*Review Article*

Various Animal Models of Genotoxicity: Zebrafish Potential Use in Genotoxicity

ABSTRACT

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| Evaluation of genotoxicity serves as a necessary safety assessment process for pharmaceuticals and cosmetics together with food additives and industrial chemicals. The evaluation demonstrates the crucial need to study DNA damage methods because these processes contribute directly to cancer development alongside infertility obstacles and neurodegenerative diseases and metabolic system disorders. Researchers widely use Zebrafish (Danio rerio) as an important model because their genes closely match human genes (around 70 percent) and their quick embryonic development combined with transparent embryo visibility enables live cell observation. Through the combination of genotoxicity testing methods that include the Comet assay together with the Micronucleus test and TUNEL assay researchers gain effective monitoring of DNA damage from environmental pollutants and pharmaceuticals and radiation. The testing methods generate mutagenic event data while running large-scale evaluations at reduced costs compared to standard mammalian experimentation protocols. Although faced with regulatory hurdles and unique metabolic characteristics of different species zebrafish models provide important benefits toward better genotoxicity research and improved safety testing in pharmaceuticals and environmental studies. Artificial intelligence and advanced transgenic techniques show promise to improve zebrafish genotoxicity research by integrating them in future research and development. |

*Keywords: Genotoxicity, Zebrafish (Danio rerio), Comet assay, Micronucleus test, TUNEL assay, Genetic similarity, Transgenic models, Oxidative stress*

1. INTRODUCTION

**1.1. Background on Genotoxicity Assessment:**

Technical evaluation of mutagens represents a vital step to determine hazards present in pharmaceuticals and cosmetics as well as food additives and insecticides and multiple industrial chemicals alongside consumer products. Mutagens make heritable alterations to DNA structures which appear in both germ and somatic cells leading to unstable genetics that results in disease development. Disease control modulation requires research on DNA repair mechanisms since they protect the genome from damage during the maintenance of genomic integrity. Multiple carcinogens trigger DNA damage to create mutations that end up favoring the development of cancer. Scientists have discovered that nuclear DNA damage shares a similar role in neurodegenerative disease development with mitochondrial DNA damage as both systems contribute to ataxias and the progression of Alzheimer's disease as well as Huntington's disease and Parkinson's disease. Knowledge about such mechanisms serves as a fundamental requirement for creating both preventative interventions and therapeutic solutions against diseases that originate from mutations. [1,2,3] The pathogenesis of infertility and major human diseases such as heart disease and early aging along with stem cell dysfunction and chronic inflammation and metabolic syndromes originates from DNA damage. As genotoxicity is a basic toxicological endpoint, it must be thoroughly evaluated during safety testing. A range of in vitro and in vivo tests are available for the identification of genotoxic hazards. Because of the carcinogenic and heritable hazards presented by these compounds, genetic toxicology testing has become a regulatory necessity for new chemical entities and other materials worldwide. [4,5]

**1.2. Zebrafish as a Model Organism:**

Since Zebrafish (Danio rerio) represent an optimal research model in toxicology and genetics because of their human-like genes and fast growth rate and clear embryology which enables laboratory observation of development and cellular changes. The similarity of zebrafish genes to human ones reaches 70% and functional matches exist between 84% of human disease-linked genes and zebrafish genes which makes this species essential for studying genetic and pharmaceutical research and disease pathologies. [8] Large-scale genetic mutation screening as well as drug activity testing and environmental toxicant examinations in zebrafish are made possible by their external fertilization method and rapid embryonic development that completes essential developmental milestones inside 24–72 hours of post-fertilization. [6] Through their transparent nature researchers can monitor developmental processes inside zebrafish embryos using non-destructive methods which apply to developmental biology along with neuroscience and toxicology research. [7] These small organisms present an outstanding option for extensive genetic research and toxicological examinations because they combine reasonable cost benefits with minimal ethical challenges relative to mammalian testing models according to [9]. In genetics research, zebrafish have been used very extensively to model neurological diseases, cardiovascular disease, and cancer due to the conservation of their genetic pathways with human pathways. [10]

