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Zebrafish: An emerging animal model for pharmacological screening

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Abstract
With 70% gene similarity to that of human diseased gene, Zebrafish is an aquatic vertebrate which has been presenting innovative ways for pharmacological research on human disease since it was first used in 1960s. This review gives an account on the use of various zebrafish models for disease, their developmental procedure and brief idea on related issues. Different models on adult and larval zebrafish are discussed in this review: Nootropic study with various behavioural models with respect to process of memory and learning, Epileptic model with various seizure inducing methods, metabolic disease like zebrafish obesity, diabetes in zebrafish, atherosclerosis model, fatty-liver disease model and account on cancer mode establishment and tuberculosis study by using *Mycobacterium marinum*, which causes infection in adult and larva zebrafish as *Mycobacterium tuberculosis* causes infections in humans. Few Transgenic, Mutant, genetically Knockout or modified zebrafish models are also discussed in above diseases. We conclude that zebrafish is expected to take lead role as a research model in the next some years due to its compelling advantages. However, this would require authentication and simplification of models and their procedures.

Keywords: zebrafish, zebrafish models, metabolic disease, learning and memory, cancer, TB

1. Introduction
Seen from past two decades, the zebrafish with scientific name *Danio rerio*, has emerged as an excellent vertebrate model for studying development and genetics [1] and in recent years, for human disease and the screening of therapeutic drugs [2, 3]. Zebrafish are freshwater fish about 4 to 5 cm long in length. They are native to streams in India and Bangladesh and are commonly kept as pets. The males are slender and torpedo in shape, having blue and black longitudinal lines and usually slight gold colouration on the belly and fins. Females are fat when fraught eggs and have and usually a slight gold colouration on the belly and fins. Females are fat when laden with eggs and have little, if any, gold colour on their undersides [4].

1.1 Advantages of Zebrafish
They are small in size, easy to handle, low cost and easy for maintenance, breading and genetic tractability. Zebrafish have fast growth rate; they become adults in 3 months and are ready to bread. They require less space for husbandry. As compared to rodents, zebrafish have more tractability in forward genetic screens and have more genetic similarity to human beings [4, 9]. Since the fish embryo is not only transparent but also developed outside the mother's body, researcher can manipulate genes to resemble human disease and observe direct change of disease or drug on it, the same cannot be done on rodents. Translucent embryos make zebrafish a more useful model organism in biomedical research, markedly improve monitoring the development of various organs, and help trace ontogenetic profiles of gene expression using various fluorescent probes *in vivo* [10]. The sequence of zebrafish genomes are highly similar to that of human beings. Approximately 70% of genes are associated with diseases in humans have functional homologs similar to those of the zebrafish [11]. They having ability to produce large number of eggs a single cross can generate 200 300 embryos. The transparent eggs were making them capable to follow during organogenesis. Development is rapid with primordial major organs forming within a day after fertilization with a beating heart and visible erythrocytes [5 12]. Compared to other vertebrate, zebrafish show more advantages.

1.2 Disadvantages/Limitations
In spite of the key advantages, zebrafish also have their limitations in research that should be taken into account. Most of the human organs are absent in the zebrafish, including breast tissue, limbs, synovial joints, cancellous bone, and lungs and prostrate. Their skin lacks some specific cellular components and heart lacks septation like human beings. They are ectothermic (cold- blooded); therefore, their physiology is not identical to humans. They had a common ancestor with humans~445 million year ago, which is far more remote from humans than other animals such as rodents (which have 96 million year divergence time from humans). They have up to 48 hpf (hours post-fertilization) of the chorion; therefore, there is large possibility of interference with drug permeability. Eggs are very small; therefore, it takes high precision to control drug delivery and dosage [13, 14]. Administering certain compounds to zebrafish is problematic in certain cases such as water immersion of highly water-insoluble compounds or uptake of a low bioavailability compounds. Also high skill level is required when administering microinjections or oral (using gavage) dosage. Zebrafish also have external fertilization and they don't show parental care of their eggs and larvae, which may limit modelling certain CNS conditions, such as separation anxiety [15].
The most obvious drawback of zebrafish, particularly for questions of human relevance (e.g., disease modelling) is that it is not a mammal but a poikilotherm (body temperature varies with the ambient temperature).

Moreover, the developing embryos lack a placenta, which will make some drugs metabolize in a different manner or, at least, at a different rate compared to mammals and can also alter their function. Zebrafish embryos exposed to drugs in their growth medium absorb these drugs directly without modification by the mother's or placenta's metabolism [15,16]. In contrast to mammals, zebrafish are poikilothermic and does not have brown adipose tissue depots. Hence, the study of thoroughfare that are activated by non-shivering thermogenesis, like β-adrenergic system, will shows more limited in zebrafish than in mammals.

On the other hand, most of the standard animal facilities have rodents at an ambient temperature substantially below the temperature associated with thermo neutrality in rodents; thus, metabolic analysis is habitually administrated exploitation animals that area unit inveterately challenged by cold stress. This condition leads to a range of physiological responses such as increased food intake, fluctuation in metabolic rate and change in sympathetic activity that could cover the metabolic phenotype under research [17].

