Review Paper:

Zebrafish as a fascinating animal model: a robust platform for *in vivo* screening for biomedical researches

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Abstract

Due to its striking similarities in anatomy, physiology and genetics with humans, the zebrafish (Danio rerio), a little tropical freshwater fish, has seen massive adoption as a model. Thus, zebrafish are a great model to study human disease mechanisms in the areas of toxicology, genetics and behaviour. Due to its shared vascular system, musculature, renal system and ocular structures with humans, the zebrafish is classified as a vertebrate. Because of the striking similarities between the human genome and that of zebrafish including the arrangement of genes and the pathways that regulate signal transduction, zebrafish might one day be used to simulate human illnesses. There are many screening models present for the determination of preclinical screening activity of the drug molecule.

The main objective of this study is to find at which model is best having low cost. A literature search was conducted on the databases namely Science direct and PubMed with the help of different keywords such as "Zebrafish", "In vitro models" and "In vivo models". The search was customized by applying the appropriate filters so as to get the most relevant articles to meet the objective of this review article: There were different research and review papers based on the Zebrafish models for the determination of different activity of new drug molecules. We found some useful models for the different activity of drugs and suggested that, if we used different methods together, then we may obtain the most relevant result in our research area.

Keywords: Zebrafish, Toxicology, Anatomy, Physiology, Genetics.

Introduction

Primarily located in Southeast Asia, the tropical freshwater fish often referred to as zebrafish (Danio rerio) was once known as Brachydanio rerio. The species is often found in water that moves slowly[1]. In order to lessen the threat of predators, zebrafish are normally located in their native environment, close to the bottom of the sea. These animals have been employed as animal models in many different research investigations due to their high rates of fecundity and fertility. Nowadays, it is believed that zebrafish are a good model to investigate immunology, behaviour, physiology, genetics, development and nutrition. Zebrafish are categorized as omnivores due to their diverse diets and omnivorous (euryphagous) eating behaviours. During experimental trials, researchers utilize different quantities of different food feeds. Both adult and larval zebrafish use the same amounts of the components. Furthermore, some labs boost zebrafish using a range of diets and feeding regimens, sometimes without doing a thorough assessment[2].

According to the zebrafish genome sequencing, which also showed that humans, mice and other species share 12719 genes, Danio rerio has 70% of the human gene pool. Therefore, when genes that cause human illness are inserted into zebrafish, fish that are developing embryos, eventually have the same illness[3]. Thus, the zebrafish model serves as a viable model for human illnesses and novel drug screening[4]. As a result, the species has been widely studied in attempts to cure hereditary illnesses including Parkinson's disease, schizophrenia and depression. Numerous hematopoietic cells (erythrocytes, myeloid cells, band T lymphocytes etc.), the central nervous system, the skeletal system complex and the cardiovascular system are among the numerous parallels between the animal's anatomy and physiology and those of humans [5].

The kidney, pancreas, adipose tissue and skeletal muscles of zebrafish are among the many main organs that have striking similarities to those of humans, making them an excellent model. Zebrafish are employed in many labs to investigate human ailments such as those pertaining to the neurological system, cancer, infectious diseases, heart disease, renal disease, diabetes, blindness, deafness, digestive disorders and hematopoiesis [6].

Reproduction of Zebrafish

Both the fertilization process and the zebrafish's development take place outside of the animal. Reproduction occurs in small groups, with or without parental guidance, by dispersing eggs under the earth. Reproduction should begin at six months for better outcomes and higher-quality embryos, even though sexual maturity typically happens between ten and twelve weeks of age[8,28]. Adults of this species are tiny, with a cylindrical body, alternating bright and dark horizontal stripes and a length of around 4-5 cm. Their diminutive size is a defining feature of the species are sexually dimorphic; females are slimmer and often silverier, while males are more rounded and usually golden on the ventral side. This becomes most apparent just before

spawning. The asynchrony of the females causes them to spawn numerous times daily, sometimes even more than once and each spawning can result in the production of up to one hundred eggs. A single female may lay 200 eggs throughout her spawn. In just two or three months, the fry can reach sexual maturity, thanks to their rapid growth rate[9].