The genetic manipulation process has become simpler through CRISPR-Cas9 tools which helped scientists create precise models for human disease research. [14] Zebrafish play a critical role in scientists' analyses of mitochondrial dysfunction along with its relationship to neurodegenerative disorders. [7] Scientists use zebrafish as fundamental test animals to evaluate environmental and pharmaceutical toxicity and their effects on drug safety as well as microplastic contamination and heavy metal exposure. [11] Zebrafish larvae research in behavioral toxicology has given scientists deep knowledge about neurotoxic activities when examining chemicals affecting locomotion and mental capacities. [12] The research of endocrine-disrupting chemicals and drugs' effects on thyroid and reproductive systems has extensively used zebrafish as an animal model. [13] Zebrafish serve as a strong and multifunctional and ethically acceptable model to push forward genetic study and toxicological analysis.

**2. Genotoxicity models in zebrafish, their applications, advantages, and limitations:**

An assessment is provided in this article about many zebrafish (Danio rerio) genotoxicity models to analyze their functional aspects and their positive features together with their constraints. The genetic correspondence between humans and zebrafish and their quick development period and clear embryonic visibility make zebrafish an excellent model organism for studying genotoxic processes in living subjects.

Different testing methods exist for genotoxic effect examination. Researchers use The Comet assay to evaluate DNA strand breaks at a cellular level according to [15] and apply the Micronucleus test to detect chromosomal damage by counting micronuclei in erythrocytes. [16] The TUNEL assay performs widely used DNA fragmentation assessment to detect apoptosis as well as measuring cellular and gene mutation effects. [17] The γH2AX phosphorylation assay functions as an effective method for detecting double-strand DNA breaks along with their repair processes. [18] The damaging effects of contaminants like heavy metals and pesticides and industrial chemicals that harm DNA in environmental studies can be fully assessed by using these assays when working with zebrafish. [29] The pharmaceutical toxicology field uses zebrafish for performing high-speed drug and safety assessment evaluation. [19] Zebrafish serve as research subjects in nanotoxicology to evaluate nanoparticle genotoxic effects in biomedicine and environmental assessment [20] and scientists use zebrafish to research DNA damage from radiation and available protective agents. [21]

Zebrafish offer several advantages as a genotoxicity model. The close genetic relationship between zebrafish and humans (70% similarity) makes them suitable for valuable biomedical investigations. [18] The quick reproductive pace of zebrafish together with their abundant offspring population supports large-scale genotoxic compound evaluations [22] and their clear embryos enable straight observation of cellular reactions to diverse genotoxic agents. [23] Research using zebrafish presents multiple benefits since it comes along with lower ethical concerns and decreased costs than using mammalian models. [24]

The benefits of zebrafish models in scientific studies are accompanied by continuous remaining challenges. Molecular assay testing depends on pooling small sample amounts because zebrafish cell counts remain low according to [15]. The metabolic differences between zebrafish and mammals create potential barriers for correct chemical metabolism and toxicity evaluations. [25] The acceptance of zebrafish models for toxicity assessments by regulatory bodies is limited since specific tests are still performed using traditional mammalian models. [26] Rodent models provide better suitability for long-term exposure studies since zebrafish live shorter lives. [23]

While zebrafish serve as highly effective research instruments for understanding DNA damage mechanisms and related toxic effects in scientific and biomedical fields.

