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Zebrafish: A Versatile Model Organism for Toxicological Research and Applications

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The field of scientific research has advanced rapidly, with the emergence of more sophisticated techniques and an increasing diversity of experimental models. Among these, the zebrafish (*Danio rerio)* has distinguished itself as a promising model. This work aims to review the literature on zebrafish as an experimental model, highlighting its characteristics, efficacy, limitations, and applications in toxicology research. Despite some limitations, such as the lack of standardization

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among strains, indirect exposure to compounds during development, and the absence of certain organs, zebrafish have proven to be highly effective. The rapid development of their larvae allows for the assessment of both acute and chronic effects of substances. In cardiotoxicity studies, zebrafish embryos have been effective in analyzing environmental pollutants and serve as models for metabolic and synaptic deficiencies caused by pesticides, as well as for neurotoxicity studies, contributing to the prevention of neurodegenerative diseases induced by oxidative stress. Furthermore, zebrafish have demonstrated their suitability for evaluating genotoxic and hepatotoxic effects. In toxicology, they can be utilized in nanosafety approaches for drug distribution, cancer treatment, and transgenic models with fluorescent organs for drug tracking. In the field of genetics, zebrafish are widely used for genome editing via CRISPR/Cas and in in vivo studies with transgenic animals. With technological advancements, it is expected that the applications of zebrafish will continue to expand, making them an essential tool in various scientific fields. Thus, the zebrafish is one of the most appropriate and efficient models for toxicology research, providing an effective alternative to traditional models.

Keywords: Biomodel; danio rerio; embryonic development; ecotoxicology; toxicokinetics.

1. INTRODUCTION

Toxicology is a science dedicated to the study of various chemical substances and their generated effects, addressing and evaluating their harmful impacts on the environment and living organisms. This science is of great importance as it has a multidisciplinary nature, encompassing themes such as biology, chemistry, pharmacology, ecology, public health, among others Bachur, T. P. R. [1]. In recent years, the field of toxicology has advanced exponentially, with the number of publications on research and reviews in the area following the same pattern [2].

This behavior has been observed for many decades, with toxicological studies, even if simple, being recorded since before the Middle Ages concerning poisons and toxic plants. This trajectory has evolved through discoveries of therapeutic agents and has matured scientifically into modern times, where numerous researchers are dedicated to understanding complex chemicals, ensuring food safety, producing pharmaceuticals, and herbal medicines, as well as gaining a deeper understanding of the effects of toxic compounds Loregian, B. [3].

This progression is marked by technological advancements, where new tools and more refined techniques provide not only greater ease and speed in conducting existing processes but also the emergence of new approaches. Thus, technological development has begun to assist researchers beyond traditional systems, with the introduction of new procedures such as in vitro testing, nanotechnology, and even nanotoxicology [4]. Computational tools such as simulations (*In silico*), epigenetics, and toxicogenomics [5].

In relation to this, another methodology that has evolved is linked to experimental models. Ferreira et al. [6] state that an experimental model is the materialization of a part of reality through a simple representation that must present precision and similarity to what is intended to be analyzed. In line with this, laboratory animals are the most commonly used. This has been the case for many years, as they provide essential mechanisms for the development of scientific work, such as the opportunity to perform in vivo procedures and represent real and natural conditions. Additionally, physiological and genetic similarities, ease of manipulation, the ability to induce diseases, and well-established protocols for the use of animal models contribute to their relevance Fagundes et al. [7].

However, despite the notable laboratory rats and mice, other species have emerged as alternatives to replace them. These include fish, which have become significant allies in science as alternative models that demonstrate equal or even superior efficiency compared to traditional systems. Certainly, these animals respond to the requirements of the Animal Ethics Committee (CEUA) concerning the principle of the 3Rs.

The concepts of reduction, refinement, and replacement are indispensable elements in scientific research. They aim to ensure that advancements are linked to ethics, with the objective of minimizing suffering and ensuring the proper management of animals in experiments Freires et al. [8]. In this regard, the

Zebrafish, emerges as one of the most commonly used samples when the methodology refers to the use of aquatic vertebrates.

