebrate models employed for studying human ailments. Their efficacy in large-scale studies of genetic variations and drug testing has been well-established. Recent advancements in CRISPR and next-generation sequencing have further boosted zebrafish-based disease modeling, enhancing our comprehension of the biological underpinnings of hereditary human diseases. Such efforts are critical for developing targeted therapies that offer innovative diagnostic and treatment options. **Materials and Methods:** We extensively examined original articles and papers from prominent databases such as PubMed, SCOPUS, Science Direct, and PubMed Central. This analysis included a comprehensive review of the articles, their citations, and cross-references. To identify relevant articles, we employed a variety of keywords, including but not limited to: Zebrafish applications, toxicity studies involving Zebrafish, CRISPR technology applications in Zebrafish research, substances utilized in developmental biology studies with Zebrafish, and various models employed in Zebrafish research. **Results:** In the medical field, zebrafish research has made strides in various areas, including developmental disorders, mental illnesses, and metabolic diseases. A growing trend involves studying an expanding spectrum of risk genes linked to neurological disorders using zebrafish as a model system. Zebrafish possess unique traits that make them an ideal choice for this purpose. For instance, their active partner growth of translucent embryos allows direct observation of the developing brain during early stages, providing valuable insights into developmental processes. Additionally, the large number of progenies zebrafish produce significantly enhances controllability, making them highly effective for therapeutic testing aimed at identifying specific molecular effectors of simple behavioral genetic traits. **Conclusion:**The exceptional attributes of zebrafish make them a potent and versatile tool for medical research. Their contributions have been instrumental in advancing our understanding of human diseases and developing potential treatments. As a result, zebrafish continue to play a pivotal role in biomedical research, propelling progress in personalized medicine and the pursuit of improved treatments for a wide range of health conditions.

**Keywords:** Human hereditary diseases, Developmental disorders, Next sequencing, Zebra fish.

**Correspondence:**
**Dr. Niladry Sekhar Ghosh**
Faculty of Pharmaceutical Science, Assam down town University, Guwahati-781026, Assam, INDIA.
Email: niladry_chem@yahoo.co.in
ORCID: 0000-0003-0099-5231

**Received:** 26-07-2023;
**Revised:** 05-09-2023;
**Accepted:** 28-02-2024.

## INTRODUCTION

Zebrafish possess substantial advantages over other vertebrate models when utilized in extensive genetic mutant and therapeutic compound screenings, as well as various biomedical research applications.[1] The utilization of zebrafish for disease modeling is revolutionizing our understanding of the molecular basis of human genetic illnesses, particularly in light of advancements in Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and next-generation sequencing technologies.[2] These initiatives play a pivotal role in shaping the future of precision medicine, offering innovative medical and diagnostic possibilities. Zebrafish serve as a powerful tool in accelerating our knowledge of human diseases at the molecular level, enabling researchers to gain invaluable insights into the complex genetic underpinnings of various illnesses. This, in turn, holds immense promise for the development of targeted and personalized therapeutic approaches, revolutionizing the field of medicine and patient care in the years to come.[3]

In the 1980s, Streisinger and other prominent researchers in the field introduced Mouse models for genetics research, marking a

**DOI:** 10.5530/ijpi.14.3.80

significant milestone in scientific exploration. However, recent advancements in the Tol2-based transgenic system within zebrafish have unlocked new possibilities for gene expression regulation in specific locations.[4] By incorporating it with other systems like GAL4/UAS or Cre/LoxP, researchers can delve deeper into understanding the impact of gene mutations on neurodevelopment, particularly in the central nervous system of zebrafish.[5] The modern tissue-clearing techniques have revolutionized the study of neural networks in the adult zebrafish brain, providing valuable insights into their complex structure and function.[6] As a result, zebrafish have emerged as a valuable model for investigating human developmental diseases, enabling researchers to explore their phenotypic characteristics through advanced molecular technologies and procedures.[7] This versatile aquatic organism has become a cornerstone in scientific research as an animal model for developmental disorders, mental diseases, and central nervous system studies.[8] The integration of zebrafish into genetics research and its cutting-edge applications in molecular technology have propelled scientific discoveries to new heights.[9,10] From exploring neurodevelopment to unraveling the intricacies of neural networks, zebrafish play a pivotal role in advancing our knowledge of human diseases and developmental processes. As a model organism, they continue to hold immense promise for further advancements in developmental biology, mental health research, and the study of the central nervous system. Additionally, their relevance in elucidating metabolic functions and cellular processes further solidifies their position as an indispensable tool in scientific exploration across various disciplines.

