Fish models in experimental pharmacology: on the mark or off the mark?

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Abstract

Fish has emerged as an alternative model organism in biomedical research for conducting experimental pharmacological and toxicological studies. As a vertebrate, they share many conserved physiological and molecular features with humans that makes them valuable model for diagnosing, investigating disease states, for testing drugs to check toxicity and their therapeutic activity against the target. Zebrafish and medaka are mainstream models that are widely employed in pharmaceutical research. This review aims to point out the probability and potential of fish as an alternative model organism in biomedical research, drug discovery and development. Further, the review appraises the limitations of fish models in experimental pharmacological and toxicological studies considering the changes in residing environment, physiology, metabolism, unpredictable inter-individual variability due to disease, variable conditioning and interspecific and intraspecific variability.

Key words: fish model, model organism, drug screening, pharmacology, toxicology

Background

Fishes are the earliest, most abundant and diverse classes of vertebrates, with approximately 34000 species that comprises of 48% the known member species of subphylum Vertebrata[1]. Although fish diverged from humans approximately 400 million years ago, there are very few differences at molecular level, justifying the selection of fish as model organism to conduct research relevant to humans [2]. The use of fish as a research model has exponentially been adopted worldwide, owing to provide insights into complex human genetic, anatomic and physiological processes as well as the pathogenesis of human disorders [3].

For more than 200 years, fish has been used in experiments as a model organism with gold fish (*Carassius auratus*) being the oldest model species, employed in toxicity studies. Afterwards, it went on to become a popular model in fields such as growth, behavioral

studies, immunology and reproduction[4]. Experiments conducted in the early 20th century sparked the emergence of medaka (*Oryzias latipes*) as a developmental genetic model organism. Medaka, like zebrafish (*Danio rerio*), has completely sequenced genome, transparent embryos, adaptation to wide range of temperatures and high fecundity, which enables them to be an extremely useful experimental animal in toxicology, developmental studies, disease modelling and environmental health sciences[5]. Zebrafish was first introduced as a biological model to study developmental genetics by George Streisinger in the 1960's. In recent years, it has successively emerged as the top research model whose applicability has extended to many other fields including physiology, toxicology, disease modelling and drug development [6]. Presently, some other fish species are also in use as model organisms in experimental pharmacology, which includes the large sized species such as rainbow trout, and the small sized ornamental fish species such as *Xiphophorus* sp, *Rivulus* sp, *Poecilia* sp., and *Cyprinodontidae* sp .

The fish cell lines are also being developed as in vitro model to complement in vivo studies for carrying out investigations in related fields. About 283 cell lines have reported to be established from finfish around the world[7]. Further, the raising potential in using cells from lower vertebrates as in vitro models for studies of DNA repair function, makes fish as a good model to monitor mutagenic and carcinogenic chemicals. [8]. The potential for the application of research outcomes to both human as well as environmental health issues makes fish species attractive, demanding and valuable alternative models in the carcinogenesis and toxicity research also [9].

Without scrutinizing the usefulness of current approaches which make most of these fish models, especially zebrafish, the present strategies have some notable limitations to be taken care of. To begin with, there is a basic anatomic and physiological difference exist between humans and fish [10]. Although fish is much similar to humans genetically, there is a limit, as human diseases caused by genes that do not exist in fish or those affecting a specific tissue or body part that fish do not have will require another animal model [4,11]. Further, there can be interspecies variation as a response to drugs, which makes it difficult to extrapolate individual level results under laboratory settings to population level and then to humans [12]. Therefore, a detailed analysis is required at first, to assess the characteristics, in order to compare the benefits and current limitations of using fish as a model organism in experimental pharmacology.

Thus, in this review, we intend to appraise the scope of using the fish as a powerful model organism in experimental pharmacology along with its possible shortcomings. To strengthen this, we concentrate on gathering base line information regarding the studies in which fish models are employed for understanding molecular and genetic mechanisms underlying human diseases, for determining drug efficacy and toxicity, drug screening and for assessing adverse effects of known drugs. Further, this review gives an overview of the usage of fish as

a model organism in pharmaceutical research, drug discovery and development and also provides an insight to fish pharmacology and toxicology. Finally, the review evaluates the limitations of fish models in experimental pharmacological and toxicological studies, considering the changes in residing environment, physiology, metabolism, unpredictable inter-individual variability due to disease, variable conditioning and interspecific and intraspecific variability.

