Abstract
Recent developments in nanotechnology has increased the market value of nanoproducts in various industries. This has increased concerns associated with potential toxicity of nanoproducts to humans and the environments. Even though, green and biosynthesized nanoparticles are considered to be less toxic than chemically synthesized nanoparticles, they still possess some level of toxicity. Conventional toxicity assessments via human cells, live animals such as rat, frog or rabbit have several drawbacks including ethical issue and challenges involving the maintenance and development of cell cultures. Zebrafish (Danio rerio) is a transparent vertebrate fish that can reproduce rapidly. Its larvae develop in 5 days up to 3-5 cm long. It also possesses about 69% similar genetic profile, molecular mechanism, cell development and organ physiology as humans. Hence, it has the potential to be utilized as an alternative to humans or live animal models for initial drug screening and toxicity tests. European Union, USFDA and ICH have approved the use of zebrafish for toxicological evaluation of pharmaceutical products including nanomedicines. The article presents for the potential of zebrafish in preclinical evaluation of the toxicity of nanomaterials. It also discusses other potential applications, including medical imaging and environmental toxicity.

Keywords:
Zebrafish; Nanomaterials; Toxicity Test; Preclinical Trials; Model Organism.

1- Introduction
Zebrafish (Danio rerio) are a tropical teleost, diploid vertebrate fish which is 3-5 cm long and reproduces rapidly [1]. They are small in size, transparent, simple to handle, species with elevated fecundity, continuous reproduction and rapid embryogenesis [2]. Due to its size, zebrafish is easy to maintain in small spaces, and their rapid reproduction capability helps in large scale studies. The embryo and larva of zebrafish, called fry, are transparent and develop in 5 days. Hence, it is possible to grow zebrafish in a petridish [3]. Several studies have reported that zebrafish can be used as a potential in vivo model to analyze the efficiency and genetic toxicity of drugs. Each stage of the zebrafish growth cycle is advantageous in certain aspects for toxicity studies especially genotoxicity [4]. Embryos of zebrafish are beneficial in fish embryo assay as they are pain-free in vivo tests, and this could be an alternative for animal experiments [5]. Shi et al. (2007) demonstrated that zebrafish embryos can be used to identify the developmental toxicity and variations in gene expression after exposing it with a persistent organic pollutant called perfluorooctanesulfonate (PFOS) [6]. The transparent larvae of zebrafish makes them excellent for fluorescent protein visualization, identifying neutrophils after 48 h of fertilization and determining the existence of innate immune system isolated from adaptive systems [7, 8]. Renshaw et al. (2006) utilized transgenic zebrafish larvae as an in vivo model to analyze genetic inflammatory response by expressing green fluorescent protein under the neutrophil-specific myeloperoxidase promoter [9]. Similarly, adult
zebrafish are considered as an exclusive model to analyze vertebrate development, especially immune system progression. Neely et al. (2002) demonstrated the effect and mechanism of bacterial infection caused by gram-positive *Streptococcus iniae* (a pathogen of fish and human), using an adult zebrafish [10].

Nanoparticles have emerged as novel small sized particles for the development of next generation materials with exclusive properties for various applications including electronics and medicine [11, 12]. There are a wide variety of synthesis methods that are available for the generation of nanoparticles and the selection of the synthesis approach is crucial to obtain the desired characteristics of nanoparticles for specific applications [11, 13]. Chemical synthesis approaches are used to generate large quantities of nanosized particles. However, the chemical and reagents used for these synthesis approaches are identified to be toxic to human and the environment. Hence, chemical-based approaches are unsuitable for environmental and biomedical applications [14, 15]. Green and biosynthesis approaches have been introduced to develop nanoparticles that are biocompatible with reduced toxicity for biomedical and drug delivery applications [16, 17]. Thus, toxicological analysis plays a significant role in validating the use of nanoparticles for medicine, bioimaging and environmental applications.

