***Review Article***

**Zebra Fish model as a promising alternative to laboratory rodents in pharmacological and biomedical research**

**ABSTRACT**

Zebra fish (*Danio rerio*) belonging to the Cyprinidae family, are useful model organisms for researching vertebrate biology because of their transparent embryos and quick growth. For high-throughput pharmacological and genetic screening, they are superior to mice, especially when studying human disease and its remedies. Zebra fish embryos are a valuable biological model since they are transparent and develop outside of the uterus. More than 80% of the proteins that causes disease have been maintained, and 70% of their genomes resemble those of humans. Because the bodily tissues and growth and development biology of Zebra fish are similar to those of humans, models of a variety of illnesses, and different thrust areas viz. cancers, hepatic, circulatory, and cardiovascular diseases where rodents are replaced by this model. Treatments for epilepsy, behavioural neuroscience, neuropharmacology, viral infections, and other topics are being studied with Zebra fish. Currently Zebra fish applications in a variety of therapeutic fields are extremely important.

**Keywords:** Cyprinidae, *Danio rerio*, mammalian models, Zebra fish.

**1. INTRODUCTION**

Zebra fish (*Danio rerio*) are a member of the Cyprinidae family, native to southern Asia, and widely distributed in many countries of the Southeast Asia region (Vargas *et al.,* 2015). The Zebra fish (*Danio rerio*), which is ideal for both developmental and genetic investigation, is a potent model organism for the study of vertebrate biology (Adhishand and Manjubala, 2023). Zebra fish develop quickly; in just 24 hours, their fundamental body plan is established, and several study instruments are available (Bashirzade et al., 2022). In the early 1980s, Streisiger used the Zebra fish (*Danio rerio*), a tiny teleost that normally inhabits pleasant waters and measures 3 to 4 cm, as an animal model for genetic research (Carina et al., 2017). Recent years have seen a sharp rise in the annual number of publications on Zebra fish as a model for biomedical research. The embryos of Zebra fish are transparent and develop outside of the uterus, which makes them a valuable biological model (Teame et al., 2019). Compared to the more well-known vertebrate genetic model, the mouse, Zebra fish provide a number of benefits for genetic screening. Because they take place in utero, early developmental processes are less accessible in mice (Figure-1).

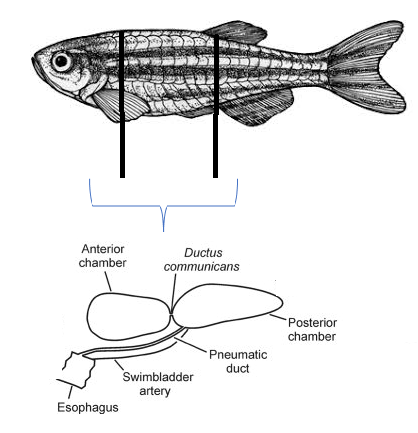


Figure-1: Zebra fish with its body parts

By practical scenario, considerably more area is needed, and breeding and maintenance are very much expensive (Adhishand and Manjubala, 2023). Furthermore, Zebra fish have been identified as distinctive vertebrate models that are suitable for high-throughput drug screening and discovery, which is particularly useful for researching human illness and related treatments and is not easily accomplished in other vertebrate models (Bradford et al., 2017). In addition, over 80% of illness-causing proteins are preserved in Zebra fish, and 70% of their genomes are similar to those of humans (Nipu et al., 2025). Significantly, Zebra fish illness models frequently accurately depict human disease, including its origin (etiology), progression, and resolution mechanisms (Bradford et al., 2017; Xia et al., 2022). Since Zebra fish are vertebrates, their body tissues and growth and development biology are comparable to those of humans. As a result, models of a variety of diseases, including malignancies (Cagan et al., 2019), hepatic disorders (Shimizu et al., 2023), circulatory disorders (Rissone and Burgess, 2018), CVS disorders (Zhao et al., 2019), and psychological and cognitive abnormalities (Fitzgerald et al., 2021; Griffin et al., 2018), have been created (Patton et al., 2021).