**3. Various Models used for Genotoxicity Assessment in Zebrafish:**

**3.1 Comet Assay in Zebrafish: An Essential Tool for Genotoxicity Studies**

The Comet assay represents a sensitive analysis technique popularly known as single-cell gel electrophoresis (SCGE) for strand break and oxidative damage detection at cellular levels. Fundamentally the assay functions by observing how electric field speed accelerates broken DNA to produce comet-shaped structures whose length and fluorescence levels evaluate DNA integrity. [27]

Laboratory procedures begin with separating cells from zebrafish tissues including embryos and gills and liver and blood and brain [15] before placing them in agarose gel before performing detergent and high-salt buffer protease removal to safeguard DNA quality. The analysis under basic pH conditions shows three distinct types of DNA damage which include single-strand breaks and double-strand breaks and alkali-labile sites through electrophoresis. [28] DNA damage analysis depends on neutralized specimens that become visible under fluorescence microscopy or confocal imaging after exposure to staining dyes which include SYBR Green, ethidium bromide and propidium iodide. The software systems CASP and OpenComet use image analysis to determine tail length and the percentage of DNA in the tail. [31]

The Comet assay holds immense popularity for studying environmental genotoxicity through examinations of DNA damage that occurs due to heavy metals pesticides and industrial chemicals. [29,32] The DNA damage evaluation of pharmaceutical chemicals including cisplatin and doxorubicin can be accomplished using this test in pharmaceutical toxicology. [19,52] The Comet assay serves in nanotoxicology by evaluating nanoparticle genotoxic effects on silver and titanium dioxide and carbon-based materials through workers who use zebrafish embryos as an efficient platform for nanomaterial safety evaluation. [20,36] Through its diagnostic capabilities the assay assesses UV-based along with X-ray and gamma radiation DNA damage while testing antioxidants as potential radioprotective agents. [21]

Due to its many beneficial features the Comet assay functions well as a fundamental technique for genetic toxicity assessments using zebrafish. This test maintains exceptional sensitivity to measure low levels of DNA damage and fits different zebrafish tissue types like blood and liver and brain. [30,15] The choice of using zebrafish advances cost-effectiveness since their management costs remain low while their high reproduction numbers drive down modeling expenses. [24] Due to their fast life cycle combined with external fertilization zebrafish serve as an excellent platform for running toxicant screenings at high speeds. [20] The ethical benefit of this assay includes the possibility to obtain biological samples without killing the animals using either blood extraction or fin clipping methods. [31]

Although it presents strong advantages the Comet assay faces various limitation points. The reproducibility of the assay becomes compromised because technical variability originates from differences in electrophoresis conditions, lysis time and staining procedures. [25] The analysis software developed by [24] helps eliminate manual subjectivity to detect DNA damage. The assay has gained extensive research use but regulatory bodies show hesitance to accept it for chemical safety testing purposes which bars its placement in standard toxicological rules. [30] The comparison of studies becomes challenging because standardization issues include differences in pH levels, electrophoresis period and score protocols. [28]

Researchers continue to rely on the Comet assay as their foundational method for zebrafish genotoxicity research because it provides essential data regarding DNA structure integrity and mutagenic changes and cellular damage responses to environmental chemicals.

**3.2 Micronucleus Test in Zebrafish: Procedure, Effectiveness, and Comparison with Other Models**

The Micronucleus (MN) test stands as a well-tested method for zebrafish (Danio rerio) genotoxicity assessment which allows scientists to detect chromosomal damage and various other genetic abnormalities in mitotic cells. The testing method identifies micronuclei which represent small cellular structures derived from damaged chromosomes or entire chromosomes after mitotic cell division fails to incorporate them. [16]

During the MN test zebrafish embryos or larvae together with adult peripheral blood cells receive exposure to genotoxic agents before obtaining cellular material from erythrocytes and tissue specimens from gills, liver and kidney for examination. The evaluation under a microscope reveals the percentage of micronucleated cells from the scored total cells by using cells that were fixed and stained with fluorescent or Giemsa dyes. [33] The assay functions efficiently for identifying mutations of chromosomal structures triggered by pollutants and radioactive materials as well as heavy metals and environmental chemicals. [34] The zebrafish MN test demonstrates widespread application in genotoxic effects screening of pesticides and industrial chemicals and pharmaceuticals leading to its significance for both environmental safety assessment and drug development evaluation. [35]