*In vitro*, *in silico* and *in vivo* analysis are the conventional methods to evaluate the toxicity of nanoparticles [18]. *In vitro* analysis includes cell lines and microbes to evaluate the toxicity of nanoparticles [19], whereas, *in silico* analysis involves computational simulations and models for toxicity evaluations [20]. Both methods are beneficial for preliminary toxicological assessment of nanoparticles for biomedical, environmental and antimicrobial applications [21]. However, *in vivo* models including live animal models are used for the validation of toxic reactions of nanoparticles. Rat, mouse, monkey, frog and cockroach are the animal models that are used as traditional *in vivo* toxicity models [22]. Even though, they provide useful results with clinical relevance, difficulty in growth and maintenance, tedious ethical clearance processes and practical/emotional difficulties in sacrificing large number of animals remain as drawbacks [23]. Thus, zebrafish is proposed as an alternative model for *in vivo* toxicological evaluation of nanoparticles. The genetic profile, cell development and organ physiology of zebrafish has 69% resemblance to humans. They also possess organs and tissues with analogous structures and functions, like humans. This includes the heart, kidney, liver, pancreas, intestinal tract and brain [24]. This article evaluates the efficiency of zebrafish as an effective *in vivo* animal model for the assessment of nanoparticle toxicity. In addition, the mechanism of nanoparticle toxicity in zebrafish and future perspectives on nanotoxicity analysis with zebrafish are also discussed.

2- Zebrafish as an *in Vivo* Animal Model

Zebrafish is proposed to be useful as an alternative to human cells in preclinical trials, toxicity analysis of medicines, complicated nerves and brain related drug development, and medical imaging applications according to Figure 1.

2-1- Preclinical Trials

The life stages of zebrafishes are widely used as an efficient animal model to evaluate drug effectiveness and screening analysis. In recent times, it has been used as an alternate vertebrate animal model for the discovery of phenotype-based drugs. Zebrafish possesses advantages such as accessible broad biology range and early toxicity insight [25]. The use of cell lines to screen drugs causes a drawback as it can screen only cell-autonomous phenotypes. The use of zebrafish offers evaluation with live animal models, having a distinct repertoire, similar to the biological processes.

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**Potential applications of zebrafish**

- Preclinical trials
- Medical imaging
- Nervous system and brain related drug development
- Environmental toxicity studies

*Figure 1. Potential applications of zebrafish as an animal model.*
in humans with a completely integrated system of vertebrate organs. Thus, zebrafish can be used for preclinical drug screening analysis to evaluate and analyze the potency of drugs for the treatment of pain, tumor metastasis, gut motility, sedation and vascular tone [26]. In addition, cell line studies do not provide adequate insights into the pharmacokinetic and pharmacodynamic characteristics of drugs such as absorption, distribution, metabolism, excretion and toxicity, whereas zebrafish has demonstrated the capacity to provide pharmacokinetic and pharmacodynamic characteristics in live models with functional livers, kidneys and blood brain barriers [27]. Parng et al. (2002) showed that zebrafish can be a potential alternative animal model to replace existing cell line based on preclinical methods to evaluate the toxicity, angiogenesis and apoptosis of drugs. About 18 chemicals were analysed to obtain lethal concentration (LC50) levels in comparison with mice models. The results revealed that zebrafish is highly beneficial in preclinical studies of drugs than mice [28]. In recent times, Wertman et al. (2016) reported that zebrafish can be utilized as a xenograft platform for novel cancer model and as a preclinical screening tool [29]. In addition, Lenard et al. (2016) showed that zebrafish are beneficial as a live model to evaluate erythroid lineage toxicity and regeneration [30]. Zebrafish has also been demonstrated to be useful for preclinical evaluation and screening of drugs for the treatment of diseases such as cancer [31], Amyotrophic lateral sclerosis (ALS) [32], neurodegenerative diseases [33], Alzheimer’s disease [34], cardiovascular and metabolic diseases [35], tuberculosis [36], Huntington’s disease [37], muscle diseases [38], polycystic kidney diseases [39] and Parkinson’s disease [40].