This review offers a comprehensive overview of the multifaceted role of zebrafish as a disease model in various biomedical applications, with a specific focus on developmental, psychological, and metabolic disorders. By harnessing the unique attributes of zebrafish, researchers have unlocked valuable insights into the molecular mechanisms underlying these complex diseases, contributing to advancements in the fields of medicine and biology.

## Zebra Fish Features in General

The Zebrafish (Danio rerio) has been an invaluable model organism in scientific research since the 1960s, offering numerous advantages for studying human genetics and diseases.[3] As a freshwater fish belonging to the minnow family, the zebrafish hails originally from South Asia and has gained popularity in the aquarium trade. With a length ranging from 2.5 to 4 cm, zebrafish exhibit a striking appearance, featuring vertical blue stripes during their adult stage. Male zebrafish typically showcase a pink or yellow coloration and possess a slender, torpedo-like shape, while females are often larger and less pink due to the eggs they carry.[11] Zebrafish's unique characteristics and genetic makeup have proven to be of great value in diverse research fields, including progressive neurodegenerative studies and investigations into developmental and disease processes, such as cancer. With a genome comprising 26,247 protein-coding genes across its 1,505,581,940 base pairs, zebrafish provide a rich resource for understanding complex biological mechanisms.[12] The zebrafish life cycle offers researchers a dynamic and well-characterized model system, enabling the investigation of various phenomena throughout its development. As the zebrafish develops, its transparent nature during the larval stages facilitates direct observation, providing insights into vital processes and responses to various environmental factors. Furthermore, zebrafish have played an instrumental role in the study of angiogenesis, nephrotoxicology, and persistent coronary syndrome, enhancing our understanding of these important aspects of human health.[13] The evolution of the brain's "foregut" has been a captivating area of exploration using zebrafish as a model organism. This research has shed light on the complexities of brain development and the mechanisms involved in neurogenesis, contributing to broader insights into human brain function and disorders.[14] In the context of oncology, zebrafish have proven to be particularly valuable preclinical models. The ability to study gene expression in specific locations through transgenic approaches has opened doors to understanding the impact of gene mutations on neurodevelopment, especially in the central nervous system of zebrafish. Modern tissue-clearing techniques have further enriched our understanding of neural networks in adult zebrafish brains, providing a glimpse into the intricacies of brain architecture.[14,15] Overall, the zebrafish's performance as a versatile and powerful model organism has accelerated scientific progress in diverse biomedical applications. As we continue to delve into the mysteries of genetics and diseases, the zebrafish will undoubtedly remain a cornerstone of scientific research, offering an invaluable tool to unlock new knowledge and therapeutic avenues.

## Development of the Zebra Fish

During zebrafish development, a solitary zygote attaches itself to the top of a large yolk cell, initiating rapid growth. Approximately 6 hr after implantation, the initial indications of gastrulation become evident, and by the second day, the eggs hatch, giving rise to free-swimming larvae. Zebrafish have a relatively long lifespan of up to 5 years, and they attain sexual maturity at around 3 months of age.[16]

