

# Zebrafish as An Emerging Model: An Important Testing Platform for Biomedical Science

Anubhav Dubey<sup>1</sup>, Niladry S. Ghosh<sup>2\*</sup>, Dr. Ranjit Singh<sup>3</sup>

<sup>1,2,3</sup>Adarsh Vijendra Institute of Pharmaceutical Sciences, Shobhit University Gangoh, Saharanpur, Uttar Pradesh, India.

## ABSTRACT

This is a good thing because animals show other organisms how to stay healthy in their environment. Biologists can learn more about a lot of different biological processes by studying animals that aren't humans. They can also get new ideas about how other organisms work. A model organism called the zebra fish (*Danio rerio*) has been used to study development and diseases like cancer and cardiovascular disease, as well as toxicology and drug discovery. Zebra fish have also been used in molecular genetics and genomic research. They are used in toxicology and biology research because they are a good example of a living thing. Because zebra fish are used to model human diseases, they have a lot more advantages than other vertebrate models. For example, zebra fish are better than other vertebrate models when it comes to testing large-scale genetic mutations and therapeutic chemicals. They are used in toxic genomics and aquaculture as genetic models. They can also be used to make human illness models based on zebra fish diseases, which can help scientists learn more about how humans get sick. For example, because it's so easy to keep and breed this little fish, it can be used in a wide range of scientific fields because it has a translucent body.

**Keywords-** Biologists, Drug discovery, Toxicology, Toxic genomics, Zebra fish, .

## Introduction

In pharmacy, scientists are constantly trying to create novel scaffolds with more potent pharmacological action than current drugs with little side effects. Preclinical trials are required after identifying a new scaffold medication. During preclinical trials, we test new scaffolds on mice, frogs, and Guinea pigs. For therapeutic trials, scientists have used zebra fish since 1960. The unknown hazards of the new medicine have scared many animals worldwide. Overall, scientists are working to create a less harmful medicine, but it still requires many sacrifices. This makes the process uncertain and costly.<sup>1</sup> So, to simplify toxicological research, many scientists chose an alternative animal model like the zebra fish.

It has been used to study human diseases for 40 years because its genome sequence is nearly 87 percent similar to the human genome and 70 percent of human genes have orthologous in the zebra fish model.<sup>2,3</sup> The zebra fish (*Danio rerio*) lives in clean water. The zebra fish is 2.5–4 cm long. It is initially transparent, but as it matures, it develops

distinctive stripes all over its body. You'll look great in bluish red. zebra fish with a pink smudge and a torpedo shape. Female zebra fish have fewer pink and blue stripes and are heavier than male zebra fish. The zebra fish lives 2-3 years in ideal conditions.<sup>4,5</sup> (Fig. 1) You can find the zebra fish in the rivers of South Asia, like India and Nepal and Bhutan and Pakistan and Bangladesh and Myanmar. It is a tropical freshwater fish. It is a bony fish (teleost) from the family Cyprinidae (Actinopterygii) (ray-finned fishes). George Streisinger used zebra fish as a model in the 1970s because

**Address for correspondence:** Niladry S. Ghosh  
Adarsh Vijendra Institute of Pharmaceutical Sciences,  
Shobhit University Gangoh, Saharanpur, Uttar Pradesh, India.

**E-mail address:** niladry.ghosh@shobhituniversity.ac.in

**Received:** 10 June, 2022

**Accepted:** 06 July 2022

**Published:** 05 August, 2022

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

**For reprints contact:** pnrjournal@gmail.com

**How to cite this article:** Dubey A, Ghosh NS, Singh R. Zebrafish as An Emerging Model: An Important Testing Platform for Biomedical Science. J Pharm Negative Results 2022;13(3):1-7

### Access this article online

#### Quick Response Code:



**Website:**  
www.pnrjournal.com

**DOI:**  
10.47750/pnr.2022.13.03.001



**Fig. 1: Zebra fish<sup>1</sup>**

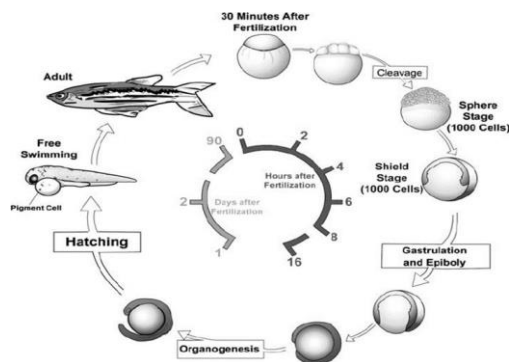
it was easier to control genetically than mice. The concept of using zebra fish embryos to investigate the development of the nervous system intrigued Streisinger's university colleagues, especially Chuck Kimmel. In the 1990s, Nobel laureate Christiane Nusslein-Volhard of Tübingen, Germany, and Wolfgang Driever and Mark Fishman of Boston, USA, used zebra fish as a model organism to create two enormous genetic mutants. Identifying mutants is an important strategy in many fields of biology. The brain, digestive tract, muscles, vasculature, and innate immune system of zebra fish are all identical to those of humans.<sup>6,7</sup> Seventy percent of human disease genes are zebra fish-like.<sup>8,9</sup>