2-2- Medical Imaging

Apart from preclinical trials, zebrafish are also used as a live animal model to evaluate the efficiency of medical imaging tools. X-ray based imaging techniques require pre-evaluation before utilization for human organ imaging, as X-ray exposure can harm cells due to their high energy [41]. Thus, zebrafish are used as a live animal model to evaluate and optimize the x-ray energy intensity for human imaging applications. Zebrafish has been used to evaluate the efficiency of Synchrotron X-ray fluorescence (SXRF) microtomography to obtain three-dimensional images of transition metals in live animal models [42]. Likewise, x-ray and light optics were used for whole-animal imaging, gene function and the zebrafish phenome project [43]. Synchrotron X-ray micro computed tomography has been used to obtain the entire body image of a hypercholesterolemic female zebrafish, as a test to demonstrate the application of the imaging technique to monitor cholesterol levels in live animal models [44]. In addition, it has been revealed that zebrafish are useful as live models to evaluate the efficiency of x-ray phase-contrast tomography for high spatial resolution muscle imaging [45], high resolution-ray vascular network imaging [46] and elemental as well as chemical specific x-ray fluorescence imaging of biological systems [47]. Laboratory-based x-ray NanoCT has been used to study the morphology of embryos [48], multifunctional green fluorescence carbon dots as fluorescent probe to detect mercury [49] and liquid metal jet x-ray sources for high resolution biomedical imaging [50] based on zebrafish as an in vivo model. Other than X-ray related imaging methods, zebrafish have also been used to examine the efficiency of two-photon time lapse live imaging of lymphatic development [51], high frequency ultrasound (45-75 Mhz) for in vivo cardiac imaging [52], optical projection tomography for in vivo label-free three dimensional vasculature imaging [53], and optoacoustic tomography for non-invasive whole-body imaging technique [54]. Zebrafishes also used as a significant in vivo model to evaluate the medical imaging methods of accelerated optical projection tomography [55], four dimensional light sheet to image cardiac development [56], functional optoacoustic neuro-tomography of calcium fluxes in adult brain [57] and carbon quantum dots based fluorescence imaging methods [58].

2-3- Nervous System and Brain Related Drug Development

Numerous studies have emphasized that zebrafish can be useful as a live animal model for the evaluation of neurotoxicity and for the determination of drug dosage to treat patients with nervous disorders [59]. Zebrafish also serve as an excellent in vivo model for rapid behavior-based identification of neuroactive small molecules as potential drugs for neurodegenerative disease treatment [60]. Zebrafish contain similar neuroanatomy and neurochemistry of the central nervous system in humans, and this is beneficial to evaluate the efficiency of drugs in treating neuropsychiatric diseases [61]. They also contain second presenilin gene, which is similar to the gene present in human Alzheimer’s disease [62]. Thus, zebrafish are recently used as a tool for research and preparation of drugs for Alzheimer’s disease [63]. Quercetin and rutin were injected into zebrafish for drug efficacy studies, and the result revealed that these drugs are beneficial in preventing scopolamine-induced memory impairment [64]. Further, zebrafish are widely used to evaluate and monitor neurogenic phenotype [65], modulatory neurotransmitter systems and behavior [66], neurogenic phenotype [65] and DJ-1 expression [67]. Zebrafish was used to evaluate the neuroprotective ability of lanthionine ketimine-5-ethyl ester against okadaic acid-induced Alzheimer’s disease [68]. Also, zebrafish have been used to effectively study accelerated brain aging towards transcriptional inversion [69], efficiency of GSK3β inhibitor, TDZD-8 [70], protective effects of quercetin [71] and gender specific expression changes in aging and Alzheimer’s disease [72]. Zebrafish have been employed in research on Parkinson’s disease [73, 74], Huntington’s disease [75, 76] and amyotrophic lateral sclerosis [77, 78] to evaluate, monitor and study the disease physiology as well as drug efficacy. Zebrafish are considered an effective live animal model to screen drugs for brain cancer [79, 80] and ischemic stroke [81, 82]. All these studies provide supporting data on the efficacy of zebrafish for in vivo evaluation and study of nerve and brain related diseases.
2-4 Environmental Toxicity Studies