George Streisinger, the founding father of zebrafish research was one of the first who work with zebrafish in the late 1960s [18].The zebrafish is becoming an popular model for research on affective disorders, disease and pharmacology due to their salient behavioural phenotypes, high compatibility with rodent experimental precedence, and overall efficiency for use as a research organism [12]. The zebrafish produced knowledge and useful model for cancer, diabetes, muscular disease, tuberculosis, brain diseases, antiepileptic and chemical toxicity.

2. Brain study

The genome and genetic pathways controlling signal transduction and development which are highly conserved between zebrafish and human beings [19]. Various molecular tools are available render zebrafish particularly promising for determining the mechanisms of action of different classes of psychoactive drugs. Zebrafish models of brain disorders will also be useful to screen potentially neuroprotective and nootropic drugs development, unfold the mechanisms crucial for new therapeutic treatments [20]. Collectively, all of this suggests that zebrafish represent a useful model in cognitive neuroscience research [20-22].

2.1 Nootropic study

Cognitive impairment or loss of memory is a common symptom in multiple brain disorders including Alzheimer's disease (AD), Huntingdon's disease, autism and schizophrenia [21]. With an increase of global elder's population and cognitive decline observed as age increased, there is an urgent need for effective treatments of age related and neurodegenerative related cognitive deficits [23]. Due to their complex nervous system and well analysed behavioural information, zebrafish are gaining popularity as an excellent intermediate model between *in vitro* and *in-vivo* mammalian models for drug screening and drug development [21].

2.1.1 Behavioural models

Various behavioural models are used to evaluate learning and memory of zebrafish such as Conditional place preference (CPP) test, plus maze test, light dark test, Y-maze test, T- maze test, novel object test and novel tank test same as used in rodents like mice for screening of nootropic dugs [24].

2.1.1.1 Conditional place preference

In the CPP test, zebrafish same as rodents show a preference to the condition that has been previously associated with a reward for example food and drug reward, thus indicating the positive-reinforcing qualities of that substance [25]. The CPP has gained identity for its use of studying the cognitive enhancing effects of nootropic compounds. For example, it was reported that chronic exposure to the Piracetam significantly improves and exposure to Scopolamine induced memory impairment in fish performance in the cued card plus maze test [26, 27], same as to the nootropic effects and memory impairment in seen in humans and rodents [24, 28, 29, 30]. For evaluation of the cognitive enhancing effect and memory impairment effect by compound, behavioural quantification was performed for the following endpoints: latency to the target arm, the number of target arms, incorrect arm and total arm entries, as well as the duration in the target or incorrect arms [21, 26].

2.1.1.2 Avoidance Learning

An active avoidance learning (means an operant procedure in which a particular response allow the animal to avoid punishment) and passive avoidance learning (means experiment in which the animal learn that a particular response leads to punishment) both have been established in zebrafish [31, 32].

This type of avoidance learning test is typically evaluated by the time spent outside our inside the chamber previously associated with an aversive stimulus. It has described that in an active avoidance task where zebrafish must travel from one compartment to the other of a tank to avoid the delivery of a shock or external stimuli. To establish passive avoidance, methods are employed similar to those used in CPP (Conditional place preference), but the US (unconditional stimuli) is must be aversive. Here again, the dependent measure is time spent outside the US combination environment. In this way screening of associative learning can be done by passive avoidance learning, short- and long-term memory in zebrafish and has been used to characterize the effects on learning of antipsychotics [31].

2.1.1.3 Spatial and Visual Discrimination

Special learning in T-maze and Y-maze learning task, hole board maze, three chamber test and visual learning by using colour /pattern. Discrimination procedure has been used to characterize operant learning in rodents and zebrafish. T-maze and Y-mazes are most common methods for arranging an operant relevance and require the acquisition of a spatial discrimination. As a brief example, if the animal makes a "correct" response (e.g. swimming down a central alley and entering a small compartment) the delivery of food or the image of non-specific is initiated then correct response or choice of correct arm and latency to engaged in the correct response can be measured and conclusion about learning and memory can be drawn [24, 31]. The hole-board arrangement consists of an open tank with a board or box with several holes at a centre one of which is rewarded (food). The location of the baited hole can change thought trials, several trials requiring for adoption of the baited arm or remaining the same across a trial which more explicitly

track a memory component [32]. The vertical Cross maze require the introducing of a single fish into it with 4 chamber stacked 2x2 in the water columns - creating bottom left and right and top left and right compartments. As with other arrangements, one compartment is rewarded with an appetitive stimulus, and spatial discrimination is measured [31, 33]. zebrafish acquire a spatial discrimination in three-chambered test in which a left or right turn out of a starting box, within a single session, for correct choice results in increase in tank space and decrease in tank space when fish chosen incorrect choice [34]. In this model, accuracy of choosing compartment and latency to respond are used to characterize acute or long- term drug effects.