### The Zebra Fish as a Model Organism has Several Distinguishing Characteristics

Scientists prefer *D. rerio* as a model organism because of its diversity of traits. The embryo grows quickly outside of the mother and is very clear, which makes it easy to play with. The blastula stage lasts only 3 hours, while gastrulation takes 5 hours. An 18-hour-old embryo has well developed ears, eyes, segmenting muscles, and a brain. By 24 hours, most basic organ systems have formed. The embryo hatches from the eggshell in 72 hours and begin seeking nourishment in 2 days. In just 4 days, the embryo transforms into a miniature adult. Rapid development simplifies genetic investigations. The adult zebra fish reaches sexual maturity at around 10 weeks and has a high fecundity rate. The zebra fish can lay up to 200 eggs each week in ideal conditions.<sup>10,11</sup> For large-scale genetic techniques to find novel genes and their specialized functions in vertebrates, the zebra fish is an ideal choice.<sup>12,13</sup> The zebra fish is a tough fish to raise. Aside from the foregoing benefits, zebra fish require little room for maintenance. For developmental, toxicological, and transgenic research, this fish is a good choice.<sup>14,15</sup>

### Life Cycle of Zebra Fish

The zebra fish goes through its entire life cycle. Zebra fish are born from such a one-cell zygote that rests on top of a big yolk cell and develops quickly. Gastrulation commences roughly 6 hours after fertilization, with the larvae releasing at 2 days after fertilization as free-swimming larvae. Zebra fish attain sexual maturity at roughly 3 months of age and can live for up to 5 years in their natural environment.<sup>16</sup>