In this context, the Zebrafish is not new to the scientific community. Researchers have focused on this species since the 1950s, when biologist George Streisinger conducted genetic analyses on the animal. By 1990, a group of scientists in Oregon hosted a meeting regarding the characteristics of this model for researchers from around the world. In Brazil, the country has been one of the largest producers of research involving Zebrafish, and the trend is towards growth, as many topics related to toxicology and behavioral neuroscience are extensively addressed here, making this fish exceptionally well-suited for such studies Costa, T. L. C. D., [9].

In light of this, the present work aims to conduct an in-depth literature review on Zebrafish as an experimental model, its characteristics, effectiveness, and limitations, as well as its applications in the study of toxicology, providing a comprehensive perspective on the subject.

2. METHODOLOGY

Searches were conducted in the databases PubMed, SciELO, and ScienceDirect using the following keywords: "Experimental fish model applied to toxicology," "Use of fish as an experimental model for toxicological studies," and "Zebrafish and in vitro experimental model." A total of 1,047 articles were found on the PubMed platform, 0 on SciELO, and 39,605 on ScienceDirect. Additionally, filters were applied in the ScienceDirect database to exclude encyclopedias, editorials, and reviews, selecting only research articles, review articles, and book chapters. Subsequently, the process of selecting and excluding articles was based on a review of titles and abstracts, eliminating those that did not address or deviated from the central theme of the article, namely, the use of fish in toxicological studies, In the end, 46 articles were included. Furthermore, the selected articles span a period of ten years, from 2014 to 2024.

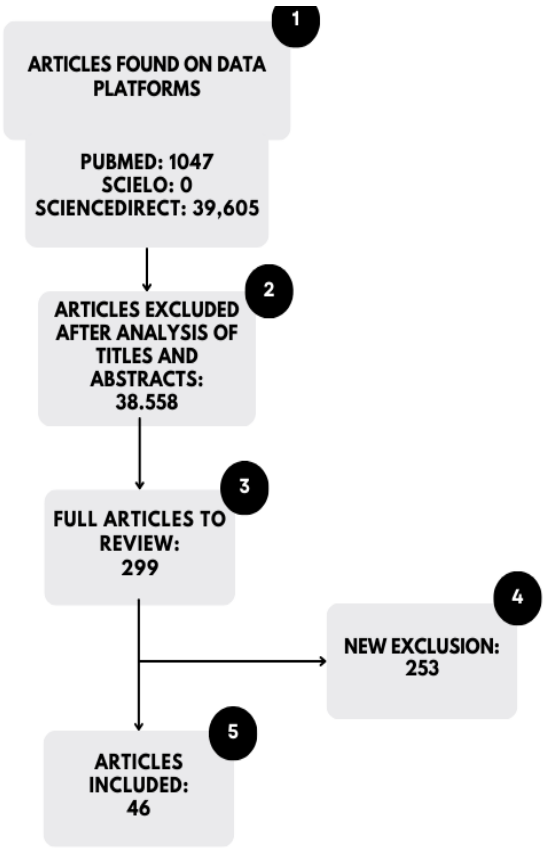

Fig. 1. Flowchart of the article screening process
Source: Authors (2024)

3. RESULTS AND DISCUSSION

3.1 Characterization of Zebrafish

Danio rerio, commonly known as Zebrafish due to the striking stripes that run along the sides of its body, is a notable teleost fish, specifically belonging to the family Cyprinidae, with origins in South Asia Ota and Kawahara, [10]. This species has a small size of approximately 2 centimeters and can reach up to about 4 centimeters [11]. In terms of its habitat, these animals are freshwater fish and typically inhabit shallow, slow, or even stagnant waters [12].

Following this, Zebrafish can exist in three stages: larval, juvenile, and adult, and can live up to five years. Its development is notably rapid, with these periods measured in hours post-fertilization (hpf) or days post-fertilization (dpf). Total embryonic development occurs at 72 hpf, and it reaches adulthood by the end of 90 dpf [13]. Furthermore, this aquatic vertebrate exhibits diurnal behavior, and the spawning of its eggs typically occurs during the early hours in the presence of light; thus, the photoperiod is an important factor in the environments where they are kept, allowing for a stable environment and a regular circadian cycle [14].