## Development of the Enteric Nervous System

Recent advancements in the zebrafish embryo model have significantly contributed to the understanding of the formation of the Enteric Nervous System (ENS). The digestive systems of various vertebrate species, including zebrafish (Danio rerio), are intricate structures comprising diverse cell types, such as epithelial, muscular, vascular, and neural cells.[16] Zebrafish, belonging to the cyprinid family, exhibit remarkable phenotypic plasticity and are the largest among schooling fishes. Stress distribution and stress response have been successfully employed to produce homozygosity in diploid zebrafish, yielding valuable results that have assisted in the genetic analysis of zebrafish. Multiple mutations have been identified, and the genes responsible for these mutations, along with their functions in zebrafish embryogenesis, have been elucidated. These findings have laid a robust foundation for future research utilizing zebrafish as a model organism for studying growth processes.[17] Zebrafish possess several advantageous traits that make them an excellent model species. Their small size, *ex utero* development of embryos, short reproductive cycles, and transparency of embryos provide numerous benefits for researchers. Moreover, zebrafish share a significant amount of genetic similarity with human beings, making them even more relevant as a research tool in various fields, including development, environmental toxicology, pharmacology, and DNA damage repair. As a result, zebrafish has become an invaluable asset in advancing scientific knowledge in these areas.[18,19]

## Genetics and early development of Zebrafish

Researchers in an increasing number of labs are now focusing on examining the genes and development of zebrafish. Despite the existence of numerous creatures already extensively studied by researchers, the significance of studying zebrafish development has risen. The current understanding of the zebrafish embryo and genome remains somewhat limited, making it an area of interest for further investigation.While the zebrafish embryo shares many similarities with embryos of other vertebrates in several aspects, its simplicity and exceptional accessibility for cellular and genetic analysis make it a preferred subject of study. The ease of studying zebrafish development sets it apart from other vertebrates, making it a valuable model organism for research.Interestingly, despite the apparent distinctiveness of fish development, researchers have observed numerous similarities between fish and other vertebrates. These similarities seem to be more fundamental and cryptic than the differences, challenging conventional expectations. Recent discoveries in zebrafish research have complemented and enhanced earlier studies conducted on tetrapods, including frogs, chickens, and mice. These parallels provide compelling evidence for the conservation of fundamental processes, particularly the genes that regulate and organize vertebrate development. Study of zebrafish development has gained significance due to its accessibility and simplicity for analysis. Moreover, the discoveries in zebrafish research have shed light on the conservation of essential developmental processes across vertebrates, enriching our understanding of embryogenesis in various species.

## Medical and Science Studies

Psychological disorders, also known as mental abnormalities, are characterized by disrupted thought processes or behaviors that cause severe emotional distress to the individual. The World Health Organization's International Classification of Diseases (ICD) is the gold standard in psychiatry for diagnosing various disorders of the mind, with over 450 differential diagnoses included.[20] Zebrafish, known for their herding and breeding activities, serve as a valuable model for studying the impact of dysfunction on mental well-being. Researchers, led by Kim and colleagues, identified a novel chemo-kine-like gene family called samdori (sam) through mutation screening, which was found to be involved in mental problems in zebrafish.[21] One of the siblings, Sam2, shares the same risk factors for intellectual disability and autism spectrum disorder. Sam2 is specifically expressed in the brain's distribution nuclei.[22] Interestingly, Sam2-knockout animals, including zebrafish and mice, exhibit defects in emotional states, particularly fear and anxiety, indicating the importance of this gene in emotional regulation.[23,24] The intricate communication between the brain and other organs involves complex interactions of the Hypothalamic-Pituitary-Gonadal axis (HPG axis) during human puberty.[25] The HPG axis plays a crucial role in the growth and regulation of various bodily systems, including reproduction. The hypothalamic hormone Gonadotropin-Releasing Hormone (GnRH) is secreted and travels to the anterior pituitary hypophyseal portal system, where it binds to receptors on the secretory cells of the adenohypophysis.[11] In response to GnRH, these cells release luteinizing hormone and follicle-stimulating hormone into the bloodstream. Consequently, adolescence is a period of significant development during which an individual body undergoes changes to become capable of sexual reproduction. Understanding these interactions is vital for comprehending the complex processes of human growth and development.[26] The zebrafish models and studies on the HPG axis provide valuable insights into the mechanisms underlying mental disorders and the intricate interactions between the brain and other bodily systems during key developmental stages, such as puberty.