**2. ACTION**

**2.1 Neuropharmacological action**

The advancements made in Zebra fish learning and memory experimental paradigms raise the possibility that this model organism might be useful in behavioral pharmacology research (Chaoul et al., 2023). Neurotransmitters, including GABA (Miller, 2019), glutamate (Pal, 2021), dopamine (Klein et al., 2018), noradrenaline (Sugama and Kakinuma, 2021), serotonin (Moncrieff et al., 2022), histamine (Qian et al., 2022), and acetylcholine (Pondeljak and Lugović-Mihić, 2020) are present in both interneuron networks and long routes, and the fundamental organization of the fish central nervous system contains each of the key areas of the mammalian brain (Martins et al., 2024). In addition, the remarkable capacity of the Zebra fish brain to regenerate, in comparison to humans, is another beneficial feature for investigating the processes behind neurological protection, neurogenesis, and the functional integration of immature neural cells (Fontana et al., 2018).

**2.2 Endocrine action**

It is generally recognized that environmental endocrine-disrupting chemicals (EDCs), a class of exogenous persistent organic pollutants, can alter hormone production, release, and metabolism in addition to the endocrine system (Tao et al., 2022). So, that the EDCs can be screened for, their effects evaluated, and their targets and methods of action studied using Zebra fish (Toso et al., 2024), because higher vertebrates have a higher degree of conservation and provide several experimental benefits, including large progenies, lower costs, embryo transparency, and adaptation to high-throughput conditions (Jarque et al., 2019). Fish have highly developed ionic and acid-base control systems that help them maintain the balance of bodily fluids (Guh and Hwang, 2017).

**2.3 Action in CVS**

The capacity of zebra fish hearts to regenerate throughout the course of their lives offers new insights into human cardiac regeneration (Bournele and Beis, 2016). In that both have a lengthy plateau period, the zebra fish and human cardiac action potentials exhibit comparable phenotypes (Vornanen and Hassinen, 2016). When testing drugs, zebra fish hearts are commonly used to assess several cardiovascular characteristics, including heart rate, cardiac output, ejection fraction, stroke volume, frequency of shortening fraction, and regularity of beating (Suryanto et al., 2022). The zebra fish heart has basic characteristics with the human heart, despite having two chambers that facilitate imaging. Forward-genetic screenings in zebra fish have revealed important mechanisms in cardiovascular disorders that mimic those of higher vertebrates, and early developmental processes and signaling pathways are maintained across species (Giardoglou and Beis, 2019).

**2.4 Action in Skeleton Biology**

With the use of accessible transgenic and mutant lines that impact certain cells or tissues, osteogenesis and osteoblast activity may be closely observed (Dietrich et al., 2021). The zebra fish skeleton, in conjunction with cellular mineralized bone material, skeletal muscles, tendon, along with other soft tissues, protects internal organs, aids in movement, and offers mechanical support (Busse et al., 2019). Recent research on the development and regeneration of the morphogenesis of the fins of the zebra fish, *Danio rerio*, indicates that some inductive cues involved in the process are comparable to those influencing the differentiation of bone and cartilage in humans and animals (Vimalraj et al., 2021). Therefore, in order to facilitate research on the intricate processes of bone loss linked to aging and unhealthy habits, a controlled experiment focusing on the changing skeletal alterations in adult zebra fish is necessary (Suniaga et al., 2018).

**2.5 Action in Cancer Biology**

A useful non-mammalian vertebrate model for studying development and illness, including more lately, cancer, is the Zebra fish (*Danio rerio*). It is possible to extrapolate study findings from fish to people due to the remarkable biological preservation associated with cancer programs between Zebra fish and humans (Hason and Bartůněk, 2019). Researchers have long recognized that treating fish with carcinogens is very simple since the chemicals may be suspended or dissolved in water, allowing the animals to be exposed for extended periods of time (Al-Thani et al., 2021). Researchers will use animal models and human cell lines to investigate the functional implications of cancer throughout the course of the next ten years. Its diverse complexity cannot be well captured by a single model; hence, it is essential to rely on many systems. Zebra fish models are being explored for understanding in vivo cancer biology because of their special qualities (Gamble et al., 2021). Because of their translucent bodies and quick cancer growth, embryos are frequently utilized to research tumor processes. They may be applied to quick initiatives like screening campaigns or imaging cancer processes. Since their immune systems and organs are fully grown, adults offer a more realistic in vivo model; yet, cancer development takes 10–14 days to 1 month (Letrado et al., 2018).