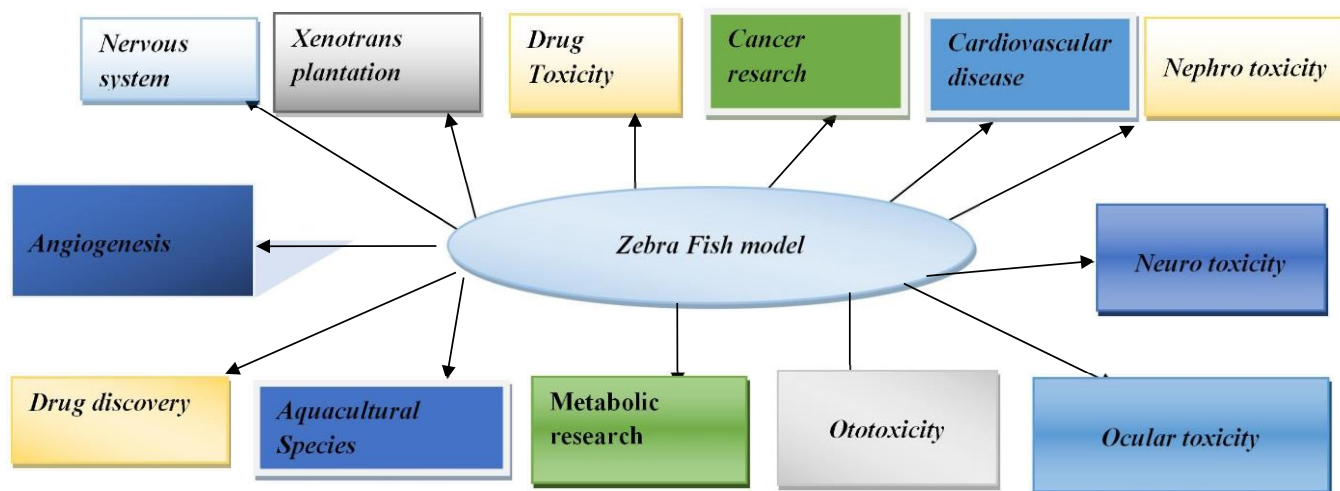
**Fig. 2: Life cycle Zebra fish**

### Zebra Fish as a Model Organism in Developmental Biology

Many human disease genes share mutations with zebra fish genes. This model can be used to study human diseases such as cancer and cardiovascular and neurological disorders. Zebra fish contain many similarities to red blood cells, neutrophils, and macrophages, as well as T and B cells. They can be used as a model for hematological disorders. Zebra fish embryos have been used a lot in cardio toxicity studies to look into gene function and mimic human diseases. Toxicological investigations using zebra fish embryos.<sup>17,18</sup> Compared to other animal models, this model is low cost, high output, and has various advantages, including reduced testing agent quantity, reduced testing time, and promising outcomes. Many pharmacological dosage forms have been withdrawn from the market due to cardiotoxicities. A pricey procedure? To reduce costs, detect probable adverse drug reactions (ADRs) before starting clinical studies. Researchers and academics are now interested in zebra fish models. Improving pre-clinical data collection by linking low-cost culture assays with high-value mammalian models Zebra fish toxicity studies give us more information about how humans work than any other in vitro model.<sup>19,20</sup> Compared to the murine model, the *Danio rerio* model has a smaller size, heavier progeny, and a faster mutation rate. Target discovery, target validation, and toxicity investigations are carried out. It produces a huge number of offspring, leading to statistical analysis confidence. It also has a lot in common with human proteins that cause cancer. Unlike other animal models, it also reduces husbandry costs. The zebra fish can absorb water-soluble drugs or compounds.<sup>21</sup>

### The Use of the Preclinical Models in Oncology-

According to the International Agency for Research on Cancer, 18.1 million new cases and 96 million deaths occurred in 2018.<sup>22,23</sup> The 1960s saw the first usage of zebra fish in cancer research.<sup>24</sup> Compared to conventional cell culture assay methods, the zebra fish model has several advantages. Both zebra fish and humans share tumor growth pathways due to similar progeny mutation rates. It produces a huge number of embryos, which is useful for statistical purposes. It turns out that more than 130 genes share a lot of the same characteristics as liver cancer cells.



**Fig. 3: Zebra fish model**

They look, change quickly, have different types of cells, and go through different stages.<sup>24,25,26</sup>

According to Zheng et al.<sup>27</sup> the zebra fish model resembles human liver cancer. There are several ways to create human carcinoma in zebra fish, such as transplanting tumor cells or creating mutants that provide precise information on tumor development. It also shows tumour migration and cancer cell metastasis.<sup>28</sup>

### Development of the Enteric Nervous System

The zebra fish model has recently made progress in the study of the development of the enteric nervous system (ENS). All vertebrates, including the zebra fish, have an extensive digestive system that includes multiple cell types, including epithelial, muscular, vascular, and neural cells. There are structural parallels between the zebra fish (teleost) and amniotes' digestive systems, but the teleost's digestive system is less sophisticated than that of amniotes. The intestinal bulb, a swollen portion of the anterior intestine in zebra fish, serves as the GI tract's only stomach. It looks like the stomachs of mammals. This intestinal bulb has motility patterns and goblet cells that make acid and neutral mucins.<sup>29,30</sup> There are no crypts or villi in the zebra fish's gut epithelium; instead, the epithelium is organized into an uneven wide fold.<sup>31</sup> Sox2, barx1, gata5, and gata6 are the genes that are involved in the development of the zebra fish's stomach, which is similar to that of amniotes.<sup>32</sup>

### Coronary Hart Disease Zebra Fish Model

Hyperlipidemia means a high level of fat. Cholesterol and triglycerides are the fats. Blood lipids and fats are major risk factors for atherosclerosis and heart disease.<sup>33</sup> According to Zhu et al.<sup>34</sup> cardio toxic medicines cause pericardial edema and circulatory disruption in zebra fish. Among the drugs he studied were aspirin, clomipramine, cyclophosphamide, nimodipine, quinidine, terfenadine, and verapamil hydrochloride. Aspirin is used to boost the heart rate.

Another medicine slows the heart rate. Microinjection of a drug into the yolk sac of a zebrafish after four to twenty-four hours, drug response is recoded by heart rate, heart rhythm, pericardial edema, circulation bleeding, and thrombosis six parameter heart rate and heart rhythm. This same drug's effect on humans and zebra fish is the same. He concludes that the zebra fish model is an excellent cardio toxicity model.

Albini et al.<sup>35</sup> utilized zebra fish to screen for cardio toxicity. He takes anti-cancer medications like 5-fluorouracil, mitoxantrone, doxorubicin, and cyclophosphamide, as well as anti-histamines like terfenadine. Dosage ranges from 0.01 m to 100 m for zebra fish. After twenty-four hours, cardio toxicity was measured by heart rate, rhythm, and circulation. Also, antibiotics like gentamicin and antiviral drugs like amantadine have similar effects on humans and zebra fish. Overall, the zebra fish model appears to be useful in preclinical research and is used a lot as a model for heart problems.