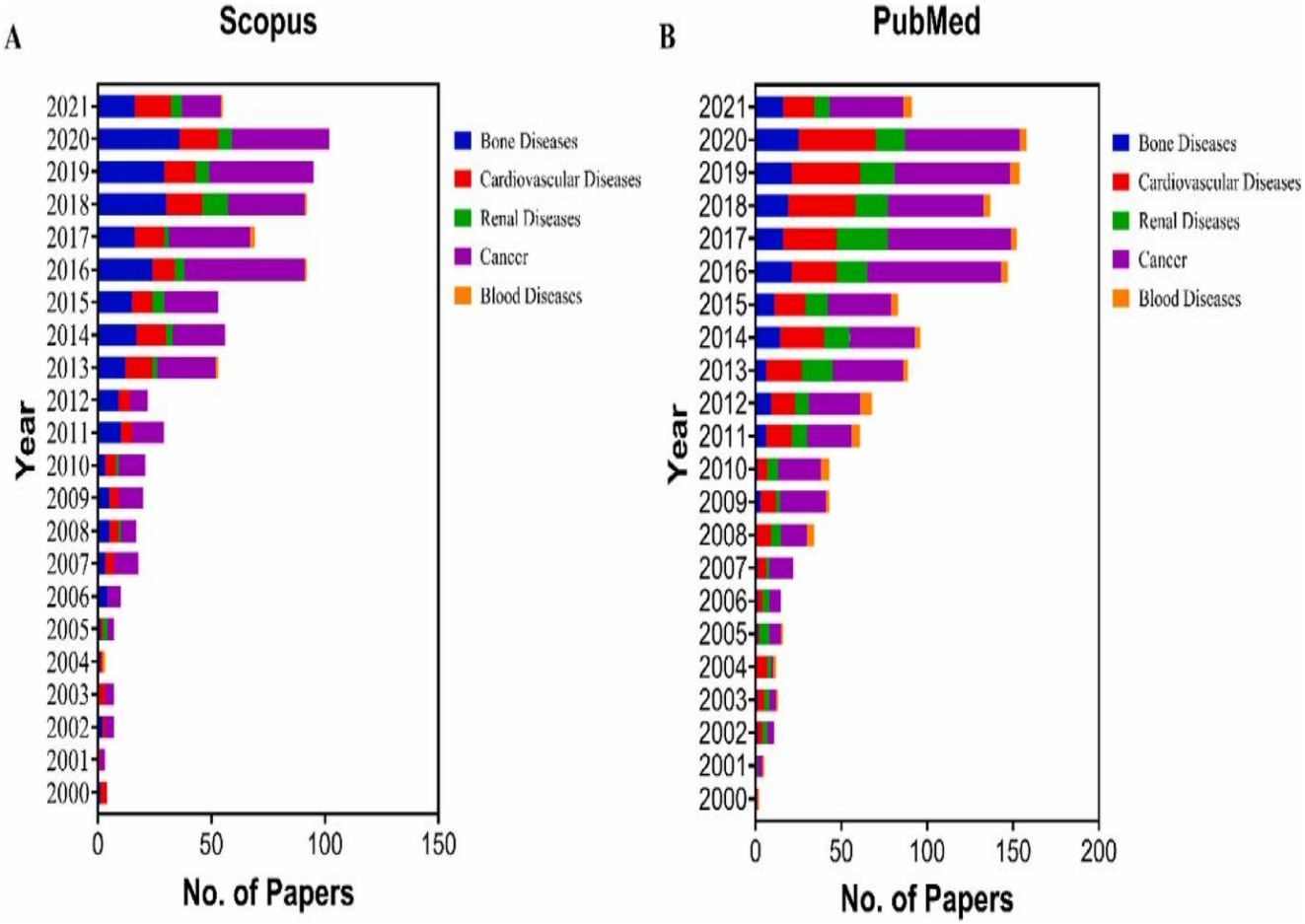

Figure 1: The figure represents the progressive use of zebrafish disease models over the years from data that has been curated from [A] Scopus and [B] PubMed databases showing few of the broadly categorized fields[2]

Taxonomy of Zebrafish
Species : D. rerio
Class : Actinopterygii
Order : Cypriniformes
Family : Cyprinidae
Phylum : Chordata
Genus : Danio
Kingdom : Animalia [7]

Figure 2: Zebra fish[9]