## Novel Substances for Study in Developmental Biology, Biology, and Medicine

Zebrafish offer valuable insights into heart development for several compelling reasons. Firstly, their heart is fully developed within just 48 hr after fertilization, despite its initially rudimentary appearance (hpf). This rapid cardiac development provides researchers with an efficient model to study the intricate processes involved in heart formation.[27] Secondly, the availability of a transgenic line with an FP-tagged heart allows scientists to observe cardiac development in real-time. This real-time visualization enables the detailed tracking of changes and interactions within the developing heart, facilitating a deeper understanding of its development.[5,27] Furthermore, zebrafish embryos possess a remarkable ability to adapt and continue developing normally even when their circulatory system is damaged. This adaptability is attributed to their capability to extract oxygen from the water via diffusion, which sustains their growth and development under adverse conditions.Moreover, zebrafish provide a simple and effective genetic mutant approach for identifying genes involved in cardiac development. One noteworthy study conducted by Walsh and Stainier focused on the zebrafish jekyll mutant, which exhibited faulty heart valves. Through this approach, they discovered the crucial role of glucose dehydrogenase in the growth of functional cardiac valves in zebrafish embryos.[28] The zebrafish model presents unique advantages for studying heart development, including its rapid and observable cardiac development, adaptability to circulatory system damage, and the simplicity of genetic mutant studies. By investigating heart development in zebrafish, researchers gain valuable insights into the fundamental processes of cardiac formation that have broader implications for understanding heart development in humans and other vertebrates.

The assessment of regulatory cis-elements in muscle development becomes more straightforward through *in vivo* transient evaluation of injected DNA fragments in model fish embryos. Among the Myogenesis Regulatory Factors (MRFs), Myf5 plays a vital role in promoting the identification and development of muscle stem cells. The transcription of Myf5 is regulated in a highly controlled manner, dependent on the developmental stage and somites. Chen *et al*. conducted an *in vivo* transient experiment and unveiled a novel cis element at 82/-62 that is crucial for specific transcriptional activity in somites.[29]

## RESEARCH INTO ORGANELLE BIOLOGY INVOLVING ZEBRAFISH

Metabolic processes in the body are regulated by various organelles, such as the Endoplasmic Reticulum (ER), mitochondria, peroxisomes, lipid droplets, and liposomes. Each cell's metabolic function contributes to the overall metabolic activity of the body, referred to as "whole-body metabolism." The successful functionality of intracellular organelles is crucial for metabolic regulation, allowing cells to respond to environmental changes and control metabolic outputs.[30] Zebrafish has emerged as a valuable model system for studying *in vivo* toxicity related to mitochondrial function due to the metabolic alterations observed after drug treatments in various studies. Recently, there has been significant interest in utilizing Zebrafish models for evaluating the effects of bioactive chemicals, including those that influence development, disease conditions, and human health, as well as anticancer treatments.[31,32] Additionally, Zebrafish has been increasingly used as an *in vivo* model for investigating gene functions related to metabolism. The CRISPR/Cas9 technique, a cutting-edge tool in reverse genetics, has been effectively employed in mechanistic studies exploring the regulation, biogenesis, degradation, and improvement of specific organelles in Zebrafish models.[29,33] This ongoing research is shedding light on metabolic processes and providing valuable insights into how organelles play essential roles in overall metabolic regulation.