### Xenotransplantation-

Xenotransplantation is a revolutionary process of designing tumors in zebra fish that has only just been found and is set to revolutionize the field of cancer research. When it comes to xenotransplantation, one of the most significant elements is that cancerous cells can be stained or marked with a fluorescent stain that clearly distinguishes implanted cells from normal cells, allowing for the detection of a positive and significant tumor's developmental phase.<sup>36</sup> The most important characteristic of xenotransplantation is that it is non-invasive. In particular, various different types of tumors have been implanted into the zebra fish's body to see how they react. Pancreatic cancer, lung cancer, ovarian carcinoma, breast cancer, prostate cancer, retinoblastoma, leukemia, and other types of cancer are among the most common.<sup>36,37</sup>

### Toxicology & New Treatment Development

The zebra fish has recently become a popular toxicology and drug discovery model. An animal's body length and shape, as

well as the shape and size of its internal organs, can be used to figure out how a drug affects growth and development. The zebra fish model has also been used to test organ function and medication effects. Toxicology in mammals is predicted using zebra fish embryos. The median lethal dose (MLD) of zebra fish embryos was found to be lower than that of mammals.<sup>38</sup> The toxicity of medicine on specific organs has also been examined in zebra fish and humans. Caffeine, camptothecin, quinine, neomycin, vinblastine, and fluorouracil were among the medicines employed to assess organ damage.<sup>39,40</sup>

### Neurotoxicology Studies Using the Zebra Fish

The zebra fish model is now commonly used to examine drug behavior and neurological disorders. Similar to other vertebrates, the CNS and major brain subdivisions are organized. The neural system of zebra fish shares many similarities with the human nervous system, including neurotransmitters like GABA, dopamine, adrenaline, serotonin, and histamine.<sup>41,42</sup> Pharmacological and toxicological activities attack them as well. The study of epilepsy requires a detailed look at brain and nerve system movement. The automatic tracking study is hot. Adult zebra fish can display social interaction and a learning mindset.<sup>43</sup> Compared to other animal models; pentylenetetrazol and picrotoxins elicit dosage-dependent increases in locomotor activity, while diphenylhydantoin and valproic acid cause decreases. The pharmacological response in zebra fish is similar to that in rodents.<sup>44</sup> In clinical trials, the anxiety-provoking effects of new compounds are assessed using the adult zebra fish model (LDT). This test is similar to the rodent model test. Performing this activity on a zebra fish model requires a dark environment.<sup>45</sup> Overall, the zebra fish model appears to be a promising neurological model. It uses the same biological target as the human body. The *in vitro* model embryo allows scientists to study genetic manipulation and conserved proteins with fluorescent tags.<sup>46</sup>

### Zebra fish as Metabolic Research Models

Risk factors for metabolic disorders include low HDL, high triglycerides, high blood glucose, high blood pressure, and abdominal obesity.<sup>47</sup> An imbalance between nutrient intake and energy expenditure can lead to metabolic disorders such as diabetes, stroke, and fatty liver disease.<sup>48,49</sup> Aside from general parallels to human metabolism, zebra fish metabolism has certain distinct features. Zebra fish embryos eat yolk for the first five days of development, then are fed to prevent starvation. Mechanistic insights into metabolic homeostasis upon energy deprivation have been developed using the feeding-to-fasting transition at 5–6 dpf.<sup>50,51</sup> The zebra fish adipose tissue composition and development is also unique. As a poikilothermic animal, zebra fish appear to lack the need for brown adipose tissue. The first adipocyte is discovered at 8 days post fertilization. Interestingly, late adipogenesis may provide an experimental setting for studying adipose tissue's role in metabolic disorders. During the larval stage,

metabolism can be modeled to mimic human illnesses. Similarly, metabolic abnormalities in adults can be modeled to study phenotypic parallels in all metabolic organs. Many metabolic similarities and differences between humans and zebra fish have been described elsewhere.<sup>52,53</sup>

### Visual Toxic Effects in Zebra Fish

There are morphological parallels between the zebra fish and the human eye, such as the cornea, lens, choroid, and retina, as well as similarities in genetic expression and tissue structure, among other things.<sup>54,55</sup> The colour vision of the zebra fish is due to the presence of a cone-dense area, which is comparable to that of humans. There are many things about zebra fish that make them a good pre-clinical model for studying drugs. Because of this, the zebra fish model has emerged as a good one. With the help of an adult zebra fish model, the optokinetic assay and dark and light alternating vertical strips were the two assays that were employed to determine ocular toxicity. Only a few medications, such as chlorpromazine, cisplatin, gentamicin, minoxidil, and vardenafil, have been shown to cause ocular toxicities in mice that are comparable to those observed in humans, with high sensitivity, specifically to 75 percent-100 percent.