Main Text

Applications of fish as a model species

Fish models can offer interesting possibilities for current and future biomedical research. This can serve as a gap-filler between in vitro assays and rodent testing.

1. As a platform for drug screening

Current drug discovery strategies utilize either an experimental phenotype- or a rational target-based approaches in which target based approach [13,14] (figure 2). Both strategies have their own strengths and weaknesses. Nevertheless, phenotype screening has the advantage of not requiring enough information on disease or a validated target and provides an insight of overall efficacy of small molecules through simultaneous activity at multiple targets[15] .

Model organisms such as zebra fish fills the gap between in vitro assays and expensive screenings using mammals. Since zebra fish is a small species and easy to maintain, the assays can be used in medium-to-high-throughput screening mode for pharmacological investigations. Direct screening as well as screening of known drugs for new insights can also be conducted in this model [16]. Screening technologies those exist as well as being developed in zebra fish, are supposed to provide details of potential off-target effects of drug molecules on the cardiac system, central nervous system, on the intestinal tract, auditory and visual functions, pro-convulsing potential and osteogenesis. In zebrafish larvae, an *in vivo* toxicology evaluation can be completed in 6 to 7 days' time. Hence zebrafish model can be considered as a useful pre-filter for the identification of safest lead candidates as early as possible in the drug discovery process [17].

Nowadays, the use of medicinal plants as complementary medicine is gaining attention, whose validation requires experimental studies on animal models. For this purpose, fish species- based models can be easily employed. The acute toxicity of Indian almond (*Terminalia catappa*) and garlic (*Allium sativum*) was evaluated in tilapia (*Oreochromis niloticus*) fingerlings and was found to be less toxic. Therefore, these herbs can be used as an alternative to treat the trichodiniasis caused by *Trichodina* sp [18]. Similarly, Rainbow trout (*Oncorhynchus mykiss*) fed with diet containing extract of *Viscum album*, *Urtica dioica* and *Zingiber officinale* showed non-specific immune responses [19]. *Eclipta alba* (Bhangra) leaf

aqueous extract showed the immuno-stimulatory effect in tested in tilapia . Studies conducted on Indian major carp (*Labeo rohita*) fingerlings using *Withania somnifera* also exhibited enhanced immunological and disease resistance properties against *Aeromonas hydrophila* infection [7]. A study conducted on the neo-tropical freshwater fish, *Prochilodus lineatus* illustrated the acute lethal and sub-lethal effects of neem (*Azadiracta indica*) extract, which caused instability in antioxidant defense system and damaged fish gills and kidney tissues. These results prove that although neem extract is less toxic compared to synthetic pesticides, it indeed causes functional and morphological changes in this species [20].

2. In the field of regenerative medicine

Regenerative medicine is the branch of medicine that develops methods to heal or replace cells, tissues and organs damaged by age, disease, or trauma and also to normalize congenital defects. Currently, the impact of this field in clinical practice includes organ transplantation, skin grafting, generation and use of therapeutic stem cells and tissue engineering [21].

Fish can be a versatile model for studying regeneration as it can regrow many tissues and organs such as fins and heart. The first use of fish as a model organism was in the field of regenerative medicine, which was about the regeneration of the fins of goldfish[22]. The research in fish regeneration biology, focused largely on the zebrafish, has broadly expanded in recent years. Studying the mechanism of regeneration and homeostasis of tissues has given promising results relevant for the development of human regenerative medicine.

Zebrafish possess a large regenerative capacity and therefore is an ideal model to carryout studies using cellular, molecular and genetic approaches. This model has advantages such as it can restore organs that poorly regenerate in mammals, regeneration can be easily followed and multiple tools for genetic manipulations are possible . Table 1 summarizes some main injury models for studying regeneration in zebrafish[23].

Other fishes used as models for regeneration studies include *Carassius auratus* (goldfish), *Cyprinus carpio* (carp), *Oncorhynchus mykiss* (rainbow trout) and *Sternopygus macrurus* (yellow-stripe knifefish) [37].

3. Assessment of genotoxicity

Genotoxicity refers to the property of certain chemical substances that can produce deleterious effects to the genetic information within a cell. They can affect germ cells and can pass genetic changes to next generation. Assessment of genotoxicity is an important component for drug development as well as toxicity studies. A genotoxic substance interacts directly or indirectly with the DNA causing strand breaks or additions or modifications[38]. Tilapia, *O. mossambicus* and Zebra fish were used to estimate the DNA strand breaks induced by mono-crotophos and the DNA damage was recorded in treated samples

(organophosphate pesticide). A significant DNA damage was observed in all the treated fish compared to the control[39-40]. The genotoxic potential of Amikacin sulphate was recorded only at higher doses and longer exposures in O. mossambicus[41].