Compared to other genotoxicity models like the Comet assay and γH2AX phosphorylation assay, the MN test is very specific to damage at the chromosomal level while the Comet assay identifies single-strand and double-strand DNA breaks. [37] Even though the MN test is less sensitive in the identification of minor damage to DNA compared to the Comet assay, it describes long-term reactions to genotoxic stress by measuring persistent changes in chromosomes. [38] Besides, the MN test in zebrafish is cost-effective, non-surgical (if blood samples are used), and accommodates high-throughput screening relative to mammalian models. [39] The assessment of micronuclei remains challenging because of subjective evaluation of nuclear fragments together with differences among individuals and the need for standardized stain methods. [33] The Micronucleus test functions as an informative and proven method for genotoxicity testing especially in aquatic toxicology as well as environmental monitoring and biomedical research since its simplicity and reliability and chromosomal aberration detection sensitivity make it applicable.

**3.3 Transgenic Zebrafish Models for Genotoxicity Testing**

Transgenic zebrafish models have revolutionized the field of genotoxicity testing by allowing real-time, non-invasive detection of DNA damage and related repair mechanisms. Survival examination of genotoxic stress in living subjects is enabled through fluorescent reporter gene systems that make use of enhanced green fluorescent protein (eGFP) alongside red fluorescent protein (RFP). These systems employ DNA damage-inducible promoters. [40] Tg(cyp1a-eGFP serves as a popular transgenic zebrafish line by using GFP to detect environmental xenobiotics like benzo[a]pyrene together with other toxicants thus advancing understanding about chemical genotoxic effects. [40]

Research using transgenic zebrafish lines now enables studies of oxidative stress and apoptosis and cell cycle arrest for toxicology examination and environmental assessment and pharmacological screening applications. [41] These entities enable quick genotoxicity evaluations while using fewer samples while surpassing the same advantages by simplifying the requirements for the lab tests such as the Comet assay and Micronucleus test with their cell isolation and staining requirements.

Working with transgenic zebrafish faces three major obstacles that include their expensive development along with maintenance requirements while deploying complex procedures for stable line development and generating hesitations due to genetic manipulation procedures. Data reliability depends largely on the consistent signals emitted from transgenes because natural discrepancies between photobleaching and transgene expression levels and metabolic factors can compromise information accuracy. [41] The challenges of transgenic zebrafish models did not stop them from reshaping genotoxicity research by providing real-time DNA damage observation within living organisms as well as automated high-throughput toxicological research potential.

**3.4 Gene Expression Analysis in Zebrafish for Genotoxicity Assessment**

The assessment of genotoxicity requires gene expression analysis because this technique reveals how DNA damage affects the molecular processes that repair genetic material. The three molecular techniques of quantitative polymerase chain reaction (qPCR), RNA sequencing (RNA-Seq), and microarray analysis are effectively used by researchers to analyze gene expression changes in zebrafish (Danio rerio) exposed to toxicants. [42]

The evaluation method allows scientists to precisely measure important DNA damage and repair elements namely p53 which controls cell cycle arrest and apoptotic responses as well as RAD51 which executes homologous recombination workflows along with double-strand break repair. [43] RNA-Seq generates complete transcriptomic data for identifying new biomarkers but qPCR maintains its position as the preferred method because it provides targeted analysis with high sensitivity coupled with specificity in genotoxic stress evaluation. [42]

The quantification of gpx1 and sod1 genes linked to oxidative stress and the measurement of atm and xrcc5 genes associated with DNA damage response in zebrafish embryos can be achieved when studying their response to pesticides and heavy metals and nanoparticles. The described model demonstrates promising capabilities for both ecotoxicology research and drug screening applications. [43] The gene expression research approach delivers extensive and early genotoxic mechanism insights beyond traditional assays like Comet and Micronucleus test which makes it an advantageous screening tool.