### Ototoxicity Model is Developed based on Zebra Fish

Aminoglycosides for life-threatening infections and cisplatin for cancer therapy can cause ototoxicity, which is often controllable but not always, reducing the patients' quality of life. Testing for ototoxicity in preclinical mammalian models can be complicated and is only done in select instances. Using the zebra fish model for preclinical safety testing helps fill these gaps.<sup>56,57</sup> The zebra fish model is successful because of its closeness to human systems in hair cell physiology, ear anatomy, and brain transmission. allowing for high-throughput regulation of the hair cell modifications of zebra fish's surface cells. Because of this fact, the zebra fish is a common ototoxicity model. There are a number of medications that are ototoxic to humans that are also ototoxic to developing, larval, and adult zebra fish. This means that the zebra fish model can be used to identify new ototoxic compounds.<sup>58,59,60</sup>

### Angiogenesis

Angiogenesis and regeneration have also been studied in zebra fish. Angiogenesis is the process of creating new blood vessels from existing vascular systems in both normal and pathological circumstances. Endothelial cells and their niche cooperate to achieve this. Abnormal remodeling causes cancer.<sup>61</sup> The zebra fish, a transparent vertebrate, has been shown to be a useful tool for studying early cardiovascular development and blood flow.<sup>62</sup> The development of the blood circulatory system could be seen in great detail in zebra fish larvae using a simple dissecting microscope and fluorescent proteins. In live specimens, confocal microscopy and time-lapse photography can show how cells change shape and how they move. Angiogenesis is vital for vascular

growth and development. Intersegment arteries are formed by angiogenic sprouting from the dorsal aorta as the embryo grows.<sup>63</sup> Mammalian cancerous cells can be xenotransplanted into zebra fish embryos and grow into tumors.<sup>64,65</sup> This led to the development of tumor angiogenesis models

### Nephrotoxicology Using the Zebra Fish as a Model

The renal system is the primary organ for detoxification. Globular filtration eliminates toxins from circulation. Toxic substances drastically impair kidney function, and causes renal failure. Use the model of the zebra fish, which is used to see how nephrotoxic compounds affect kidney function and histology. In comparison to other animal models, the kidney function and structure of zebra fish is very similar to that of humans. Because of this, zebra fish (*Danio rerio*) is a good alternative to other mammalian models for genetic experiments.<sup>66, 67</sup> This model was used to examine cisplatin nephrotoxicity. Cisplatin is used to treat cancer, but it has a serious adverse effect called nephrotoxic acute renal injury. In zebra fish, intraperitoneal cisplatin administration induced a reduction in kidney proximal tubular functioning. A simplified but powerful model to explore the pathophysiology of cisplatin nephrotoxicity, adult zebra fish are ideal for pharmacological screening and genetic manipulation.

### Conclusion

Preclinical experiments using the zebra fish models have shown promising results in 2018. Among some of the 3000 scholarly publications is a zebra fish model, which has tripled in the last decade. Since no other animal model is as effective and promising as the zebra fish model, it has replaced a few animal toxicity investigations. It is used to determine drug toxicity to limit the use of animals in drug toxicological research. It can also be used to find new targets for cancer, cardiovascular disease, diabetes, obesity, neurology, xenotransplantation, eye or ear and renal illnesses etc. Compared to mammalian models, the zebra fish has the convenience and variety of in vitro and in vivo experiments with the low cost and easy maintenance of in vivo experiments. The zebra fish as a model organism appears to have a bright future. In the coming years, more reports on the use of zebra fish as a bio indicator are required to be approved.

### Acknowledgements

In recognition of their kind support for my study, we express our gratitude to Prof. (Dr.) Ranjit Singh, Vice-Chancellor, Shobhit University, Gangoh, Saharanpur, Uttar Pradesh, and his team. We would like to express our gratitude to the technical staff members at the Department of Pharmaceutical Sciences. We would also like to express our gratitude to the following individuals: Mr. Debashis Purohit, Mr. Ravi Pratap Pandey,.

### Declarations

**Ethical Approval** - This article does not contain any experiments involving human subjects or animals that were conducted by the author.

**Funding:** None.

**Conflict of Interest** - No potential conflicts of interest are declared by the authors.

**Informed Consent**- The authors took all the necessary permission to reproduce (no.) figures and tables for the manuscript.