MODELS OF ZEBRAFISH-

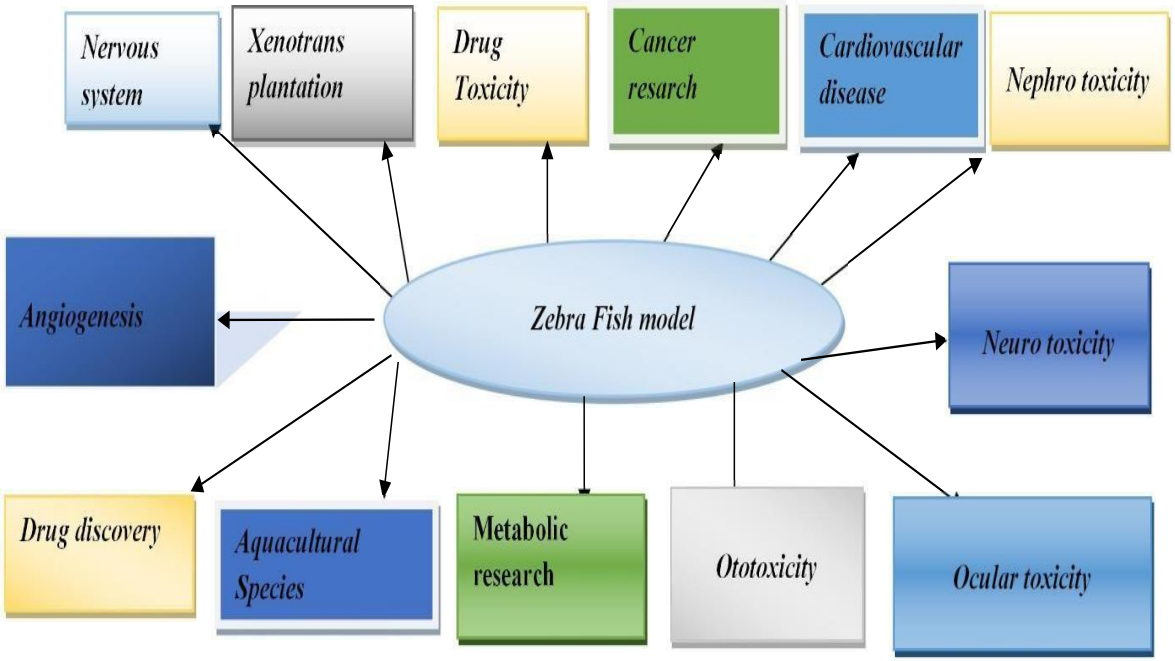

Figure 3: Zebra fish models[9]

The zebrafish model's advantages over its animal companions: When studying vertebrate growth and illness, zebrafish are far superior to rodent models. The growing embryo's optical clarity enables real-time observation at the organism level and a single clutch can include hundreds of embryos[10]. More and more, researchers in the field of biomedicine are turning to zebrafish (Danio rerio) as their experimental model of choice. When most people think of animals used in medical research, these models likely are not the ones that spring to mind. Researchers from all around the globe are showing a growing interest in this little tropical freshwater fish. Its embryos are transparent and can develop outside of the mother's body. Fantastically, this feature eliminates the need for intrusive treatments while still allowing researchers to examine vertebrate embryonic development in great detail. The fact that embryogenesis may be completed in as little as 72 hours and that zebrafish can lay 200–300 fertilized eggs weekly is an additional perk[11].

Because of its small size, it is able to store a lot of data and does not require a lot of infrastructure, unlike the animal homes needed for mice. It also has a high level of genetic similarities to humans, particularly in the central nervous system and is highly manipulable. When compared to the yearly cost of producing mice, the cost of using zebrafish is lower [12]. The researchers employ zebrafish models since one of the problems with utilizing animals is that they are often disturbed. Some human characteristics cannot be completely exploited or trusted in these situations. When scientists were looking for alternatives to using animals in experiments, they came across zebrafish[29].

Zebrafish as another animal model in human and animal vaccination analysis: One advantage of testing vaccines in zebrafish is that, in comparison to other vertebrates, zebrafish have a number of desirable biological traits including a fast reproductive rate, the ability to undergo external fertilization, excellent eyesight and a quick maturation period. Zebrafish also have an advanced immune system that is strikingly comparable to the human immune system. This finding provides more evidence that the vast majority of the chemicals and signaling pathways that play a role in the immune response in humans, are present and behave similarly in fish. Researchers can study how infections work in fish because they are easily infected by many types of germs such as Gram-negative and Gram-positive bacteria, protozoa, viruses, fungi and mycobacteria[13].