**2.6 Action in GIT**

One of the best vertebrate model species for researching illnesses and mutations specific to diseases is the Zebra fish. The intestine of Zebra fish is generated from a basic gut tube, in which the pancreas and liver separate and grow on their own (San et al., 2018). Because the gastrointestinal system of larval Zebra fish (*Danio rerio*) is physiologically and functionally comparable to the human gut, it has become a potent tool for studying bacterial gastrointestinal illnesses. Zebra fish have a number of known genes linked to IBD susceptibility, according to recent research that employed larval Zebra fish to find new anti-inflammatory treatments for the disease (Flores et al., 2023).

**2.7 Action in Kidney**

Major organ systems in the Zebra fish emerge soon after conception, and within 48 hours following fertilization, the pronephros is operational, performing tasks including osmoregulation and blood filtration. More than 150 gene mutations have been found to cause genetic renal disorders, which impact all kidney functions (Gehrig et al., 2018). The cellular makeup and nephron segment pattern of these organisms are similar to those of nephrons in mammals (Wen et al., 2018). Furthermore, Zebra fish, rodent, and big animal models of acute kidney damage, as well as kidney organoids produced from human pluripotent stem cells, have advanced throughout the last 10 years (Hukriede et al., 2022). In contrast to the mammalian kidney, which grows in stages with more developed kidneys (pronephros, mesonephros, and metanephros), the embryonic Zebra fish only develops a pronephros, the most immature form of a kidney. In contrast, the mammalian kidney has millions of nephrons (Haruhara et al., 2023).

**3. DIFFERENCE BETWEEN LAB RODENTS AND FISH MODEL**

The use of animal models in biomedical research helps researchers understand the cellular and molecular pathophysiology of human disease and provides platforms for developing and testing new therapies. The remarkable convergence between mammalian genomes and the numerous commonalities in areas ranging from morphology to cell biology and physiology have made mammalian models—like the mouse—the industry standard for simulating human illnesses (Sur et al., 2023). If we talk about molecular biology, Zebra fish possess 26,206 protein-coding genes (Ding et al., 2024), more than any animal that has been previously sequenced, and their genome has more species-specific genes than those of humans, mice, or chickens (Klein et al., 2018, Nipu et al., 2025). In comparison with mouse, mice and other animal models, zebra fish possess some advantageous reasons as a model of human diseases. Firstly, within a few weeks a pair of zebra fish can produce hundreds of fertilized eggs and require very minimal space to grow them. While mouse or mice or other vertebrates requires months to give birth to babies and also needs the high maintenance. Secondly, to check the progress of the disease inside the organ, the animal has to sacrifice for the sake of surgery or postmortem. Whereas the transparency of zebra fish helps the scientist to observe the growth of disease inside the fish and are allowed for the real-time imagining of internal organs. Lastly, the studies can be done using zebra fish very after the fertilization of fish eggs and on embryos, while to start the study on any animal models, the animal should gain a mature age and requires a specific body weights and other physical specifications (Brown et al., 2023). In case if there is any disease affected animal in a group of animals, other animals of the laboratories can be saved by isolation that diseased animal (Rosada et al., 2024). While if any zebra fish population might be affected by the onset of sickness, which could potentially lead to the population's demise. Therefore, it is crucial to avoid sickness in an entire community. This becomes extremely important in an older colony since it is difficult to replace sick fish and the introduction of a deadly infection or water pollutant can ruin years of hard work (Keller and Keller, 2018). Multiple Sclerosis (MS) animal models provide important information about the pathophysiology of the illness and possible treatment options. Zebra fish models such as EAE (Experimental Autoimmune Encephalomyelitis) can be used to examine the cellular processes of demyelination and remyelination, even if they do not precisely resemble multiple sclerosis. New zebra fish models have recently been created, which helps in understanding disease pathways and screening possible treatments (Burrows et al., 2019). The balance between energy production and utilization is maintained by various organs like the brain, intestines, liver, skeletal muscle, and adipose tissue (Figure-2). Zebra fish are a suitable model for studying metabolic dysfunction due to their conserved lipid storage system and all organs involved in energy homeostasis and metabolism, including appetite and insulin regulation (Teame et al., 2019). The researchers employ zebra fish models since one of the problems with utilizing animals is that they are quickly disturbed, making it impossible to fully utilize or trust certain human characteristics in certain situations. When looking for new experimental designs to lessen, enhance, and eventually replace the usage of animal models, the zebra fish were found (Kandasamy et al., 2022). Additionally, because of its tiny size, the zebra fish model has a drawback in that it is difficult to get many organ samples in separate procedures; but, because of its bigger organ size, it has an advantage in animal models including rats or mice (Zhong et al., 2022). In contrast to other animal models (mammals and invertebrates) utilized in drug development and disease modelling, zebra fish offer a practical option that strikes a compromise between ease of use and simplicity on the one hand, and human relevance on the other. In essence, the zebra fish model bridges the gap between the intricate mouse/human models and the crude warm/fly ones (Ghada and Nada, 2018).