### References

- Blomme, E. and Will, Y., (2015) Toxicology Strategies for Drug Discovery: Present and Future. *Chemical Research in Toxicology*, 29(4), pp.473-504.
- Hay M, Thomas DW, Craighead JL, et al. (2014) Clinical development success rates for investigational drugs. *Nature Biotechnology* 32(1),40-51.
- Santoriello C, Zon LI (2012) Hooked! Modeling human disease in zebra fish. *The Journal of Clinical Investigation* 122(7),2337-2343.
- Gore AV, Monzo K, Cha YR, Pan W, Weinstein BM, (2012) Vascular development in the zebrafish. *Cold Spring Harbor Perspectives in Medicine*.2 (5),a006684.
- Kanungo J, Cuevas E, Ali SF, Paule MG, (2014) Zebrafish model in drug safety assessment. *Current Pharmaceutical Design*.20(34),5416-5429.
- Kalueff AV, Stewart AM, Gerlai R. (2014) Zebrafish as an emerging model for studying complex brain disorders. *Trends in Pharmacological Sciences*.35 (2),63-75.
- Guyon JR, Steffen LS, Howell MH, Pusack TJ, Lawrence C, Kunkel LM. (2007) Modeling human muscle disease in zebrafish. *Biochimica et Biophysica Acta*, 1772(2),205-215.
- Weinstein B. (2002) Vascular cell biology in vivo: A new piscine paradigm? *Trends in Cell Biology*. 12(9),439-445.
- Lieschke GJ, Oates AC, Crowhurst MO, Ward AC, Layton JE. (2001) Morphologic and functional characterization of granulocytes and macrophages in embryonic and adult zebrafish. *Blood*. 98(10),3087-3096.
- Zhao S, Huang J, Ye J. A,(2005) Fresh look at zebrafish from the perspective of cancer research. *Journal of Experimental & Clinical Cancer Research*.34:80. DOI: 10.1186/s13046-015-0196-8.
- Santoriello C, Zon LI. Hooked! (2012) Modeling human disease in zebrafish. *The Journal of Clinical Investigation*;122(7),2337-2343.
- Hill AJ, Teraoka H, Heideman W, et al. (2005) Zebra fish as a model vertebrate for investigating chemical toxicity. *Toxicological Sciences* 86(1), 6-19.
- Pritchard VL (2001). Behavior and morphology of the zebra fish (*Danio rerio*).
- Mahesh B. Manke, Zebra fish model as an alternative to animal testing: A review. *Prac Clin Invest* 4(1), 34-43.
- Pelegri F. Mutagenesis.(2002) In: Nusslein- Volhard C, Dahm R, editors. *Zebrafish: A Practical Approach*. Oxford: Oxford University Press; 2002. pp. 145-174.

