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A NOVEL SCIENTIFIC APPROACH: ZEBRAFISH AS AN INFORMATIVE IN-VIVO TESTING PLATFORM IN PHYSIOLOGICAL INVESTIGATION

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Abstract The zebrafish (Danio rerio), a small tropical freshwater fish, has been widely embraced as a model organism due to its notable anatomical, physiological, and genetic resemblances to humans. Zebrafish serve as an excellent model organism for investigating the processes of human diseases in the fields of toxicology, genetics, and behaviour. The zebrafish is categorized as a vertebrate due to its similar circulatory system, musculature, renal system, and ocular components to humans. The potential utilization of zebrafish as a model organism for simulating human diseases arises from the notable resemblances observed between the human genome and that of zebrafish, encompassing gene organization and signal transduction pathways. Numerous screening models are available for assessing the preclinical screening efficacy of medicinal molecules. The primary aim of this study is to identify the optimal model that offers the most cost-effective and time-efficient approach for determining various tasks. A comprehensive review of the literature was performed using the databases Science Direct and PubMed, including several keywords like "zebrafish," "in vitro models," and "in vivo models." In order to align with the purpose of this review article, the search was tailored by using suitable criteria to find the most pertinent articles. Various research and review publications have been conducted using zebrafish models to assess the distinct actions of novel pharmacological compounds. Based on the findings of our investigation, we have identified several valuable models pertaining to the diverse actions of medications. It is proposed that by employing a combination of various methodologies, we might potentially achieve the most pertinent outcomes within our research domain.

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. Due to their high rates of fecundity and fertility, many different research investigations have employed these animals as animal models. Nowadays, it's believed that zebrafish are a good model to investigate immunology, behavior, physiology, genetics, development, and nutrition. Zebrafish are categorized as omnivores due to their diverse diets and omnivorous (euryphagous) eating behaviors. 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 develop the same illness [3]. Thus, the zebrafish model serves as a viable model for human illnesses and novel drug screening [4]. Consequently, researchers have extensively studied the species to treat hereditary illnesses such as 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 haematopoiesis [6].

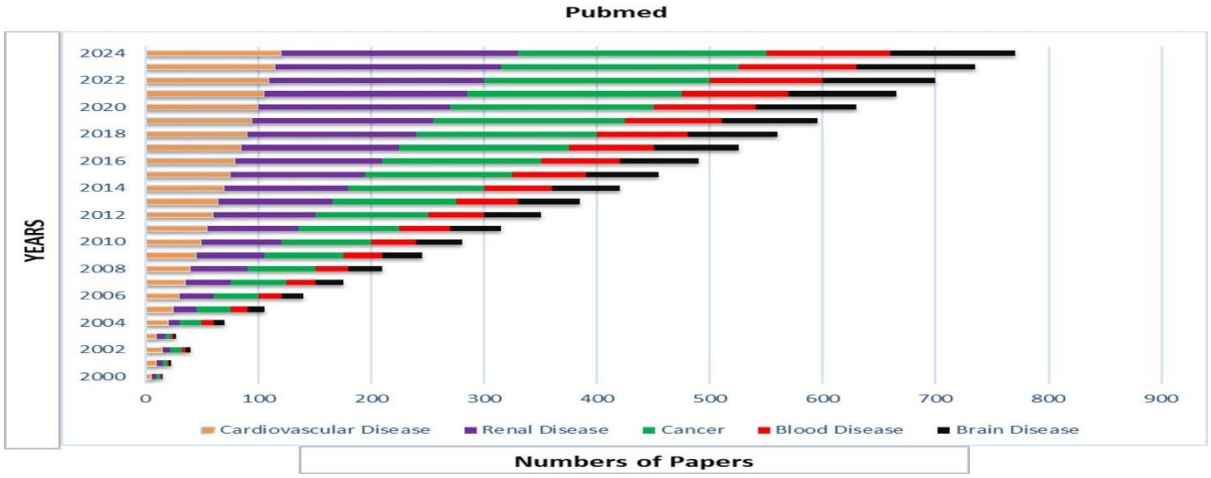

Figure-1 The image shows the growing usage of zebrafish illness models over time using PubMed highlighting a few main sectors where they are beneficial [2].