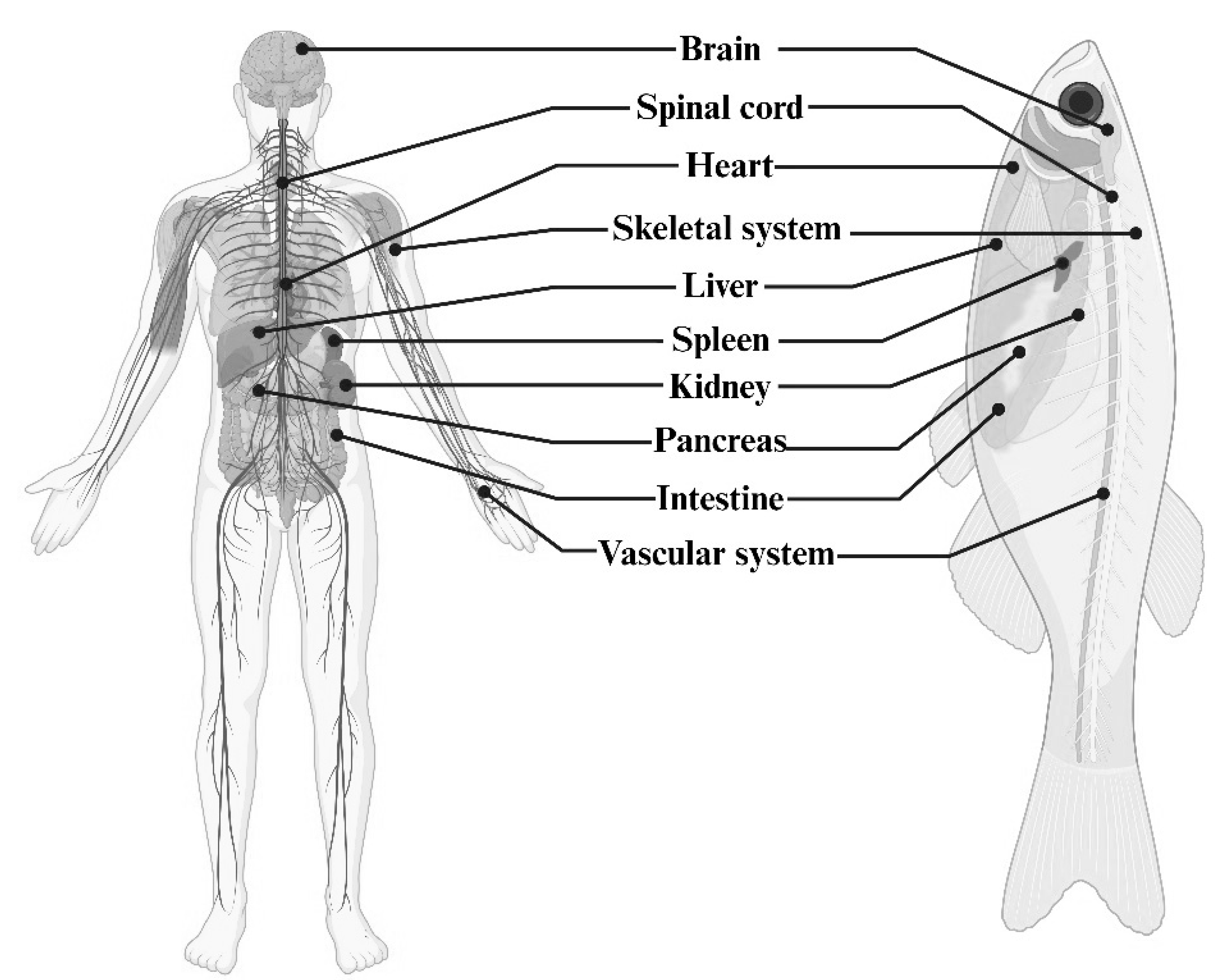


Figure-2: Similarities between body parts of human and Zebra fish

**4. SIMILARITIES BETWEEN ZEBRA FISH AND HUMANS**

The Zebra fish is a novel model system for investigating human illness and a significant model system for understanding vertebrate development. Understanding how the genomes of Zebra fish and humans are related can help understand the roles of human genes acquired from Zebra fish mutations, as well as Zebra fish models for genes associated with human disorders (Gerlai, 2023). Numerous investigations have shown that the Zebra fish genome is a readily available resource for identifying human gene homologs, and the fish can assist with the functional examination of these genes (Maliha et al., 2024). Zebra fishes have similarities with humans and other animals in their ocular and visual functions, which is consistent with neurology and genetic descriptions (Richardson et al., 2016). Compared to Drosophila and nematodes, two of the most commonly used non-vertebrate model animals in genetics and neurology, Zebra fish are significantly more sophisticated and evolutionarily closer to humans (Shams et al., 2018), and it is also found in research that, unlike mammals, Zebra fish have the unusual capacity to regenerate neurons along their rostrocaudal brain axis throughout their lives (Saleem and Kannan, 2018). Since the anatomy, function, and regulatory processes of Zebra fish and humans are so similar, the Zebra fish, *Danio rerio*, is used as an elective model organism in the study of vertebrate skeletal biology (Carnovali et al., 2019), those are found similar in human foetus, which is