Synodontis clarias and *O. niloticus* were sampled from various locations/seasons of river Anambra and season, species of fish and geographical location affected the micronuclei profile of the fish [42]. A similar study was performed on *Astyanax altiparanae* to assess the geno-toxicity of samples from Jordão River with different levels of metal contamination. Micronucleus indices in fish erythrocytes after exposure to contaminated samples were measured and correlated with environmental parameters. An increase in water concentrations of metals was observed in samples collected from urban zone causing higher geno-toxicity and hence more impacts [43]. Fish cell lines are an alternative to whole fish for assessment of geno-toxicity in many toxicity studies. The cytotoxic effect of the organophosphorus pesticide, 'Parathion' was determined using FG-9307, a cell line derived from gills of *Paralichthys olivaceus*. This study confirmed that the fish cell lines can be applied in *acute in vitro cytotoxicity* studies [44].

4. As a disease model

Fish as a biological model for diseases can be useful to elucidate some biological mechanisms involved in the pathogenesis of disorders common to both fish and humans. Small sized freshwater fish species that can be easily bred and maintained in large numbers at a lower cost are most preferred. These species such as zebra fish, Medaka, platy fish and swordtail fish are amenable to various molecular techniques, such are markedly valuable for modeling human disorders. Since at the molecular level, there are very few differences, the application of a non-mainstream fish species as disease model can also be explored with the aid of new and improved gene sequencing systems. Fish models can offer numerous experimental advantages; chiefly ease of genetic manipulation, for the investigation of disease mechanism and other characteristics [45].

Zebra fish has been widely used as a candidate species for modeling human disease. Zebra fish share a notable genetic similarity to humans with an approximate of 70% of human disease genes having functional homologs in zebra fish. Myocardial infarction was modeled in zebra fish using cryoinjury. Regeneration of cardio-myocytes was studied in zebra fish heart and a protein, *thymosin b4*,was found to trigger the formation of new cardiac tissue. Zebrafish strains with mutations in the dystrophin gene were found to have a phenotype similar to the human disease, Duchenne muscular dystrophy (DMD). Since there is no cure for DMD, Zebrafish dmd mutants have been used to screen potential compounds that can ameliorate the disease pathology [46]. A few studies report the use of zebra fish as a model for Parkinson's disease since it can mimic the phenomenology of different movement disorders. Zebrafish, being a highly social animal, lives in groups and therefore particularly useful to

model disorders of human social behaviors such as aggression and autism. Continuous application of stress can develop zebrafish models of anxiety and depression due to their physiological and genetic homogeneity with human responses to stress. Zebrafish is also used for assessing addiction, attention deficit hyper-activity disorder and obsessive-compulsive disorder [47].

Medaka is complementary to zebra fish for modeling human diseases. Nonalcoholic steato-hepatitis model in medaka (*O. latipes*) was developed by feeding the fish a high fat diet; which exhibited hyperlipidemia and hyperglycemia and degeneration of hepatocytes. Influence of n-3 polyunsaturated fatty acids on disease progress was also studied [48]. Medaka can be a well-suited Parkinson's disease (PD) model as symptoms can be identified through behavioral analysis and verification of the neuron loss. Both toxin- and mutation-induced PD models were developed in Medaka fish [49].

The emerging fish models are evolutionary mutant models in which disease developed is either adaptive or true illness. Antarctic fish, *Notothenia coriiceps* naturally exhibits reduced bone mineralization. Therefore, notothenioid genes, responsible for the natural osteopenia in this ice fish are identified and characterized to have a better understanding of the mechanism involved in the pathogenesis of human bone diseases. The white-blooded icefish, *Chaenocephalus aceratus* is a species that has no erythrocytes or hemoglobin, but have special cardiovascular adaptations for an adequate supply of oxygen in tissues. This can be a model for human anemia as well as can provide information regarding human red blood cell formation. Toadfish (*Opsanus tau*) provides models for hepatic encephalopathy and sickle cell anemia by its characteristics of excessive urea production and similarity with mutant human sickle hemoglobin under hypoxia. Fish models, which develop same disease as in humans include platy and swordtail (melanoma), eels (bone demineralization, childhood kidney cancer), and damselfish (neurofibromatosis) etc [2].