Zebrafish as an alternative animal model for cancer: This is possible because fish have parts of both innate and adaptive immunity. Exploring zebrafish as a potential cancer model, genetic engineering can be successfully applied to zebrafish. It is shown that forward genetics can be helpful in finding novel cancer indicators. Cancer models have been constructed using either spontaneous mutations or trans genetics that imitate the mutations observed in human malignancies. Because of its see-through anatomy, the zebrafish provide a unique opportunity to study cancer cells in action and the environmental responses to them including angiogenesis and inflammation screening. Furthermore, they are small and easy to maintain. Overexpressing proto-oncogenes, which are out of balance in people with cancer, were used to make zebrafish models of leukemia[14].

The zebrafish as a potential new epilepsy model: The nervous systems of zebrafish and humans are quite similar;

in fact, 85% of the epilepsy genes in humans have a known equivalent in zebrafish. Zebrafish are a great model organism for studying genetic engineering. Embryos of the zebrafish species are able to absorb drugs straight from the water they swim in. It is important to screen for genes that either enhance or decrease seizure susceptibility in zebrafish since these fish are easy to keep in large populations[15].

The zebrafish as a potential new diabetes model: The morphogenesis of the pancreas was one of several organogenesis studies that made use of zebrafish. By studying zebrafish, we can learn how molecules from the outside world, like retinoic acid, FGF and Shh[16], affect genes that are inside the cell. Zebrafish have recently emerged as a valuable alternative model for investigating the mechanisms of diabetes mellitus development and potential treatments. When exposed to high glucose levels, zebrafish become hyperglycemic and when their blood sugar levels remain consistently high, they develop retinopathies[17].

Obesity and disorders associated with it were studied in zebrafish by feeding them high-calorie, high-fat diets that mimicked human metabolism. The levels of froctosamine in the eyes increased by 41% and the quantities of mRNA for insulin receptors in the muscle were reduced in zebrafish immersed in a 111 mM high-glucose solution [18]. Researchers have developed a model of type 2 diabetes mellitus in zebrafish by overfeeding them with a high-calorie diet. Pancreatic gene expression patterns reveal a common mechanism for the onset of type 2 diabetes mellitus in zebrafish and humans.

According to research on the correlation between age and type 2 diabetes mellitus, zebrafish that are 4 to 11 months old take longer to develop hyperglycemia, a condition where glucose concentrations rapidly increase, compared to older zebrafish [19]. Blood glucose levels as high as 400 mg/dL were seen in adult zebrafish after 24 hours of immersion in a 1% glucose solution. Insulin resistance in skeletal muscle, a result of transgenic expression of the IGF-I receptor, is one of the two models of insulin resistance in transgenic animals. The second strategy included achieving insulin resistance by using CRISPR/Cas9 to specifically knock down the insulin receptor gene in the liver[20].

Non-alcoholic fatty liver disease and other liver disorders can be studied using zebrafish as an alternative animal model: The zebrafish liver is quite similar to the human liver in terms of its genetic composition, cellular composition and function. This discovery led to the study of the zebrafish liver as a model for human liver development, illness and possible therapies by delving into the intricate genetics and embryology of the human liver. To begin with, zebrafish were recognized as a valuable biological model due to their ability to form liver tumors when exposed to carcinogenic compounds. This allowed researchers to compare gene expression in zebrafish cancers to that of human liver tumors. Hepatic steatosis mimics the symptoms observed in humans with high-carbohydrate diets, as observed in zebrafish treated with 6% fructose through various feeding strategies[21].

Overfeeding zebrafish hastens carcinogenesis and leads to fatty liver development. The hormone leptin, which is responsible for obesity, was also shown to be uncontrolled in the oncogenic and overfed zebrafish [22].

Zebrafish as an alternative animal model for cardiotoxicity: One of the main worries in medication development is cardiotoxicity; zebrafish provide an alternate animal model for this phenomenon. Research on cardiotoxic chemicals in zebrafish embryos has shown pathways that are strikingly comparable to human embryos. Treatment with clomipramine and terfenadine affected cardiac functioning, produced edema and hemorrhaging and eventually caused the heartbeat to cease in zebrafish. Researchers used a transgenic zebrafish model to examine small chemicals that control heart rate[23].

Zebrafish as an alternative animal model for lipid-related diseases: Because it shares many characteristics with mammals in terms of lipid processing, metabolism and absorption, the zebrafish is an ideal model to investigate disorders associated with lipids [24]. Zebrafish are a good model for atherosclerosis because they can be used to study macrophage lipid accumulation, lesion formation and changes at the cellular level in the arterial wall. Researchers research obesity in zebrafish because the fish's melanocortin system reacts to leptin and its energy balance is comparable to that of mammals including the control of fat levels in humans. A transcriptional regulator of cholesterol metabolism in zebrafish is SREBP, which stands for sterol-regulatory element-binding protein. This system is comparable to the liver X receptor in mammals. The abnormalities caused by the mutated genes in fish are similar to diseases that affect humans.