## DISCUSSION

Zebrafish, as vertebrates, play a pivotal role in advancing scientific research, especially in the realm of in-depth cellular investigations of embryogenesis and genetic studies. The molecular characterization of genes involved in development has emerged as a prominent area of focus in scientific inquiry. To achieve a comprehensive understanding of the intricacies of developmental processes, it is imperative to conduct examinations and manipulations of embryos, genes, and molecules within the same organism. The groundbreaking contributions of researchers like Christi and Niisslein-Volhard, along with their colleagues, have demonstrated the significance of studying developmental processes in a single organism, as seen in their brilliant work on unraveling the establishment of the body axes in Drosophila. Such comprehensive studies pave the way for profound insights into the underlying mechanisms of embryonic development and genetic regulation, advancing our knowledge of fundamental biological processes.

In the realm of model organisms, fruit flies and nematode worms like *C. elegans* have been instrumental in unraveling crucial aspects of animal evolution. However, vertebrates, particularly zebrafish, possess a wealth of their own secrets waiting to be discovered. What sets zebrafish apart is the striking similarity in early developmental processes and the underlying molecular signals that govern them when compared to distantly related vertebrates, including frogs, chickens, and mice. These remarkable parallels offer a unique opportunity to delve into the evolutionary processes that underpin not only zebrafish but also our own development as higher organisms. Zebrafish, with their diverse biological and genetic characteristics, serve as an alluring and straightforward paradigm for studying the intricate web of evolutionary changes that have shaped complex life forms. By examining the molecular pathways and regulatory networks shared among these vertebrates, we can gain profound insights into the fundamental mechanisms that have driven the evolution of life on Earth. Thus, zebrafish's significance extends beyond its role as a model organism; it emerges as a gateway to unraveling the profound mysteries of evolutionary biology and our place in the grand tapestry of life.

The scientific community's fascination with zebrafish stems from its unique potential to offer profound insights into the core aspects of embryogenesis and genetic regulation. By unraveling the shared mechanisms between zebrafish and other vertebrates, researchers can unlock the secrets that underlie the fundamental processes governing life's development. This intriguing species acts as a crucial bridge, bridging the knowledge gap between invertebrate models and mammals, providing a clearer and more comprehensive understanding of vertebrate development as a whole.

Zebrafish's remarkable significance lies in its ability to serve as a versatile and dynamic model organism, offering a window into the intricate workings of vertebrate embryogenesis and genetic regulation. Its genetic similarity to other vertebrates, including humans, paves the way for vital comparative studies that shed light on our shared evolutionary history. By probing the intricacies of zebrafish development, researchers can make valuable connections to the mechanisms at play in more complex organisms, ultimately enhancing our comprehension of human embryogenesis and developmental biology.In the pursuit of unlocking nature's secrets, zebrafish plays an integral role in understanding the evolution of vertebrates, including humans. This fascinating species allows researchers to explore the ancient genetic pathways that have been conserved across species, enabling us to comprehend the evolutionary steps that have shaped life on our planet. The study of zebrafish continues to pave the way for groundbreaking discoveries, as it serves as an indispensable model organism for investigations into the intricate interplay of genes, cellular processes, and developmental outcomes.

As scientists delve deeper into the zebrafish's genetic landscape, they uncover vital clues that offer a glimpse into the genetic blueprints governing vertebrate development. Its ability to rapidly develop, produce large numbers of offspring, and its optical transparency during early stages of development make it a powerful tool for observing embryonic processes in real-time. Moreover, the ease of genetic manipulation in zebrafish allows for targeted investigations, giving researchers unprecedented control over experimental conditions. Zebrafish stands as a vital and versatile ally in the pursuit of understanding vertebrate embryogenesis and genetic regulation. By bridging the knowledge gap between invertebrates and mammals, this remarkable model organism offers an unparalleled opportunity to unravel the mysteries of developmental biology and its profound implications for human evolution. As research in this field advances, zebrafish's influence as a powerful model organism is set to expand, continuing to unlock the intricate complexities of vertebrate development and evolutionary history.