Nowadays, the efforts to identify the disease pathogenesis as well as to reveal novel drug targets using model organism research have gained immense popularity. The application of dihydroorotate dehydrogenase inhibitors such as anti-inflammatory drug leflunomide in melanoma formation was identified using zebra fish model. There are many more drug discovery studies using the zebrafish model that have gone to advanced stages of clinical studies. All-trans retinoic acid (ATRA) was found to suppress the transcription factor c-myb, a driver of adenoid cystic carcinoma using a pluripotent zebrafish blastomere culture system. The findings led to the initiation of a second phase II clinical trial for testing the compound in patients with recurrent metastatic adenoid cystic carcinoma of the head and neck. ProHema is another compound that has currently being evaluated in an ongoing phase II stage clinical trial in hematologic malignancies. Moreover, another research approach revealed that Rosuvastatin, used for treating hypercholesterolemia, has been repurposed as

an antiangiogenic drug after a study on the zebrafish model. Furthermore, the morphological limb defects of thalidomide as in humans were detected in zebrafish models [45].

Mycobacterium marinum (Mm), causes a tuberculosis-like disease in fish, which can be employed as a cost-effective surrogate model for human tuberculosis (TB). Therefore, the use of medaka (*O. latipes*) to model the chronic TB offers platforms for the identification of anti-TB drugs [50].

5. Translation to clinical trials

Zebrafish has recently been proposed to develop 'avatar models'; an emerging approach in precision medicine in oncology. In this, the cancer cells from patient's tumor are xenotransplanted into zebrafish 'avatars' for drug efficacy studies and the results obtained can be translated to patients' trails. This ensures best personalized drug treatment for the patients. Recently, in an experiment conducted in zebrafish embryo, clotrimazole co-treatment with Lonafarnib has been acknowledged as a potential cure for melanoma. Besides, xenotransplanted humanized models for respiratory diseases like SARS-CoV-2 infection can be developed in zebrafish owing to their swim bladders as buoyancy organs. Further, high throughput screens using zebrafish models have helped to discover new therapeutic candidates in many cases where the results of clinical trial support the preclinical conclusion [51,52].

Currently used fish models in experimental pharmacology

Zebra fish is the widely accepted versatile in vivo model organism for studying a broad array of topics ranging from developmental biology and morphogenesis to neurosciences, regeneration and aging. Zebra fish has shown potential for drug discovery in various disease models. Zebrafish model was used to identify drugs that can limit the cardiotoxicity of doxorubicin. A large number of compounds were screened, and visnagin and diphenyl urea were identified as cardioprotective compounds, as they reduced the effects of doxorubicin on the zebrafish heart [13]. Medaka, (*Oryzias latipes*), another small, egg-laying, freshwater, bony fish, can be regarded as a second laboratory fish model in parallel to the zebra fish. This is one of the small fish species of choice in many carcinogenesis bioassays [5]. Rainbow trout (*Oncorhynchus mykiss*), a widely distributed fresh water fish species, is one of the widely studied fishes in many research areas including carcinogenesis, toxicology, comparative immunology, disease ecology, physiology and nutrition. Rainbow trout as a large fish model in aflatoxin-induced hepatocellular carcinoma and medaka as the small fish species in diethyl nitrosamine (DEN) induced hepatocellular carcinoma show the potential of fish species as alternative models in carcinogenesis and toxicity research. Further, trout hepatocytes spheroids are identified to be a promising in vitro model to study xenobiotic metabolism and drug efflux through assessing the expression and functionality of genes related to xenobiotic metabolism [9]. Besides this, many pharmaco-kinetic and pharmaco-dynamic researches have