Apart from medical and pharmaceutical evaluations, zebrafish are extensively used in environmental risk assessment studies. It has been reported that zebrafish, specifically embryos, can be used, not only to study acute toxicity, but also to evaluate teratogenicity, identifying mode of action, toxicokinetics, toxicodynamics and prediction of adverse and long term effects of environmental toxicity, especially toxicity due to heavy metals exposure towards an organism [83]. Further, zebrafish are widely explored as a potential animal model for the evaluation of environmental toxicity of chemicals via fish embryo toxicity test [84]. They also served as an effective vertebrate model for investigating chemical toxicity [85], dioxin developmental toxicity [86], structure-activity relationship assessment of perfluorinated chemicals [87], and proteotoxicity and embryotoxicity carrier solvents towards the environment [88]. In addition, the toxicity of heavy metals such as mercury, copper, nickel, lead and cobalt towards embryos and larvae of organisms have been evaluated using zebrafish [89]. Similarly, the toxicity of lead and tin based perovskite solar cells [90], chlorpyrifos, nickel chloride [91], and cadmium induced deformities in aquatic animals [92] have been evaluated using zebrafish. In recent times, zebrafish are used to investigate the time-response characteristics and potential biomarker identification of heavy metals [93], influence of microplastics on the accumulation and chronic toxicity of cadmium [94], and elevation of heavy metal toxicity via polyaspartic acid [95]. Thus, it is noteworthy that toxicity analysis of chemicals using zebrafish models will be an essential test in future to evaluate their toxicity mechanisms.

3- Zebrafish for Nanotoxicity Evaluation

The use of zebrafish as a tool to evaluate the toxicity of nanoparticles towards living organisms has received significant interests. The toxicity of nanoparticles has become a topic of interest since the synthesis of most nanoparticles involves the use of toxic chemicals. For example, gold nanoparticles are synthesized using cetyl trimethyl ammonium bromide (CTAB), which is proven to be toxic to human cells and tissues [96]. Several methods have been employed to evaluate the toxicity of nanoparticles in vitro using microbes [97], cell lines [98] as well as in vivo methods using animal models such as mice [99], cockroach [100] and drosophila [101]. However, each method poses some drawbacks that may be resolved using a combination of models. In vitro toxicological analysis of nanoparticles is not sufficient to evaluate their toxicity in animals as they represent only a specific type of cells which may differ in animals due to the combination and accumulation of nanoparticles in their metabolic pathway [102]. Thus, it is necessary to use in vivo toxicity analysis via live animals. Major challenges associated with the use of live animal models include the need for ethical clearance, animal safety clearance, hands-on training in animal dissection and errors in toxicology result and restrictions in the number of animals that can be used [103]. Thus, zebrafish, which is a simple fish model with easy approval process for ethical clearance and animal safety, are used as an effective tool for evaluating the toxicity of nanoparticles in live animal models [104]. In addition, zebrafish can turn into a fertile adult in two or three months and can lay about 200 eggs [105]. This high reproductive ability and fertility along with simple maintenance procedures contributes to the positive attributes of zebrafish for use in nanotoxicological analysis. The toxicity of almost all types of nanoparticles including metal [106], metal oxide [107], carbon [108], polymer nanoparticles [109], nanocomposites [110], zero-dimensional [58], one-dimensional [111], two-dimensional [112] and three-dimensional nanoparticles [113] have been evaluated directly using zebrafish with promising results as shown in Table 1. Zebrafish are also used to evaluate the genotoxicity of nanoparticles, as it is easy to analyze the genome of zebrafish compared to larger animals [114]. Zebrafish are emerging as an effective replacement of mice models for toxicological evaluation and screening of nanoparticles.