## CONCLUSION

In recent years, preclinical studies utilizing zebrafish models have yielded highly promising and encouraging findings. The scientific community has witnessed a remarkable surge in the utilization of zebrafish models in research, with a staggering threefold increase in their appearance in journal articles over the past decade. Zebrafish has risen to prominence as a preferred and highly effective model system, gradually replacing conventional animal toxicity studies. This transition to zebrafish models not only contributes to the refinement of research practices but also aligns with ethical considerations, reducing the number of animals required for toxicology studies while effectively assessing drug toxicity.

Beyond its application in toxicology, zebrafish models hold immense potential for diverse disease research areas, spanning from cancer to cardiovascular disease, diabetes, obesity, neurology, xenotransplantation, ocular, otologic, and renal disorders. Researchers have come to rely on zebrafish as a valuable tool to explore novel targets for the treatment of various diseases, presenting unparalleled opportunities for drug development and precision medicine.One of the most significant strengths of zebrafish lies in its ability to combine the advantages of both *in vivo* and *in vitro* experiments. This unique characteristic grants researchers the cost-effectiveness and ease of maintenance associated with *in vivo* studies, while also providing the convenience and versatility of *in vitro* and *in vivo* investigations. Zebrafish's exceptional qualities as a model organism make it a compelling alternative to traditional mammalian models, potentially revolutionizing research practices in diverse fields of study. Despite the promising trajectory of zebrafish as a model system, it is essential to validate its reliability further as a bioindicator. Rigorous and comprehensive studies will be required to substantiate zebrafish's standing as an invaluable and effective model organism. These studies will undoubtedly undergo rigorous review processes to establish zebrafish's credibility as a versatile and powerful tool in the scientific community.

As scientific research continues to advance, zebrafish's impact on driving discoveries in medical and biological fields is poised to grow exponentially. Its unique characteristics and genetic similarities to humans offer unparalleled insights into complex biological processes, facilitating groundbreaking discoveries and innovative therapeutic approaches. With its undeniable potential and growing relevance, zebrafish stands as an indispensable ally in the pursuit of scientific advancements and betterment of human health.

## ACKNOWLEDGEMENT

We are thankful to Prof (Dr.) Ranjit Singh Vice-Chancellor Shobhit University, Gangoh, Saharanpur, Uttar Pradesh, for their kindly support for my work. We are grateful to the technical staff members of the Department of Pharmaceutical Sciences.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**CRISPR:** Clustered Regularly Interspaced Short Palindromic Repeats; **Cas9:** CRISPR-associated protein 9; **ICD:** International Classification of Diseases; **HPG axis:** Hypothalamic-Pituitary-Gonadal axis; **GnRH:** Gonadotropin-Releasing Hormone; **FP-tagged:** Fluorescent protein-tagged; **MRFs:** Myogenesis

Regulatory Factors; **Myf5:** Myogenic Factor 5; **ER:** Endoplasmic Reticulum.