However, challenges such as high costs, technical complexity, and the need for bioinformatics expertise limit its widespread adoption. Gene expression profiling transforms zebrafish-based genotoxicity research by supplying enhanced detection of chemical and environmental genotoxicants and pharmaceutical-related genotoxicants.

**3.5 Other Emerging Models in Zebrafish Genotoxicity Research**

Academic research in zebrafish genotoxicity testing has established two advanced test models through CRISPR/Cas9 gene editing technology and HTS methods. The CRISPR/Cas9 system has revolutionized zebrafish research by allowing scientists to make exact genetic modifications which enhance their research capacity for studying DNA damage repair and its repair methods as well as gene-environment relationships. [44] DNA repair genes like p53, ATM and BRCA1 have recently been made knockoutable by researchers which provides researchers with innovative tools for studying hereditary cancer syndromes and mutagenesis. [45]

The implementation of HTS platforms represents a major breakthrough because these platforms combine automated imaging with robotics and artificial intelligence systems to speed up zebrafish embryo and larva genotoxic effect assessment. [46] The technological systems permit extensive chemical evaluations by permitting researchers to examine thousands of compounds for their ability to induce mutagenic and teratogenic effects within a brief time frame. Zebrafish genotoxic agent assessment through single-cell sequencing technologies allows scientists to examinutational signatures along with transcriptomic modifications in these fish exposed chemicals. [20]

The advanced models bring multiple difficulties even though they show promising potential. The implementation of CRISPR methods needs substantial verification work for minimizing unintended consequences yet automation systems require developed analytical systems to process data efficiently. These methods still have high operational costs together with technical requirements which make them difficult to use at scale. The novel tools continue to enhance zebrafish genotoxicity research by delivering high-precision approaches and efficient as well as scalable examination techniques for analyzing DNA damage and repair within living organisms.

**4. Applications of Zebrafish Genotoxicity Models**

Due to their valuable characteristics zebrafish (Danio rerio) serve as an accepted laboratory model for inspecting toxicity in the environment and exploring pharmaceutical compounds as well as studying damage from radiation in genetic investigations. The assessment of pollutants and industrial chemicals along with nanomaterials on genetic integrity is conducted extensively by using these organisms. The combined effects of heavy metals and pesticides and microplastics result in DNA strand breaks and oxidative stress and chromosomal aberrations according to results from Comet and Micronucleus assays reported in [47]. Zebrafish embryo screening technology provides fast detection of genotoxic water pollutants which makes it a powerful tool for environmental monitoring. [48]

The evaluation of genotoxic hazards in new compounds including anticancer agents along with antibiotics and hormone-based drugs depends heavily on studies using zebrafish as research subjects. The DNA damage along with mutagenic effects and apoptotic changes in zebrafish become detectable in preclinical studies before scientists move their work to mammalian models. [49] Zebrafish embryos provide clear visibility of fluorescent reporter gene expression because of their transparent state which makes them essential for molecular-level drug-induced genotoxic effect assays. [15]

Research on the biological effects of ionizing and non-ionizing radiation grows significantly from zebrafish studies. The study of DNA damage along with repair capabilities and the mutations arising from radiation can be analyzed through these experiments. [50] The assessment of embryonic development affected by gamma radiation along with radioprotective compound testing takes place through the use of zebrafish models. [51]

The advantages of zebrafish models are their many benefits but two major obstacles stem from how species differ in metabolic processes and restricted regulatory protocols for human risk assessments. Zebrafish promote genotoxicity research through their economic benefits and efficient research methods while maintaining ethical appropriateness for toxicology testing and drug discovery when compared to mammalian models.