An alternate zebrafish model for tumor development: Research on cancers caused by environmental carcinogens has made use of fish species as a vertebrate model. Research into embryogenesis, organogenesis and tumorigenesis has shown that zebrafish are the most useful model organisms[25]. Zebrafish have orthologous oncogenes and tumor suppressor genes (TSGs) that are quite similar to humans. Zebrafish and humans show higher histological similarities regarding chemically produced cancers. There were differences in gene expression patterns at different phases of tumor aggressiveness between zebrafish and humans, according to investigations of hepatic gene expression[26].

Zebrafish as a potential substitute for rats in the study of renal diseases: An essential function of the kidneys in freshwater fish like zebrafish is to excrete water and maintain osmoregulation. Having said that, zebrafish kidneys are better models for kidney research than human kidneys because of the many functional similarities between

the two. Injuries to the kidneys (AKI), nephronophthisis, PKD, renal tubular clearance and glomerular filtration are some of the processes that zebrafish can help researchers learn more about[27].

Conclusion

When it comes to biomedical research, zebrafish are the gold standard. Investigating mutagenesis, carcinogenesis and genome sequencing in zebrafish can contribute to the creation of novel medications for human use. For the purpose of studying different illnesses and testing drugs, zebrafish might be a useful model. Zebrafish are useful in studies of embryogenesis and organogenesis. It also helps with the development of different instruments for genetic study. In biomedical research on many human illness situations, zebrafish models are utilized as an alternative to animal models because of their benefits.

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References

1. Adamson K.S., Eamonn S. and Andrew J.S., Use of Zebrafish Models to Investigate Rare Human Disease, *Journal of Medical Genetics*, **55(10)**, 641–49 **(2018)**

2. Adhish M. and Manjubala I., Effectiveness of zebrafish models in understanding human diseases—a review of Models, *Heliyon*, **9(3)**, e14557 **(2023)**

3. Archer A., Gilbert L., Giselbert H., Agneta M. and Jan-Åke G., Transcriptional Activity and Developmental Expression of Liver X Receptor (Lxr) in Zebrafish, *Developmental Dynamics*, **237(4)**, 1090–98 **(2008)**

4. Bailone R.L. et al, Zebrafish as an Alternative Animal Model in Human and Animal Vaccination Research, *Laboratory Animal Research*, **1(7)**, 30-36 **(2020)**

5. Capiotti K.M., Régis A.L., Wilges K., Maurício R.B., Carla D.B. and Rosane S.D.S., Persistent Impaired Glucose Metabolism in a Zebrafish Hyperglycemia Model, *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, **171(7)**, 58–65 **(2014)**

6. Choi T.Y., Tae-Ik C., Yu-Ri L., Seong-K.C. and Cheol H.K., Zebrafish as an Animal Model for Biomedical Research, *Experimental & Molecular Medicine*, **53(3)**, 310–17 **(2021)**

7. Cluskey M.C., Braedan M. and Ingo Braasch, Zebrafish Phylogeny and Taxonomy, *The Zebrafish in Biomedical Research*, **5(8)**, 15–24 **(2020)**

8. Connaughton V.P., Cassandra B., Lauren F., Emily G. and Carly S., Alternate Immersion in an External Glucose Solution Differentially Affects Blood Sugar Values in Older versus Younger Zebrafish Adults, *Zebrafish*, **13(2)**, 87–94 **(2015)**

9. Dubey A., Ghosh N.S. and Singh R., Zebrafish as An Emerging Model: An Important Testing Platform for Biomedical Science, *J Pharm Negative Results*, **13(3)**, 1-7 **(2022)**

10. Dubey A., Ghosh, N.S. and Singh R., An in-depth and in vitro evaluation of the antioxidant and neuroprotective activity of aqueous and ethanolic extract of Asparagus racemosus Linn seed, *Res. J. Chem. Environ.*, **27(10)**, 46-66 **(2023)**

11. Dubey A., Ghosh N.S. and Singh R.S., Role of Aqueous and Ethanolic Seed Extract of Asparagus racemosus on Acr- Induced Neurotoxicity in Adult Zebrafish: Emergence of Neuroprotective Results, *Egyptian Journal of Aquatic Biology & Fisheries*, **27(6)**, 285-296 **(2023)**