## REFERENCES

1. Blomme EA, Will Y. Toxicology strategies for drug discovery: present and future. Chem Res Toxicol. 2016;29(4):473-504. doi: 10.1021/acs.chemrestox.5b00407, PMID 26588328.
2. Hay M, Thomas DW, Craighead JL, Economides C, Rosenthal J. Clinical development success rates for investigational drugs. Nat Biotechnol. 2014;32(1):40-51. doi: 10.103 8/nbt.2786, PMID 24406927.
3. Santoriello C, Zon LI. Hooked! Modeling human disease in zebrafish. J Clin Invest. 2012;122(7):2337-43. doi: 10.1172/JCI60434, PMID 22751109.
4. Gore AV, Monzo K, Cha YR, Pan W, Weinstein BM. Vascular development in the zebrafish. Cold Spring Harb Perspect Med. 2012; 2(5):a006684. doi: 10.1101/cshper spect.a006684, PMID 22553495.
5. Kanungo J, Cuevas E, Ali SF, Paule MG. Zebrafish model in drug safety assessment. Curr Pharm Des. 2014;20(34):5416-29. doi: 10.2174/1381612820666140205145658, PMID 24502596.
6. Kalueff AV, Stewart AM, Gerlai R. Zebrafish as an emerging model for studying complex brain disorders. Trends Pharmacol Sci. 2014;35(2):63-75. doi: 10.1016/j.tip s.2013.12.002, PMID 24412421.
7. Guyon JR, Steffen LS, Howell MH, Pusack TJ, Lawrence C, Kunkel LM. Modeling human muscle disease in zebrafish. Biochim Biophys Acta. 2007;1772(2):205-15. doi: 10.101 6/j.bbadis.2006.07.003, PMID 16934958.
8. Weinstein B. Vascular cell biology *in vivo*: a new piscine paradigm?Trends Cell Biol. 2002;12(9):439-45. doi: 10.1016/s0962-8924(02)02358-9, PMID 12220865.
9. Lieschke GJ, Oates AC, Crowhurst MO, Ward AC, Layton JE. Morphologic and functional characterization of granulocytes and macrophages in embryonic and adult zebrafish. Blood. 2001;98(10):3087-96. doi: 10.1182/blood.v98.10.3087, PMID 11698295.
10. Zhao S, Huang J, Ye J. A fresh look at zebrafish from the perspective of cancer research. J Exp Clin Cancer Res.2015;34(1): 80. doi: 10.1186/s13046-015-0196-8, PMID 26260237.
11. Hill AJ, Teraoka H, Heideman W, Peterson RE. Zebrafish as a model vertebrate for investigating chemical toxicity. Toxicol Sci. 2005;86(1):6-19. doi: 10.1093/toxsci/kfi1 10, PMID 15703261.
12. Dubey A, Yadav P, Peeyush VP, Kumar R. Investigation of proapoptotic potential of Ipomoea carnea Leaf extract on breast cancer cell line. J Drug Deliv Ther. 2022;12(1):51-5. doi: 10.22270/jddt.v12i1.5172.
13. Lele Z, Krone PH. The zebrafish as a model system in developmental, toxicological and transgenic research. Biotechnol Adv. 1996;14(1):57-72. doi: 10.1016/0734-9750 (96)00004-3, PMID 14536924.
14. Peterson RT, Nass R, Boyd WA, Freedman JH, Dong K, Narahashi T. Use of non-mammalian alternative models for neurotoxicological study. Neurotoxicology. 2008;29(3):546-55. doi: 10.1016/j.neuro.2008.04.006, PMID 18538410.
15. Caballero MV, Candiracci M. Zebrafish as screening model for detecting toxicity and drugs efficacy. J Unexplored Data. 2018;3(4):3-4. doi: 10.20517/2572-8180.2017.15.
16. Stanton MF. Diethylnitrosamine-induced hepatic degeneration and neoplasia in the aquarium fish, Brachydanio rerio. J Natl Cancer Inst. 1965;34:117-30. doi: 10.1093/jn ci/34.1.117, PMID 14287275.
17. Zheng W, Li Z, Nguyen AT, Li C, Emelyanov A, Gong Z. Xmrk, kras and myc transgenic zebrafish liver cancer models share molecular signatures with subsets of human hepatocellular carcinoma. PLOS ONE. 2014;9(3):e91179. doi: 10.1371/journal.pone. 0091179, PMID 24633177.