**5. Advantages and Limitations of Zebrafish Genotoxicity Models**

Research on genotoxicity conducted with Zebrafish (Danio rerio) has become widespread because of multiple favorable characteristics. The human genetic similarity of 70% enables researchers to conduct pertinent toxicity tests. [46] The efficient development of zebrafish embryos with transparent characteristics enables investigators to track DNA damage through combination of Comet assays with Micronucleus tests and fluorescent reporter-based assays. [52] The affordability of zebrafish makes them practical to use because they need less caretaking resources than rodents in toxicological research settings and their smaller space requirements help accommodate more experiments. [46] The animal research regulations exclude zebrafish embryos and larvae from most protective measures allowing for an ethical advantage when using this research model. [24] The genotoxicity results in Zebrafish models might undergo changes because of xenobiotic biotransformation difference with mammals. [15] Zebrafish models report a brief lifespan which creates difficulties to study persistent toxic effects of exposure much longer than rodent models can determine. [19] Pooled samples become necessary to obtain sufficient statistical power because the embryo sample quantity is restricted which reduces availability of cells for molecular assessments. [15] The use of zebrafish continues as a strong toxicological research model although researchers can enhance their usefulness by developing new transgenic tools and CRISPR/Cas9 applications.

**6. Future Perspectives in Zebrafish Genotoxicity Models**

The progression of zebrafish genotoxicity models will be remarkable because of novel combinations between artificial intelligence (AI), upgraded transgenic lines and high-throughput screening (HTS) platform technologies. AI-powered image analysis systems must integrate for standardized genotoxicity testing which ultimately automates assays while removing human element in Comet and Micronucleus testing and gene expression analysis. [53] The development of advanced transgenic zebrafish that incorporate DNA damage response gene biosensors (p53, ATM, BRCA1) will enable precise real-time detection of genotoxic stress response inside living organisms based on fluorescent signal outputs. [54] Scientists apply personalized medicine through PDX models with patient-derived cancer tissue that predicts customized therapeutic drug responses in cancer therapy due to zebrafish similarities to human organisms. [55] Zebrafish models show promise as alternative test subjects to mammalian models for chemical safety evaluations according to regulatory bodies although species authentication remains a significant point. [52] The main impediment to broader zebrafish genotoxicity applications remains standardization of experimental protocols that produces both reproducible and acceptable regulatory results. [55] Researchers need to pursue works that standardize methods and develop better analytical methods for pathways while establishing zebrafish-human data connections to implement zebrafish models in toxicology testing frameworks. Technology improvements in zebrafish genotoxicity tests when incorporated with AI analytical tools and automation techniques will enhance their predictive analytical performance while improving their regulatory value in approaching the future regulatory frameworks.

**7. Conclusion**

Zebrafish (Danio rerio) serve as a flexible research model for genotoxicity studies that offers researchers important advantages of human genetic similarity along with rapid development rates and affordable operations and extensive testing functionality. The review identifies multiple studies of zebrafish genotoxicity through Comet assays and Micronucleus tests and TUNEL assays and transgenic fluorescence reporters that evaluate DNA damage with mutational data and genomic repair dynamics. [15] Zebrafish show broad applicability in chemical hazard identification because they demonstrate valuable use in environmental toxicology and other fields including pharmaceutical safety testing and nanotoxicology and radiation research. [47] Mutant metabolic processes in zebrafish differ from those observed in mammals combined with inconsistent test protocol standards and insufficient studies on prolonged chemical exposures pose ongoing obstacles for researchers. [19] Future research in zebrafish genotoxicity studies will benefit from artificial intelligence (AI) automated image analysis combined with transgenic model improvements and personalized medicine applications as per [53]. Zebrafish needs additional research for regulatory-standard genotoxicity model acceptance which should focus on method standardization while enhancing mammalian model validation and developing precise metabolic profiling techniques. [52] The resolution of present obstacles enables zebrafish models to fill the gap between in vitro and in vivo genotoxicity testing which ultimately results in better predictive toxicological evaluations within upcoming years.

Competing interests

The authors declare that there is no conflict of interest related to this work.

**COMPETING INTERESTS DISCLAIMER**:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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