associated with severe phenotypes (Brecht et al., 2023). Fish lack significant lymphoid accumulations, in contrast to the skin of mammals, and particularly human skin, which contains several active defense systems. Actually, not much is known about the lymphoid cells' native position in the fish integument or if inflammation or an immunological response attracts them (Campos‐Sánchez and Esteban, 2020). Humans and Zebra fish have comparable brain anatomy. Zebra fish have the forebrain, midbrain, hindbrain, and spinal cord. Zebra fish's optic tectum, thalamus, and cerebellum are similar to human brains (Gawel et al., 2019). These findings can be used to discover pharmacotherapies that may lessen the toxicity of potential treatments, reveal toxicity processes, and rank safer chemicals for testing in mammals (Cassar et al., 2019).

5. **LIMITATIONS OF ZEBRA FISH MODEL**:

Though the application of Zebra fish models in biomedical and pharmacological research is much more effective but, in some areas the model is not much suitable, viz., in the case of research on mammalian physiology due to the lack of all the organ systems as required for the mammalians. Even in complex research areas like neurological and endocrine functions determination, the model shows limitations (Roper and Tanguay, 2018).

**6. SCIENTIFIC DATA EVIDENCES ON ZEBRA FISH:**

There are several works that have been carried out from the past a decade and all are scientifically validated with wide acceptability. Recently the works on this Zebra fish are focused with high demand with their many molecular level studies. Some of the recent activities are highlighted in the present review:

Zebra fish have shown increasing utility in biological psychiatry, particularly in studying aggression. They can be used to analyze aggression in models that are not fully established in other species, such as the mirror test, which records simple behavioral traits without physical harm. Studying multiple Zebra fish agonistic behaviors can reveal novel neural mechanisms underlying aggression and social hierarchies in this species. Compared to mammals, fish are simpler creatures that may be more morally appropriate for evaluating stressful aggressive behaviors since they injure fewer conspecifics during conflicts. This supports the wider application of Zebra fish models to probe aggression biology and pathobiology, as well as their relevance to human aggressive behavioral disorders and other neuropsychiatric illnesses (Zabegalov et al., 2019). Zebra fish can be used to study disease states, mutations in targeted genes, and basic renal physiology. By implementing loss of function models and morpholinos, researchers can understand the impact of these genes on human kidney function. Zebra fish can also assess the pathogenicity of genetic variants in patients with kidney disease. Overall, Zebra fish is a valuable model for studying renal function and disease (Outtandy et al., 2018). Zebra fish have been a popular model for infection biology, with recent research focusing on GI tract-pathogen interactions. The Zebra fish's ease of intravital imaging and statistical power make it suitable for addressing complex questions. It may help uncover new cellular microbiology, identify novel virulence factors, and aid in the discovery of effective therapies. Future studies using Zebra fish to study host-microbiome interactions will continue to identify host-intrinsic and host-extrinsic factors and selective pressures critical for shaping the host microbiome, potentially aiding in the development of novel therapies for combating microbiome-associated diseases (Flores et al., 2020). In addition to offering important insights into infection biology, Zebra fish models of bacterial infection can make significant strides in our knowledge of cellular immunity. This model will be further supported by high-resolution microscopy and advanced gene editing tools to better understand pathogenesis and basic cellular processes. Due to its adaptability and speed of discovery, the Zebra fish infection model is useful for studying the cell biology of newly developing pathogens in vivo and uncovering unexpected features of the host-pathogen interaction. Preclinical drug development and toxicity testing are increasingly being conducted using Zebra fish species (Torraca and Mostowy, 2018). In Zebra fish embryos, nimatrelvir had no effect on survival rates or morphological abnormalities. Higher dosages, however, resulted in less development, hatching, and pericardial edema. To evaluate pharmaceutical safety during pregnancy, more research is required (Zizioli et al., 2022). A novel model for tracking glycosylation processes in vertebrate development is provided by the Zebra fish *Danio rerio*, which can provide light on human disorders brought on by abnormalities. Designing Zebra fish lines with specific glycosylation profiles is made possible by the accurate glycomic profiles of eight organs, which display exact patterns of protein glycosylation and glycosphingolipid production (Yamakawa et al., 2018). Spinal deformity and poor osteogenesis can result from the loss of Mapk7, which is essential for bone growth and homeostasis. By causing damaged osteogenesis through RPS6KA3 and its substrates, a decrease in MAPK7 pathway activity and gene expression might deepen our understanding of bone homeostasis (Zhou et al., 2018). Although they are frequent contaminants in freshwater and marine environments, the toxicity of microplastics (MPs) and nanoplastics (NPs) is not well recognized. Dose-dependent increases in accumulation and changes in behavioral responses were observed in Zebra fish larvae exposed to 50 and 200 nm polystyrene nanoparticles. Although exposures had no effect on malformations, mortality, or hatching rate, transcriptome analysis revealed motor dysfunction and neurodegeneration. The study offers a thorough grasp of how NP affects the health of people and animals (Pedersen et al., 2020). According to the study, oxidative stress and dysfunctional mitochondria are important factors in controlling hepatic lipid metabolism, which is enhanced by Cd through modifications in lipogenesis and lipolysis (Pan et al., 2018). Cost, quick development, transparency, and survival without a completely working circulatory system are some benefits of using Zebra fish as a conserved model system for cardiovascular illness. It is essential to medication research and can assist in identifying patients' concerns about drug safety. Future research ought to look into the molecular targets, pathways that result in comparable phenotypes, and new targets that have an impact on cardiovascular development (Zakaria et al., 2018). According to the study, medication toxicity may be assessed using Zebra fish embryotoxicity assays, which could be a good substitute for lab animals like rats, mice, and rabbits. Compared to adult Zebra fish, Zebra fish embryos and larvae are more vulnerable to poisons. The Zebra fish model can assist in finding novel drugs for human illnesses and shed light on the toxicity processes of medicinal plants (Chahardehi et al., 2020). According to the study, medication toxicity may be assessed using Zebra fish embryotoxicity assays, which could be a good substitute for lab animals like rats, mice, and rabbits. Compared to adult Zebra fish, Zebra fish embryos and larvae are more vulnerable to poisons. The Zebra fish model can assist find novel drugs for human illnesses and shed light on the toxicity processes of medicinal plants (Ortega-Recalde et al., 2019). The Zebra fish, which resembles the human embryonic yolk sac in structure, is a good model to research toxicant alterations in early embryonic feeding. Since of its metabolic activity, the yolk is a focus for investigations in developmental toxicology since it can store hydrophobic toxicants and promote exposure during the period when the yolk is used for feeding (Sant et al., 2018). Significant genetic and neurochemical alterations in Zebra fish exposed to VPA last throughout adulthood and impair social behavior. These results support the use of Zebra fish as a model organism for studying neuropsychiatric illnesses and emphasize the significance of the histaminergic system in these conditions (Baronio et al., 2018). High intergenotype diversity in phenotypic expressivity is revealed by this study's analysis of skeletal phenotypes in Zebra fish with various type I collagen variants. According to the results, Zebra fish may help discover the genetic modifiers and underlying mechanisms of phenotypic heterogeneity in Ehlers-Danlos syndrome and osteogenesis imperfect (Gistelinck et al., 2018).