been done on different species of fish using several groups of drugs such as tetracyclines, penicillins, macrolides, quinolones, sulfonamides, immunostimulants, anticancer agents, herbal drugs and vaccines. Lamprey eels (predator family of jawless fish) have been employed as model organism in spinal cord research. A novel protein was identified from the supraneural body of adult lamprey (LIP) that shows cytocidal activity against human MCF-7 and K562 cells with target cell specificity and LIP was found to have strong cytocidal effects against tumor cells both *in vitro* and *in vivo*. Silver nanoparticles are used for their antimicrobial properties, whose cytotoxic effect was assessed in the Indian carps, *Catla catla* heart cell line, and gill cell line and *Labio rohita* gill cell line, using MTT (3-(4,5-Dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and neutral red assay. Goldfish (*C. auratus*), was also successfully used for screening antibiotic drugs for neurotoxicity. Further, the molecular evolution of opioid receptor family was studied using cDNAs isolated from the teleost fish, *Catostomus commersoni* and the study pointed out that the function of opioid receptors in lower vertebrates is to suppress pain as in mammals or they have developed an antinociceptive function during the course of evolution. Other fishes which have been used in biomedical research include: *Channa punctatus*, *Clarias gariepinus*, *Fundulus heteroclitus*, *Myoxocephalus scorpius*, *O. mossambicus* and *O. niloticus*, and *Salmo trutta* and *S. iredius* [7]. Zebrafish is the mostly studied and preferred species to model humans, taking advantage of the orthologous genes of interest and the suitability for medium and high throughput screening. Sequencing of zebrafish genome reveals that approximately 70% of human genes have at least one zebrafish orthologue, which makes it suitable for studying genetics of organogenesis, embryo development as well as human physiology and disease [53]. Currently, the clinical side of biomedical research has introduced a trend known as personalized medicine that involves translating knowledge from preclinical models to humans. Co-clinical trial with zebrafish 'avatars' enables faster analysis of local and systemic effects of drug treatment for cancer and therefore, can be used for first line therapy, in spite of having technical challenges yet to overcome [51].

It's essential to understand that there is no single fish model ideal for addressing all biomedical questions. Each species has unique strengths and weaknesses. Gold fish, as a model system, has advantages in understanding of skeletal and organ morphology and coloration of vertebrates. Further, it is easy to collect molecular components from the blood and are useful for micromanipulation experiments and establishing disease models [54]. In comparison with zebrafish, medaka is tough, less prone to diseases and has distinctly defined sex chromosomes [55]. Further, studies of medaka can provide information regarding additional phenotypes, useful for disease modelling. Therefore, medaka can be a potential, complementary model organism in developmental biology and genetics[2]. Annual killifish has short life span as an advantage for being a human ageing model [56]. Zebrafish is a successful and versatile model to study developmental biology, diseases including cancer, toxicology,

drug discovery, and molecular genetics [,57]. Therefore, fish models have promising role for advancing researches in future (figure 1).

Fish models in pharmacological and toxicological studies: are they feasible?

Model organisms are living, non- human species used during the human medical investigations to gain knowledge about the disease; its prevention, diagnosis, and treatment. The ease of experimenting under controlled situations and mimicking disease conditions reinforced the development of such models and applied in various biological fields. To meet the need of restricting the use of animals in research and thereby minimizing their suffering, lower organisms such as fish were proposed to be an alternative model in research. Considering their short life span and high fecundity, they have entered the fray as a model organism for conducting experimental pharmacological and toxicological studies to understand the behavior of the higher vertebrates[2]. It is well known that, fish are comparatively more affordable, easier to keep with a short life cycle and faster to rise with high fecundity than mammals [1,3]. The eggs can be fertilized externally and can be easily manipulated and customized to the experimental requirements. Most of the egg stages are transparent so that embryonic development can be easily monitored [58]. These physiological adaptations will facilitate the use of fish as a convenient model to diagnose and investigate disease states and to test drugs for toxicity and their therapeutic activity against the target more effortlessly than in mammals [1,3]. Further, the close genetic similarities between fish and humans offer to carry out genetic manipulation employing diverse methods, and to study vertebrate development and human diseases [58]. Zebrafish is the emerging star among fish models, widely used in disease research and drug screening. Cytochrome p450 is a family of hemoproteins those codes for enzymes in drug metabolism. Zebrafish have 32 genes which are direct orthologs of that of humans. Notably, metabolic genes are well conserved than other genes between human and fish.[59] Resveratrol was found to improve age-related retinal neuropathy in zebrafish via activation of the AMPK, SIRT1, and mTOR signaling pathways that are involved in human aging [60]. Large genetic screens of this species have applications in transgenesis, mutagenesis and early development studies.