18. Wallace KN, Akhter S, Smith EM, Lorent K, Pack M. Intestinal growth and differentiation in zebrafish. Mech Dev. 2005;122(2):157-73. doi: 10.1016/j.mod.2004.10.009, PMID 15652704.
19. Holmberg A, Olsson C, Hennig GW. TTX-sensitive and TTX-insensitive control of spontaneous gut motility in the developing zebrafish (Danio rerio) larvae. J Exp Biol. 2007;210(6):1084-91. doi: 10.1242/jeb.000935, PMID 17337720.
20. Charlton H. Hypothalamic control of anterior pituitary function: a history. J Neuroendocrinol. 2008;20(6):641-6. doi: 10.1111/j.1365-2826.2008.01718.x, PMID 18601683.
21. Vadakkadath Meethal S, Atwood CS. The role of hypothalamic-pituitary-gonadal hormones in the normal structure and functioning of the brain. Cell Mol Life Sci. 2005;62(3):257-70. doi: 10.1007/s00018-004-4381-3, PMID 15723162.
22. Boehm U, Bouloux PM, Dattani MT, de Roux N, Dodé C, Dunkel L, *et al*. Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism--pathogenesis, diagnosis and treatment. Nat Rev Endocrinol. 2015;11(9):547-64. doi: 10.1038/nrendo.2015.112, PMID 26194704.
23. Kim HG, Ahn JW, Kurth I, Ullmann R, Kim HT, Kulharya A, *et al*. WDR11, a WD protein that interacts with transcription factor EMX1, is mutated in idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. Am J Hum Genet. 2010;87(4):465-79. doi: 1 0.1016/j.ajhg.2010.08.018, PMID 20887964.
24. Kyritsis N, Kizil C, Zocher S, Kroehne V, Kaslin J, Freudenreich D, *et al*. Acute inflammation initiates the regenerative response in the adult zebrafish brain. Science. 2012;338(6112):1353-6. doi: 10.1126/science.1228773, PMID 23138980.
25. Peterman EM, Sullivan C, Goody MF, Rodriguez-Nunez I, Yoder JA, Kim CH. Neutralization of mitochondrial superoxide by superoxide dismutase 2 promotes bacterial clearance and regulates phagocyte numbers in zebrafish. Infect Immun. 2015;83(1):430-40. doi: 10.1128/IAI.02245-14, PMID 25385799.
26. Basu S, Sachidanandan C.Zebrafish: a multifaceted tool for chemical biologists. Chem Rev. 2013;113(10):7952-80. doi: 10.1021/cr4000013, PMID 23819893.
27. Parng C. *In vivo* zebrafish assays for toxicity testing. Curr Opin Drug Discov Devel. 2005;8(1):100-6. PMID 15679177.
28. Choi TY, Choi TI, Lee YR, Choe SK, Kim CH. Zebrafish as an animal model for biomedical research. Exp Mol Med. 2021;53(3):310-7. doi: 10.1038/s12276-021-00571-5, PMID 33649498.
29. Chen Z, Berquez M, Luciani A. Mitochondria, mitophagy, and metabolic disease: towards assembling the puzzle. Cell Stress. 2020;4(6):147-50. doi: 10.15698/cst202 0.06.222, PMID 32548571.
30. McGrath P, Seng WL. Use of zebrafish apoptosis assays for preclinical drug discovery. Expert Opin Drug Discov. 2013;8(10):1191-202. doi: 10.1517/17460441.2013.825244 , PMID 23964640.
31. Baxendale S, van Eeden F, Wilkinson R. The power of zebrafish in personalised medicine. Adv Exp Med Biol. 2017;1007:179-97. doi: 10.1007/978-3-319-60733-7_1 0, PMID 28840558.
32. Horzmann KA, Freeman JL. Making waves: new developments in toxicology with the zebrafish. Toxicol Sci. 2018;163(1):5-12. doi: 10.1093/toxsci/kfy044, PMID 29471431.
33. Yoganantharjah P, Gibert Y. The use of the zebrafish model to aid in drug discovery and target validation. Curr Top Med Chem. 2017;17(18):2041-55. doi: 10.2174/15680 26617666170130112109, PMID 28137236.

**Cite this article:** Gautam MK, Panda PK, Dubey A, Kumari M, Ghosh NS. Zebrafish as a Fascinating Animal Model: A Robust Platform for *in vivo* Screening for Biomedical Research. Int. J. Pharm. Investigation. 2024;14(3):696-701.