Additionally, despite its natural habitat, this tropical fish is commonly sold commercially for use in personal aquariums but is primarily utilized in scientific research, living in breeding facilities.

3.2 Advantages Vs. Limitations

Regarding the use of Zebrafish as an experimental model, this fish presents various advantages that make it an excellent asset for research. Firstly, in experiments aimed at evaluating toxicity, the effects of a metabolite, or even those intending to generate new medications for human use, it is essential that the sample possesses a certain degree of compatibility so that the generated effects do not deviate from reality. In this regard, Zebrafish has its genome completely sequenced, as reported by Howe et al. [15] in 2013, with 70% homology to that of humans, allowing for the modeling of many human diseases in these animals [16].

Additionally, there are anatomical similarities with mammals, as this fish has structures such as the heart, kidneys, liver, pancreas, bones, muscles, and central nervous system (CNS). Furthermore, there is correspondence regarding the immune system, sensory system, and signaling profiles. In other words, the similarities are not limited to a single factor but are evident at physiological, molecular, anatomical, and genetic levels [17].

Regarding reproduction, females exhibit high fecundity [18] and are capable of producing approximately 200 to 300 eggs per week, with embryogenesis completed after 72 hpf and reaching new reproductive maturity by the end of 3 months [19]. This characteristic of rapid development is highly valuable for experimentation, as it allows researchers to avoid relying on extended growth periods, enabling them to observe all stages of the fish and quickly focus on tests and analyses.

In this regard, the process of embryonic maturation occurs *Ex vivo*, meaning outside the organism, and the embryo is transparent during the early stages of development. This factor facilitates the visualization of responses, enabling the monitoring of inflammatory processes, imaging-based tracking of treatment impacts or the effects of a chemical compound, as well as the visibility of fluorescent markers and morphological changes [20,21,22].

Moreover, due to their small size as vertebrates, they can be stored together in larger quantities compared to other biomodels such as primates, while still avoiding stressful conditions. For this reason, their breeding and maintenance within laboratory aquariums become less costly [23]. Additionally, swimming profiles, social behavior, light-dark preference, anxiety, and aggressiveness are observable in Zebrafish and can be utilized as assessment tools in neurotoxicological testing [24].

Despite this significant number of advantages, there are still some limitations regarding the use of this animal. The lack of standardization and firm establishment of Zebrafish strains is a concern, as this heterogeneity can lead to variations in experimental studies utilizing them. Their size also necessitates more refined and sensitive techniques. Since they develop externally and are protected by a chorion until approximately 72 hours post-fertilization (hpf), the exposure of the fish to the absorption of a compound may be indirect and relatively minimal. Lastly, the absence of organs such as lungs and the lack of proper limbs, joints, and mammary glands disqualify them from research that may involve these targets [25,26].

3.3 Traditional Model and Alternative Model

When discussing scientific research involving animal experimentation, the first species that come to mind are the well-known rats (*Rattus norvegicus*) and mice (*Mus musculus domesticus*). However, in contemporary science, questions have arisen regarding animal pain and suffering, leading to an increased need for the application of alternative models.

As a result, despite being proven effective, the need arose to establish a model that required low maintenance while maintaining efficacy. Zebrafish have been used in research for quite some time, with George Streisinger pioneering the field of Zebrafish research in the 1970s [27]. He sought a model that could be used in genetic studies and, as an aquarist, recognized the advantages of Zebrafish. Its rapid development, small size, and ease of maintenance were some of the characteristics that led to Zebrafish being considered a model organism [28].

It was around the 1930s that Zebrafish were introduced as a model organism for developmental and embryological studies. Consequently, it was applied as a genetic model following advances in genetic methodologies, including cloning, mutagenesis, and transgenesis [29]. Thus, Zebrafish were proposed as a model system for understanding and studying vertebrate development. Shortly thereafter, the Zebrafish genome was sequenced, revealing that 70% of human genes are homologous to those in Zebrafish [28].

From that point on, Zebrafish established themselves as a model for understanding human diseases.