**7. FUTURE ASPECTS AND OPINION**

With high-throughput genetic and pharmacological screening for epilepsy treatments, Zebra fish research is expanding quickly to study epileptic convulsions. When it comes to human brain disorders, imaging technologies offer previously unheard-of data about neurological growth and function. Zebra fish have the potential to be used in epilepsy research because of their conservation of neurological and developmental pathways (Yaksi et al., 2021). Although Zebra fish research in behavioral neuroscience is still in its early stages, technological developments will result in more sophisticated testing, novel medications, and possible applications in ingredient and food safety studies (Bailone et al., 2019). By examining the similarities and differences between the Zebra fish and human immune systems and doing comparative analysis, future research should improve Zebra fish models for investigating viral infections (Fajar Sofyantoro et al., 2024). Numerous regulatory elements are pertinent to study in neuropharmacology and neurotoxicology, and they are somewhat unique to Zebra fish and other aquatic species. Zebra fish have a bright future even if we still don't fully understand their behavioral and physiological activity (de Abreu et al., 2019). As of yet, Zebra fish are not regarded as a typical model for studies in nanomedicine. To completely describe the model, analyze possible uses, and determine its predictive usefulness for research involving higher animals, more research is required. Accurate pharmacokinetics or therapeutic efficacy prediction necessitates accurate physiological characteristics (Sieber et al., 2019). In the future, the effectiveness of a whole medication delivery system may also be assessed using Zebra fish. Since persistent angiogenesis is a crucial change in cell physiology in neoplasia, cancer treatment, which targets tumor cells or angiogenesis pathways, is a significant area nowadays (Rothenbücher et al., 2019). As new genetic engineering and cutting-edge screening methods continue to be developed, their success, appeal, and usefulness will only increase (Katarzyna et al., 2022). Particularly in neurodevelopmental research, *Danio rerio* has given researchers a novel platform that has produced insightful results. This species has been beneficial for a number of neurological illnesses, including Parkinson's disease, Alzheimer's disease, and Huntington's disease. In contrast to primates and rodents, Zebra fish have made molecular investigations feasible because of their transparent embryos, ease of genetic modification, and brief life cycle (Razali et al., 2021). Zebra fish is an excellent aspect for the research of genotoxicity and DNA studies (Canedo and Thiago, 2021). There is still some need to establish a benchmark for Zebra fish husbandry and serve as a forum for facility managers, husbandry staff, veterinarians, and other interested parties to talk about potential advancements in the field (Aleström et al., 2019). By using the Zebra fish model, in the future, personalized medicines can be developed for disease and treatment for the betterment of the population in worldwide scenario (Jaanus et al., 2020). The field of Zebra fish study is always growing as new instruments complement established methods (Crouzier et al., 2021).

**8. CONCLUSION**

Zebra fish are valuable vertebrate biology models due to their quick growth and translucent embryos, which accurately represent human illnesses. They offer benefits over mice for high-throughput drug screening and genetic screening and can be used to study various illnesses like cancer, liver problems, and psychiatric issues. Zebra fish are useful for understanding the pathophysiology of human diseases and developing novel treatments, as they have a short development period, transparency, and the ability to track disease spread. They can also be used to study energy balance, metabolic dysfunction, and multiple sclerosis. Zebra fish provide a balance between usability, simplicity, and human relevance, making them an ideal choice for vertebrate skeletal biology research. Their genomes can assist in the functional analysis of genes linked to human illnesses and identify human gene homologs. Zebra fish research is expanding to study epilepsy treatments, behavioral neuroscience, viral infections, neuropharmacology, and neurotoxicology. Their transparent embryos, ease of genetic modification, and brief life cycle make molecular investigations feasible. Zebra fish husbandry is crucial for setting benchmarks and promoting advancements in the field.

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