12. Dubey A., Ghosh N.S. and Singh R., A Toxicological Study on Seed Extracts of Asparagus Racemosus Linn (Ethanolic and Water) in Experimental Animals, *Journal of Advanced Zoology*, **44(2)**, 71–78 **(2023)**

13. Fatma S., Ravindra K., Anshuman D. and Rajeeb K.S., Expression of Two Uncharacterized Protein Coding Genes in Zebrafish Lateral Line System, *The International Journal of Developmental Biology*, **65(10)**, 11–12 **(2021)**

14. Ferrari J.T. et al, Experimental Model of Hepatic Steatosis by Fructose in Adult Zebrafish: A Pilot Study, *Clinical & Biomedical Research*, **38(2)**, 151–54 **(2018)**

15. Fornabaio G. et al, Angiotropism and Extravascular Migratory Metastasis in Cutaneous and Uveal Melanoma Progression in a Zebrafish Model, *Scientific Reports*, **8(1)**, 51-56 **(2018)**

16. Gerlai R.M., Lahav S., Guo and Rosenthal A., Drinks like a Fish: Zebra Fish (Danio Rerio) as a Behavior Genetic Model to Study Alcohol Effects, *Pharmacology Biochemistry and Behavior*, **67(4)**, 773–82 **(2000)**

17. Gleeson M., Connaughton V. and Arneson L.S., Induction of Hyperglycaemia in Zebrafish (Danio Rerio) Leads to Morphological Changes in the Retina, *Acta Diabetological*, **44(3)**, 57–6**(2007)**

18. Gonzales J.M. and Sheran H.L., Feed and Feeding Regime Affect Growth Rate and Gonadosomatic Index of Adult Zebrafish (*Danio Rerio*), *Zebrafish*, **10(4)**, 532–40 **(2013)**

19. Han Y., Zhang J.P., Qian J.Q. and Chang-qin H., Cardiotoxicity Evaluation of Anthracyclines in Zebrafish (*Danio Rerio*), *Journal of Applied Toxicology*, **35(3)**, 241-52 **(2014)**

20. Hölttä-V., Maarit, Veijo T.V., Salo L.N., Christian B., Annika E.P.P. and Elina I., Zebrafish: Gaining Popularity in Lipid Research, *Biochemical Journal*, **429(2)**, 235–42 **(2010)**

21. Hortopan G.A., Matthew T.D. and Scott C.B., Zebrafish as a Model for Studying Genetic Aspects of Epilepsy, *Disease Models & Mechanisms*, **3(8)**, 3–4 **(2010)**

22. Kinkel M.D. and Victoria E.P., On the Diabetic Menu: Zebrafish as a Model for Pancreas Development and Function, *Bioassays*, **31(2)**, 139–52 **(2009)**

23. Lieschke G.J. and Peter D.C., Animal Models of Human Disease: Zebrafish Swim into View, *Nature Reviews Genetics*, **8(5)**, 353–67 **(2007)**

24. Mohesh M. and Rao B.N., Danio Rerio (Zebrafish): A Cost Effective Animal Model for Anti-Tuberculosis Drug Research, *Annals of SBV*, **4(20)**, 29–32 **(2015)**

25. Mueller T., The Adult Central Nervous Cholinergic System of a Neurogenetic Model Animal, the Zebrafish Danio Rerio, *Brain Research*, **1011(2)**, 156–69 **(2014)**

26. Noble S., Ismail A., Rafael G., Yanwei Xi and Marc E., Zebrafish Parla- and Parlb-deficiency Affects Dopaminergic Neuron Patterning and Embryonic Survival, *Journal of Neurochemistry*, **122(1)**, 196–207 **(2012)**

27. Patton E.E., Zon L.I. and Langenau D.M., Zebrafish disease models in drug discovery: From preclinical modelling to clinical trials, *Nature Reviews Drug Discovery*, **20(8)**, 611–628 **(2021)**

28. Sabarinath C.P., Sudhakar and Shanmuganath C., Phytochemical and Antibacterial Screening on Leaves of Solanum Torvum, *Asian Journal of Research in Pharmaceutical Science*, **8(3)**, 130 **(2018)**

29. Teame T. et al, The Use of Zebrafish (Danio Rerio) as Biomedical Models, *Animal Frontiers*, **9(3)**, 68–77 **(2019)**.

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