3.4 Applications in Toxicology Studies

Zebrafish as an experimental model represents a viable alternative to the mammalian models currently used in toxicity testing and other biological research. Since Zebrafish are easier and less expensive to house and care for than popular rodent models [21]. Additionally, the rapid development of Zebrafish larvae allows for the evaluation of both acute and chronic effects of substances on their organism [30]. Features such as their small size, transparent embryos, rapid external development, and high reproductive capacity [31] have contributed to making Zebrafish an attractive model for developmental studies.

3.4.1 Cardiotoxicity

Although Zebrafish and humans have distinct structures for their cardiovascular systems: such as the two-chambered heart with one atrium and one ventricle in Zebrafish, compared to the four-chambered heart in humans-Zebrafish are still used as an alternative model for studying the cardiovascular system. This is due to their advantages, including low cost, external development, transparent embryos, the possibility of genetic manipulation, and genetic homology with humans [27].

An example is the study by Arman and İşisağ Üçüncü, [32], which evaluated the cardiotoxicity of Zebrafish embryos exposed to acrolein, a pollutant found in the environment that can be derived from the incomplete combustion of organic substances, industrial discharges, power plant emissions, among others. Another example is the work by Ming Li et al. [33], which analyzed muscone, one of the most physiologically active compounds of natural musk, frequently used to treat heart diseases. The results showed that the compound is toxic to Zebrafish embryos, causing mortality, reduced hatching rates, pericardial edema, and decreased heart rate.

Anthracyclines are an effective chemotherapy for antineoplastic treatment; however, cumulative cardiotoxicity is the primary side effect with a poor prognosis, and currently, there is no therapy that reverses anthracycline-induced cardiotoxicity. The article by Lu et al. [34] aimed to demonstrate the cardioprotective effect of Calycosin against anthracycline using Zebrafish as an embryonic model.

3.4.2 Neurotoxicity

Neurotoxicity occurs when exposure to toxic substances alters the normal activities of the nervous system, including neural transmission, connectivity, and survival [35]. In the work of Faria et al. [36], it was demonstrated that fenitrothion, an organophosphate insecticide, caused neurotoxicity in fish after 24 hours of exposure. The toxicological effects were analyzed through behavior and the expression of gap43a, gfap, atp2b1a, mbp, and syt1a.

Another example is Azevedo et al. [37], whose study investigated the metabolic and synaptic

deficiencies triggered by pyriproxyfen, a pesticide with various applications, including antiparasitic medications for pets and domestic/agricultural pest control.

Additionally, the study by Prabha et al. [38] evaluated the antioxidant and neuroprotective capacity of the RW20 peptide. This peptide is derived from histone acetyltransferases and was analyzed with the aim of being a protective agent against neurodegenerative diseases caused by oxidative stress.

3.4.3 Genotoxicity

Genotoxicity generally refers to DNA damage, which includes single-strand breaks, double-strand breaks, and the formation of adducts, as well as alterations in the genes/proteins involved in repair. Zebrafish have been suggested as a suitable model for evaluating these effects [27]. Derikvandy et al. [39] used Zebrafish to analyze the toxicological effects of untreated wastewater from the ethyl alcohol industry. Their results showed that after 21 days of exposure to 2% wastewater, the expressions of the genes *Sod1*, *Gstp-1a*, and *Gpx1a* were increased in hepatocytes. Thus, the authors concluded that the analyzed water caused significant increases in the expression of antioxidant and detoxification-related genes in the liver of Zebrafish.

Chowdhury et al. [40] investigated the concentration of amoxicillin residue (AMXR) in raw and pasteurized milk samples from cows with mastitis. The study was conducted using the standard HPLC method compared to pure amoxicillin, examining the effect of the residue on developmental toxicity and genotoxicity in Zebrafish. The results showed DNA damage caused by AMXR.

3.4.4 Hepatotoxicity

The liver is the largest gland in the bodies of vertebrates. It plays an important role in the metabolism of carbohydrates, fats, proteins, vitamins, and hormones, as well as in detoxification regulation. Consequently, the liver is the primary target organ for toxic reactions. Hepatic injury due to toxic substances, or hepatotoxicity, is primarily caused by drugs and poisons that induce liver damage, including oxidative stress and apoptosis [41].