However, the environment affects life over the course of evolution, from aquatic to terrestrial life, and such adaptation changes structures and functions of organ systems. Comparison of fish and humans in terms of anatomy and physiological processes showed marked superficial differences, as well as conserved genetic similarities[10]. Though mammals resemble humans better, fish can be an alternative, as they can offer to reduce the number of mammals sacrificed while supporting the results obtained through in vitro studies. Moreover, fish offers flexibility in experiments; in case a particular species becomes unfit for the experiment, there is always a choice to choose other fish species or to shift to a mammalian model. For example, in developmental toxicity studies, fish models can only assess the direct effects of agents regarding reproduction and development as it does not

include a placenta [61]. Further, there are regulatory challenges to using fish as a model system. The institutional animal care and use committee (IACUC) follows the guide for the care and use of laboratory animals, which ensures that the research animals are cared for and managed according to the highest possible standards. However, the guide contains little information relevant to fishes and other aquatic animals. In addition, the regulators, who are laboratory animal care professionals, might be less familiar with fish as a research model. Hence, it's necessary to introduce fish-related issues to IACUC in order to familiarize with the need as well as a direct interaction between fish users and regulators can be proposed in order to bridge the gap. Moreover, enhanced utilization of presently available data, as well as compilation into a universal authoritative reference, can be recommended [62]. Presently, laboratory fish welfare needs more attention intending to preserve fish health and to reduce inter-individual variability. Further, it is noticeable that even the species who share close evolutionary relationship with human differ in important ways, which can affect the quality of using fish as a stand-in model for human. There is a basic difference in metabolism. Humans, being an endotherm, can keep their body temperature stable whereas fish, an ectotherm, vary body temperature depending on the environment. There can be numerous and unpredictable inter-species variability in terms of drug pharmaco-kinetics and pharmacodynamics [10,12]. Moreover, the influence of water temperature needs important consideration in heterotherms such as fish, as both pharmaco-kinetics and drug activities are temperature dependent. Acclimatization is possible, provided, they are kept at their respective acclimatization temperature in the laboratory [12]. The laboratory fish welfare can be another challenge as the intensification of fish used in laboratory research especially that of zebrafish raises ethical concern [62]. It is also customary to monitor the water quality and other biological requirements when maintaining them in captivity as it can influence their health and resulting research. Moreover, it won't be wise to ignore their inter-individual variability due to disease, conditioning protocols and interspecific and intraspecific variability [63].

Studies can become complicated due to skewed sex ratio in cohorts of zebrafish as sex determination in fish is often flexible, reversible, and difficult to define by genetic factors. Moreover, influence of environmental factors is also not clearly understood [57]. For many genes in humans, there can be multiple, additional forms in fish such as two copies in zebrafish and four copies in gold fish. This significantly complicates the generation of knock out strains [64]. Moreover, only zebrafish genome has been fully sequenced, for which a complete, comparative genetic analysis with that of human being has yet to be carried out.

Another drawback is the lack of placenta in ovo-viviparous fish models, which results in direct interaction between chemicals or drugs and relevant tissues in contrast to indirect interaction through the placental connection in humans. Last but not least both differ from a physical standpoint, that is fish possesses gills and human being have lungs. Besides, fish

have two heart chambers while humans have four, which brings about the complexity of the circulatory system as fish have a single circulatory system whereas the latter have double circulation. Therefore, care should be taken while modelling the complex congenital heart diseases in fish [65]. However, there is no perfect model exist other than the human himself. All model organisms have their benefits and downfalls. Therefore, developing alternative model organisms is carried out that can address the biomedical questions in particular research.

Conclusion

No model organism can perfectly mimic human biology. However, fish is widely used as an alternative model organism in preclinical studies, which could bridge the gap between in vitro analysis and the expensive in vivo screening using mammals. Fish model system is powerful yet not much explored in experimental pharmacology and provides an opportunity to have a better understanding of a disease, its therapeutic targets, drug toxicity, and mechanisms of drug action. The practical use of fish model deals with different branches of science such as regenerative medicines, genotoxic analysis and toxicity assessments. Fish models have been documented as a potential candidate for imitating human diseases. Starting from zebra fish and medaka, a number of fish species have been employed to identify different pharmacological targets. However, the current focus of fish models in pharmacology is mainly on nano-medicines, biosensors and genetic manipulations. Presently, zebra fish and medaka are the common small fish models in use, researches are moving on in broader ranges, with a view to identify more suitable fish models. Genome editing technologies such as CRISPR and TALENs provide further possibilities to create genetic manipulation. However, sequence annotation is not yet complete and further research may enhance orthology. For fish models, replacing mammals completely as a preclinical model is limited in practical use due to the changes in residing environment, physiology, metabolism, unpredictable inter-individual variability due to disease, variable conditioning and interspecific and intraspecific variability. Currently, they can offer a better alternative model for addressing the biological questions from a comparative point of view. The exploitation of individual fish species, being aware of the strengths and limitations of each one, should also be encouraged. Moreover, the understanding of the impact on environment, behavioral, nutritional, genetic and epigenetic factors on research outcome can be extremely challenging. Further, it is anticipated that future investigations may reveal more possibilities for manipulation of this model for pharmacological applications.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

Supriya Raja Harikumar: Collection of information, compilation and preparation of manuscript

Reshma Janardhanan: Writing, reviewing and editing.