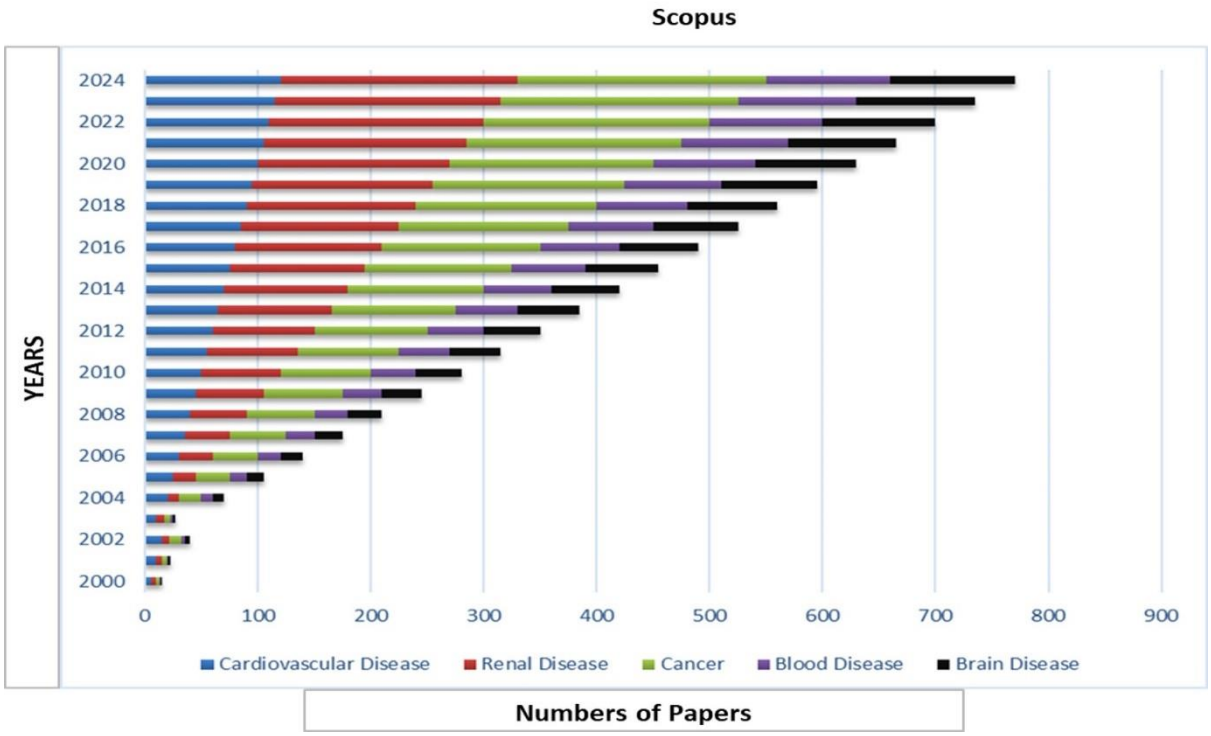

Figure-2 The image shows the growing usage of zebrafish illness models over time using Scopus &SCI, ESCI highlighting a few main sectors where they are beneficial [2].

Taxonomy of zebrafish

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

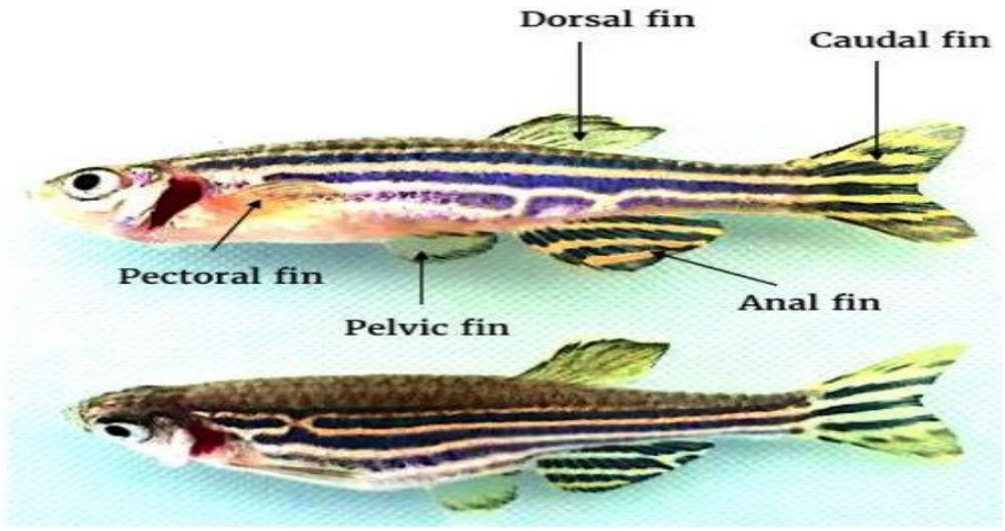

Figure-3 Male and female Zebra fish [9,30]

Table-1 Advantage and disadvantage over rodents for developing disease model [2]