16. Lele Z, Krone PH. (1996) The zebrafish as a model system in developmental, toxicological and transgenic research. *Biotechnology Advances*.14(1),57-72
17. Peterson RT, Nass R, Boyd WA, et al. (2008) Use of non-mammalian alternative models for neurotoxicological study. *Neurotoxicology* 29(3), 546-555.
18. Alberto R, Shawn MB (2018) Rare genetic blood disease modeling in zebra fish. *Frontiers in Genetics* 9: 348.
19. Munos B (2009) Lessons from 60 years of pharmaceutical innovation. *Nature Reviews Drug Discovery* 8(12), 959-968.
20. Caballero MV, Candiracci M (2018) Zebra fish as screening model for detecting toxicity and drug's efficacy. *Journal of Unexplored Medical Data* 3 (4),35-40.
21. Martina H, Petr B. (2019) Zebra fish models of cancer-new insights on modeling human cancer in a non-mammalian vertebrate. *Genes* 10(11), 935.
22. Anubhav Dubey, Deepanshi Tiwari, Yatendra Singh, Om Prakash, Pankaj Singh. (2021) Drug repurposing in Oncology: Opportunities and challenges. *Int J of Allied Med Sci and Clin Res.* 9(1),68-87.
23. Dubey A, Yadav P, Peeyush , Verma P, Kumar R. (2022) Investigation of Proapoptotic Potential of Ipomoea carnea Leaf Extract on Breast Cancer Cell Line. *JDDT* 12(1),51-5.
24. Stanton MF (1965) Diethylnitrosamine-induced hepatic degeneration and neoplasia in the aquarium fish, *Brachydanio rerio*. *Journal of the National Cancer Institute* 34, 117-130.
25. Zain ZZ, Fatiha MB, Gheyath KN, et al. (2018) Using zebra fish for investigating the molecular mechanism of drug- induced cardiotoxicity. *BioMed Research International* 4: 1-10.
26. Patricia L, Irene M, Iranzu L, et al. (2018) Zebrafish: Speeding up the cancer drug discovery process. *Cancer Research* 78(21),6048-6058.
27. Zheng W, Li Z, Nguyen AT, et al. (2014) Xmrk, kras and myc transgenic zebra fish liver cancer models share molecular signatures with subsets of human hepatocellular carcinoma. *PLOS One* 9(3),e91179.
28. Wallace KN, Akhter S, Smith EM, Lorent K, Pack M. (2005) Intestinal growth and differentiation in zebrafish. *Mechanisms of Development.*;122,157-173
29. Holmberg A, Olsson C, Hennig GW. (2007) TTX-sensitive and TTX-insensitive control of spontaneous gut motility in the developing zebrafish (*Danio rerio*) larvae. *The Journal of Experimental Biology*.210:1084-1091.
30. Ng AN, de Jong-Curtain TA, Mawdsley DJ, White SJ, Shin J, Appel B, et al. (2005) Formation of the digestive system in zebrafish: III. Intestinal epithelium morphogenesis. *Developmental Biology*. 2005;286:114-135.
31. Wallace AS, Burns AJ. (2005) Development of the enteric nervous system, smoothmuscle and interstitial cells of Cajal in the human gastrointestinal tract. *Cell and Tissue Research.*;319,367-382.
32. Muncan V, Faro A, Haramis AP, Hurlstone AF, Wienholds E, van Es J, et al. (2007) .T-cell factor 4 (Tcf7l2) maintains proliferative compartments in zebrafish intestine. *EMBO Reports.* ;8,966-973.
33. Dubey Anubhav, Tiwari Mamta, Kumar Vikas, Srivastava, Sing Akanksha. (2020) Investigation of Anti-Hyperlipidemic Activity of Vinpocetine in Wistar Rat, *International Journal of Pharmaceutical Research* -12(2),1879-1882. DOI:https://doi.org/10.31838/ijpr/2020.12.02.250.
34. Zhu JJ, Xu YQ, He JH, et al. (2014) Human cardiotoxic drugs delivered by soaking and microinjection induce cardiovascular toxicity in zebra fish. *Journal of Applied Toxicology* 34(2),139-148.
35. Albini A, Pennesi G, Donatelli F, et al. (2010) Cardiotoxicity of anticancer drugs: The need for cardio-oncology and cardio-oncological prevention. *Journal of the National Cancer Institute* 102(1), 14-25.
36. Smith AC, Raimondi AR, Salthouse CD, Ignatius MS, Blackburn JS, Mizgirev IV, et al. (2010) High-throughput cell transplantation establishes that tumorigenic cells are abundant in zebrafish T-cell acute lymphoblastic leukemia. *Blood*. 2010;115(16),3296-3303
37. Zhao S, Huang J, Ye J. (2015) A fresh look at zebrafish from the perspective of cancer research. *Journal of Experimental & Clinical Cancer Research*.34:80. DOI: 10.1186/s13046-015-0196-8
38. Kari G, Rodeck U, Dicker AP.(2007). Zebrafish: An emerging model system for human disease and drug discovery. *Clinical Pharmacology and Therapeutics*;82(1),70-80
39. Zhang C, Willett C, Fremgen T. (2003) Zebrafish: An animal model for toxicological studies. *Current Protocols in Toxicology*. DOI:10.1002/0471140856.tx0107s17.
40. Wu X, Zhong H, Song J, Damoiseaux R, Yang Z, Lin S.(2006) Mycophenolic acid is a potent inhibitor of angiogenesis. *Arteriosclerosis, Thrombosis, and Vascular Biology*;26:2414-2416.
41. Fan CY, Cowden J, Simmons SO, et al. (2010) Gene expression changes in developing zebra fish as potential markers for rapid developmental neurotoxicity screening. *Neurotoxicology and Teratology* 32 (1), 91-98.
42. Lee J, Peterson SM, Freeman JL (2016) Alzheimer's disease risk genes in wild-type adult zebra fish exhibit gender-specific expression changes during aging. *Neurogenetics* 17(3), 197-199.
43. Xi Y, Nobel S, Ekker M (2011) Modeling neurodegeneration in zebra fish. *Current Neurology and Neuroscience Reports* 11(3),274-282.
44. Uday PK, Yatinesh K, Iekhsan O, et al. (2017) Zebrafish as a model for epilepsy-induced cognitive dysfunction: A pharmacological, biochemical and behavioral approach. *Frontiers in Pharmacology* 8, 515.
45. Fei L, Jia L, Xiuyun L, et al. (2018) Characterization of the locomotor activities of zebra fish larvae under the influence of various neuroactive drugs. *Annals of Translational Medicine* 6(10),173.
46. Kysil EV, Meshalkina DA, Frick EE, et al. (2017) Comparative analyses of zebra fish anxiety-like behavior using conflict-based novelty tests. *Zebrafish* 14(3),197-208.
47. Park, W. Y., Choe, S. K., Park, J. & Um, J. Y. (2019) Black raspberry (*Rubus coreanus* Miquel) promotes browning of