| Nanoparticles | Description | Reference |
|---------------|-------------|-----------|
| **Metal nanoparticles** | | |
| Gold | Adult and embryo | [115, 116] |
| Silver | Embryo and adult | [117, 118] |
| Copper | Embryo | [119] |
| Selenium | Embryo | [120] |
| Platinum | Embryo | [121] |
| Aluminium | Adult | [122] |
| **Metal oxide nanoparticles** | | |
| Zinc oxide | Embryo | [123] |
| Copper oxide | Cells, embryos and instars (fry) | [124] |
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Titanium dioxide Embryo [125]

Cerium dioxide Early life stages [126]

Magnesium oxide Embryo and larval stages [127]

Silica Embryo [128]

Iron oxide Life stages [129]

| Carbon-based nanoparticles |
|--------------------------|
| Multiwalled carbon nanotubes Embryo [130] |
| Single wall carbon nanotubes Embryo [131] |
| Graphene quantum dot Embryo [132] |
| Carbon dots Embryo [133] |
| Pristine graphene Embryo [134] |
| Graphene oxide Larvae [135] |
| Fullerene Embryo and adult [136, 137] |

| Polymer nanoparticles |
|-----------------------|
| Chitosan Embryo [138] |
| Hydroxy apatite Embryo [139] |
| Poly (lactic-co-glycolic acid) (PLGA) Life stages and adult [140] |
| Anionic and cationic polyamidoamine (PAMAM) and poly (propylene imine) (PPI) Embryo [141] |
| Bismuth-asparagine Embryo [142] |
| Redox polymer Embryo [143] |
| Iron chelator starch-deferoxamine Embryo [144] |

| Nanocomposites |
|----------------|
| Graphene oxide-titanium dioxide Embryo and larvae [145] |
| Chitosan-zinc oxide Early stages of zebrafish [146] |
| Starch-magnetite Adult [147] |
| Titanium dioxide-multiwalled carbon nanotube Embryo [148] |
| Gold-gadolinium doped-carbon quantum dot Embryo [149] |
| Titanium dioxide-chondroitin-4-sulfate Embryo and life stages [150] |

4- Future Perspective

It is noteworthy from Table 1 that the embryo of zebrafish is widely used for the toxicity evaluation of nanoparticles to determine the effects of nanoparticles on reproduction during development stages. The larval, life stages and adult of zebrafish are extensively used to examine the genotoxicity of nanoparticles towards live animal models. In future, zebrafish can be developed into potential live animal model for in vivo screening and evaluation of nanoparticles toward various diseases. There are several reports in recent times which demonstrate the capacity of zebrafish for screening and evaluating nanomedicines for the treatment of kidney diseases [151], cancer [152], diabetes [153], T-cell acute lymphoblastic leukemia [154] and human aging related diseases [155]. Improvements in the zebrafish embryo tests will further benefit researchers to examine the toxicity of nanoparticles in live embryos for various biomedical applications [156]. Zebrafish are considered as a promising alternative for nanotoxicological evaluation and drug discovery analysis to screen, evaluate and obtain benign nanomaterials for various applications.
5- Conclusion

The main objective of this article is to provide an account to support the use of zebrafish for toxicological assessment of nanoparticles. Strict regulations and further development in zebrafish-related nanotoxicological assays such as fish embryo tests will enhance the efficacy of zebrafish in toxicity studies. Zebrafish can also be used in preclinical studies of nanomedicines due to their advantages over conventional in vitro and in vivo studies. However, there exists certain drawbacks, such as uncertainty in the result and misinterpretation of genotoxicity as well as classification of specific life stages in zebrafish, which should be addressed via specific physiological studies to enable a wide-spread application of zebrafish in toxicological analysis. Thus, zebrafish can become an alternative to conventional in vitro and in vivo models used in toxicological studies to facilitate the application of nanoparticles in biomedical and environmental applications.

6- Conflict of Interest

The authors declare no conflict of interest.

7- Ethical Approval

This work did not involve the use of animals and therefore ethical approval was not necessarily required.

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