Basis	**Zebrafish Models**	**Rodent Models**
Size	Very small	Relatively bigger
Animal care costs	Quite low	Quite high
Housing Requirements	Less space needed	Takes up a lot of space
Development	External	Internal
Number of offspring	100-300 per week	5-12 per month
Life span	3-5 years	2-3 year
F0 reverse genetic screens	Easily available and highly affordable	Not readily available and difficult to afford
F3 reverse genetic screens	Moderately available	Rarely available
External visibility of skeletal development	Easily viewable due to transparency at developmental stages	Not possible due to development in the uterus
Possibility of in-vivo fluorescence imaging	Highly possible	Low possibilty
Orthologues count per human gene	1 or >1	1
Genome-wide screening	Possible	Not possible
Genetic manipulation	Can be done easily	Harder process
Phenotyping	Rapid	Cumbersome
Neuroregeneration	Possible	Not possible
Telomer size	Short like human with the age related programe of decline	Long
Drug administration	Easier	Relatively difficult
3R strategy	Developed	Yet to be developed
Xenograft engraftment	Rapid	Slow
Experimental cycle	Short	Long
Ethical constraints	Very low	Quite a low

MODELS OF ZEBRAFISH-

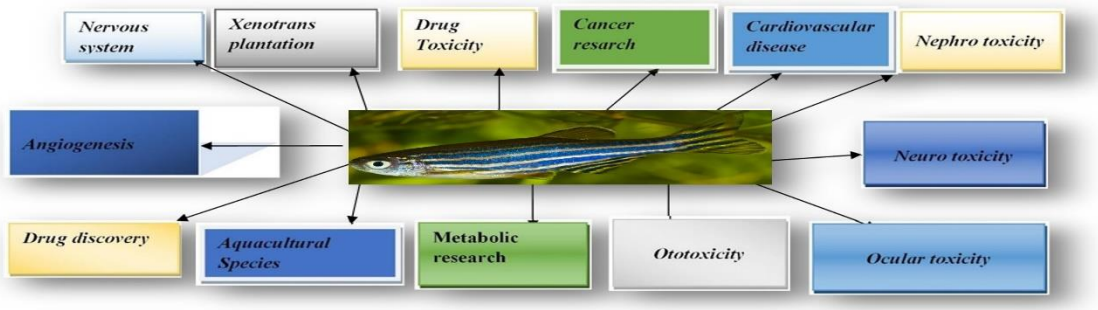

Figure -4 Zebra fish models [9]

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].

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 aren't the ones that spring to mind. Researchers from all around the globe are showing a growing interest in this little tropical freshwater fish, and one of the main reasons why is because 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 doesn't 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 use zebrafish models to address the issue of animals' frequent disturbance. In these situations, it becomes impossible to fully exploit or trust certain human characteristics. When scientists were looking for alternatives to using animals in experiments, they came across zebrafish [29].

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].

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 Zebrafish can successfully undergo genetic engineering. Forward genetics has demonstrated its usefulness in identifying novel cancer indicators. Researchers have constructed cancer models using either spontaneous mutations or trans genetics, which mimic the mutations found in human malignancies. Because of its see-through anatomy, the zebrafish provides 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, was used to make zebrafish models of leukaemia [14].

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]

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]. We studied obesity and associated disorders in zebrafish by feeding them high-calorie, high-fat diets that mimicked human metabolism. Immersing zebrafish in a 111 mM high-glucose solution led to a 41% increase in froctosamine levels in the eyes and a reduction in mRNA for insulin receptors in the muscle. Researchers have created 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]. Adult zebrafish, after 24 hours of immersion in a 1% glucose solution, showed blood glucose levels as high as 400 mg/dL. 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].

Hepatoprotective 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, delving into the intricate genetics and embryology of the human liver. Initially, researchers recognized zebrafish as a valuable biological model because they can develop liver tumors when exposed to carcinogenic compounds. This allowed researchers to compare gene expression in zebrafish cancers to that in 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].

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].

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]. Researchers can study macrophage lipid accumulation, lesion formation, and changes at the cellular level in the arterial wall using zebrafish as a good model for atherosclerosis. 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. SREBP, or sterol-regulatory element-binding protein, is 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 for tumour analysis:

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 when it comes to chemically produced cancers. There were differences in gene expression patterns at different phases of tumour aggressiveness between zebrafish and humans, according to investigations of hepatic gene expression [26].

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 research in the field of biomedicine, zebrafish are considered to be the very best. Through the study of mutagenesis, carcinogenesis, and genome sequencing in zebrafish, it is possible to contribute to the development of innovative drugs that are intended for use in humans. It is possible that zebrafish might serve as a valuable model for the purpose of researching various diseases and testing various medications. Research pertaining to embryogenesis and organogenesis can benefit from the use of zebrafish. Additionally, it contributes to the establishment of various tools for the purpose of genetic research. For the purpose of conducting biomedical research on a wide range of human illnesses, zebrafish models are employed as an alternative to animal models, attributable to the numerous advantages they offer.

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Conflict of interest:

The authors declare no conflict of interest.

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Ethical approvals

This study does not involve experiments on animals or human subjects.

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