- preadipocytes and inguinal white adipose tissue in cold-induced mice. *Nutrients* 11, <https://doi.org/10.3390/nu11092164>.
48. Jung, U. J. & Choi, M. S. (2014). Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. *Int J. Mol. Sci.* 15, 6184–6223 (2014).
  49. Flynn, E. J. 3rd, Trent, C. M. & Rawls, J. F. (2009) Ontogeny and nutritional control of adipogenesis in zebrafish (*Danio rerio*). *J. Lipid Res.* 50, 1641–1652.
  50. Minchin, J. E. N. & Rawls, J. F. (2017) A classification system for zebrafish adipose tissues. *Dis. Model Mech.* 10, 797–809 .
  51. Seth, A., Stemple, D. L. & Barroso, I. (2013) The emerging use of zebrafish to model metabolic disease. *Dis. Model Mech.* 6, 1080–1088 .
  52. Schlegel, A. & Gut, P. (2015). Metabolic insights from zebrafish genetics, physiology, and chemical biology. *Cell Mol. Life Sci.* 72, 2249–2260.
  53. Salmi, T. M., Tan, V. W. T. & Cox, A. G. (2019). Dissecting metabolism using zebrafish models of disease. *Biochem Soc. Trans.* 47, 305–315.
  54. . Sanes JR, Zipursky SL (2010) Design principles of insect and vertebrate visual systems. *Neuron* 66(1): 15-36.
  55. Richardson R, Tracey-White D, Webster A, et al. (2017) The zebra fish eye-a paradigm for investigating human ocular genetics. *Eye* 31(1): 68-86.
  56. Steven C, Isaac A, Jennifer LF, et al. (2020) Use of zebra fish in drug discovery toxicology. *Chemical Research in Toxicology* 33(1): 95-118.
  57. Uribe PM, Sun H, Wang K, et al. (2013). Aminoglycoside-induced hair cell death of inner ear organs causes functional deficits in adult zebra fish (*Danio rerio*). *PLOS One* 8(3), e58755.
  58. Lanvers-Kaminsky C, Zehnhoff-Dinnesen AA, Parfitt R, et al. (2017). Drug-induced ototoxicity: Mechanisms, pharmacogenetics, and protective strategies. *Clinical Pharmacology & Therapeutics* 101(4): 491-500.
  59. Coffin AB, Ramcharitar J. (2016) Chemical ototoxicity of the fish inner ear and lateral line. *Advances in Experimental Medicine and Biology* 877: 419-437.
  60. Zhao Z, Tong JW, Zhang JP, et al. (2011). Zebra fish model for the study on drug ototoxicity of aminoglycoside antibiotics. *Acta Pharmaceutica Sinica* 46(8): 928-935.
  61. Pandya N, Dhalla N, Santani D. (2006) Angiogenesis—A new target for future therapy. *Vascular Pharmacology.*;44:265-274. DOI: 10.1016/j.vph.2006.01.005.
  62. Chávez MN, Aedo G, Fierro FA, Allende ML, Egaña JT. (2016) Zebrafish as an emerging model organism to study angiogenesis in development and regeneration. *Frontiers in Physiology.* 7:56. DOI: 10.3389/fphys.2016.00056.
  62. Schuermann A, Helker CS, Herzog W. (2014). Angiogenesis in zebrafish. *Seminars in Cell & Developmental Biology*;31:106-114. DOI: 10.1016/j.semcdb.2014.04.037.
  63. Haldi M, Ton C, Seng WL, McGrath P. (2006) Human melanoma cells transplanted into zebrafish proliferate, migrate, produce melanin, form masses and stimulate angiogenesis in zebrafish. *Angiogenesis.* 9:139-151. DOI: 10.1007/s10456-006-9040-2
  64. Tobia C, De Sena G, Presta M. (2011) Zebra fish embryo, a tool to study tumor angiogenesis. *The International Journal of Developmental Biology.* 2011; 55:505-509. DOI: 10.1387/ijdb.103238ct
  65. Elmonem MA, Berlingerio SP, Heuvel VD, et al. (2018) Genetic renal diseases: The emerging role of zebra fish models. *Cells* 7(9): 130.
  66. Gehrig J, Pandey G, Westhoff JH. (2018) Zebrafish as a model for drug screening in genetic kidney diseases. *Frontiers in Pediatrics* 6: 183.
  67. Meyoung JK, Daeun M (2020) Cisplatin nephrotoxicity is induced via polymerase activation in adult zebra fish and mice. *American Journal of Physiology-Regulatory* 318(5), R843-R854.