An example is the work of Hu et al. [42], which investigates Iprodione, a broad-spectrum fungicide used for the early control of fruit trees and vegetables. Due to rainfall, it may pose a risk of toxicity to non-target individuals, and the authors specifically focus on liver development. They concluded that Iprodione can result in hepatotoxicity in Zebrafish and may potentially represent toxicity risks for other aquatic fish, organisms, and even humans.

Shaw et al. [43] analyzed the effect of shikimic acid, a mycosporine-like amino acid, against stress induced by hexavalent chromium, a toxic heavy metal, in the hepatocytes of Zebrafish. The work of Guidony et al. [44] studies the toxicological effect of the bactericide Triclosan on the hepatocyte cells of Zebrafish.

3.5 Future Perspectives

In light of all the above, Zebrafish is poised to emerge as an appropriate experimental model with many applications yet to be tested. Regarding toxicology, this animal could be incorporated into nanoscale approaches for the distribution and absorption of drugs, tested for cancer treatment using inhibitor medications, and there is also the possibility of generating transgenic models with fluorescent organs for tracking and drug screening [45].

Moreover, beyond the field of toxicology, this biomodel is expected to expand in applications in genetics, where it has already proven convenient for genome editing using CRISPR/Cas to study functional aspects of pathologies [46], as well as for generating transgenic animals and their application in in vivo studies [18]. In the realm of neurobehavioral studies, they can be assessed for cause and effect of neurotoxins generated by environmental pollutants [47], which is a relevant theme considering contemporary conditions. Additionally, studies on chronic stress, anxiety control, and other complex brain disorders [48,49,50] are also possible.

Thus, Zebrafish is expected to see its applications increasingly expanded across various and relevant fields of scientific knowledge, with technological advancements serving as significant contributors to the rapidity of this occurrence, leading to the development of ever more sophisticated and sensitive techniques.

4. CONCLUSION

In light of the information presented, a positive outlook can be observed regarding the use of an alternative model to those commonly employed in scientific experimentation. Zebrafish has proven to be one of the most suitable and efficient samples when the goal is to conduct research in the field of toxicology. Its physiological, anatomical, embryonic, and genetic characteristics explain this. Additionally, its affordable cost and alignment with the 3R's criteria of animal experimentation, by being a potential substitute for the rat and mouse models, offer the advantage of providing statistically significant results that are both reliable and efficient. Zebrafish stands out in toxicology subfields such as cardiotoxicity, neurotoxicity, genotoxicity, and hepatotoxicity. In these areas, it plays a key role in investigating drugs and toxic substances through exposure and absorption analyses.

Despite all these advantages, limitations such as its small size and the need for refined techniques still exist. However, Zebrafish continues to emerge as a promising experimental model in science. Studies focusing on nanoscale applications and screenings, the use of transgenic models to mimic human disease models, and the application of CRISPR gene editing to create mutations are just a few of the new themes and perspectives advancing, positioning Zebrafish as a suitable target biomodel. It is hoped that more studies will be developed using this animal model, that its advantages will be further applied, its limitations reduced, and new investments made in the use of this biomodel.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1. The generative AI technology used was ChatGPT, which is based on large language models (LLMs) developed by OpenAI. The applied version is the most recent in the series, belonging to the GPT-4 (Generative Pre-trained Transformer 4) family. All of the generative artificial intelligence behind ChatGPT was developed and trained by OpenAI.
2. The technology was used strictly to assist in the translation of the manuscript from Brazilian Portuguese to English for the purpose of publication in the current journal. After the translation, the text was reviewed again by team members to identify and correct any potential translation errors.
3. The input prompt used was: "Translate the text from Portuguese to English, without altering the meaning of the sentences."
4. Example of using gpt chat:

   Original language: O campo da investigação científica tem avançado exponencialmente nos últimos anos, surgindo técnicas mais sofisticadas e crescendo também a diversidade de modelos experimentais.

   Tradução para o inglês com auxílio da tecnologia: The field of scientific research has advanced exponentially in recent years, with more sophisticated techniques emerging and the diversity of experimental models also growing.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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