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Table 1. Injury models for organ regeneration in fish

Organ	Type of Injury	Reference
Caudal fin	Amputation	24
	Cryoinjury	25
Heart	Resection (ventricle- 20%)	26
	Genetic ablation	27
	Cryoinjury	28
Kidney	Chemical	29
Brain, Spinal cord and Eye	Spinal cord resection	30
	Stab lesion	31
	Optic nerve crush	32
Liver	Chemical	33
	Resection	34
Pancreas	Genetic ablation	35
Skin	Laser	36

Figures

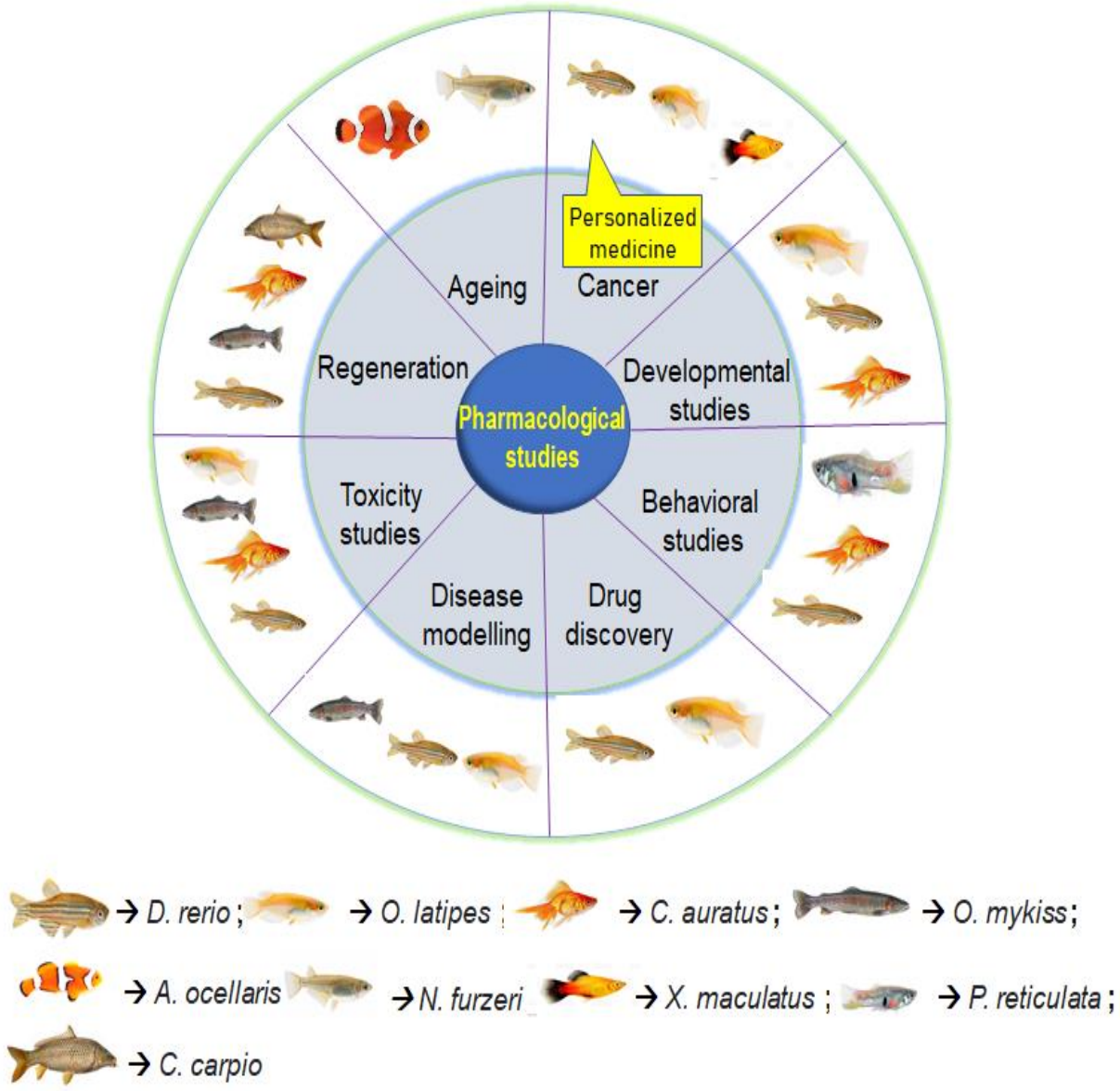

Figure 1. Applications of fish as model organism in pharmacological studies

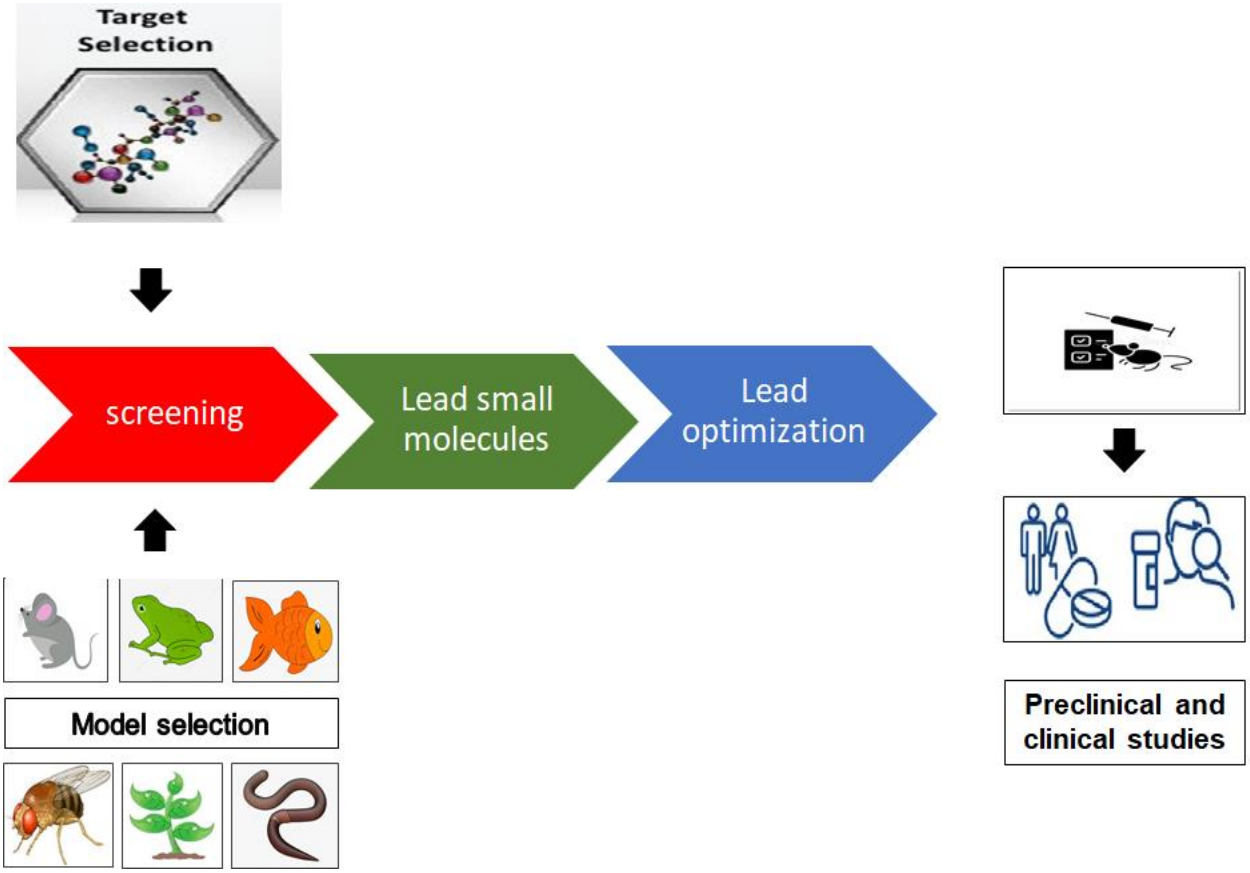

Figure 2. Drug screening strategies via target based and phenotype- based approaches