2.2 Epilepsy study

Epilepsy is a neuronal disorder allied with distinct neurological and behavioral alterations characterized by recurrent spontaneous seizures. A seizure is a sudden alteration of behaviour due to temporary change in the electrical function of brain. The first zebrafish model in the area of epilepsy research was reported in 2005 [35]. There are mainly two types of epilepsy models and they are further divided in to two more types as shown in fig. 1

2.2.1 Chemically induced epilepsy models

To develop a chemically-induced zebrafish seizure model, zebrafish are exposed to different doses or concentrations of a novel or already know consultant drugs. Often the drugs are added to the swimming/tank water [35, 36]; this method is being most suitable for high-throughput purposes. Another route of drug administration can be injection, intra-peritoneally for adult and for embryonal and larval stage in to yolk sac [38]. Seizures generated by this method are acute and are regularly described as recurrent clonus-like convulsions; they may also be preceded by high-speed (circular) swimming and followed by a loss of posture. This was usually observer in drug induce models the larval and adult PTZ seizure, adult kainate and larval allylgycine model. Also seizures have been described as spasms, twitching, jerking, or tremor all this indication are shown by larval domoic acid seizure model, the adult strychnine seizure model, picrotoxin and caffeine seizure model and larval ginkgotoxin, pilocarpine model, linopirdine and XE991 model and the adult 1,3,5-trinitroperhydro-1,3,5-triazine (RDX) model also display episodes of freezing [35, 37, 38, 39].

2.2.2 Hyperthermia induced seizure model

For a hyperthermia-induced zebrafish seizure model, fish have been exposed to different temperatures and rates of temperature elevation. This can be done by means of a controlled heating device, and should be precisely monitored in proximity of the zebrafish. Yet, only one zebrafish model of hyperthermia-induced seizures has been characterized [40]. It was measured that epileptic form discharges from the forebrain of agar-immobilized 3-7 dpf (day after fertilization) larvae were exposed to an increase in well temperature [40]. The agar temperature rose from ±22 to ±33.5°C in 4-5 min and after that the temperature slowly decreased to baseline. At a temperature of 25.5°C the first epileptic form was observed and was a large-amplitude, polyspiking and long-duration event. After that discharges occurred and lead into high-frequent, small-amplitude, short-duration events. These acute electrographic seizures longs for only for 2-6 min and were age-dependent with a peak at 5 DAF [49, 40]. Compared to rodent models this zebrafish model may be easily used to study hyperthermia-induced seizures in the developing brain.

2.2.3 Genetic and Morphant Zebrafish Models of Epilepsy

The majority of genetic zebrafish epilepsy models were generated using morpholino oligonucleotides (MOs) [41]. MOs have the small size and neutral charge; MOs diffuse through the embryo after giving microinjection in the 1-2 cell-stages [42]. Other models such as one ZFNs-based model [43] and one CRISPR/Cas9-based mutant were studied [44] and the majority of current genetic zebrafish epilepsy models were generated by using MOs [41]. An exponential increase in the number of genetic epilepsy models is to be expected given the successful use of CRISPR/Cas9 genome editing [39]. Observation shows that genetic stable mutant and morphant zebrafish is an excellent model for clinically-relevant epilepsies as they tautologise key features of the human condition.

In last few years, MOs were condemned for their capability to induce off-target effects. Therefore, generation of a morpholino-based model requires its validation with the adequate controls for correct interpretation of obtained results. In brief, MO dosing should be standardized for optimal efficacy, without overt off-target effects, a p53 MO can be co-injected to overcome the common off-target effect of p53-dependent neural toxicity and proper control MOs should be used [39].

3. Zebrafish for metabolic disease

Over the past 3 decades, the prevalence of the common metabolic diseases obesity, type II diabetes (T2D), atherosclerosis and non-alcoholic steatohepatitis (NASH) has soared and these conditions threaten to decrease in lifespan [45]. It is mention that some of the recent studies using zebrafish to model human metabolic diseases and discuss recent progress in using zebrafish to model the interrelated conditions of the metabolic syndrome, which include obesity, diabetes, fatty liver disease and atherosclerosis.

3.1 Zebrafish as Obesity model

Obesity is a result of positive energy balance. Regulation of energy intake and expenditure involves many organ systems including the skeletal muscle, brain, intestines, and adipose tissue. That's why; whole animal models are needed for better analysis of the development and progression of metabolic dysfunction. Zebrafish have adipose tissues, digestive organs and skeletal muscle are the organs that are important for regulation of energy homeostasis and metabolism in mammals which makes it excellent model to study metabolic dysfunction [46, 47]. Excessive nutrients in zebrafish cause rise in plasma triglyceride levels and hepatic steatosis and exhibit deregulation of pathways which control lipid metabolism, including SREBF1, PPARs, NR1H3, and LEP [48]. The conservation of these metabolic pathways which play important role in adipocyte differentiation, energy homeostasis, and cholesterol metabolism demonstrates zebrafish as a suitable model for human lipid metabolism and metabolic disease study [47].

Adipose hypertrophy and hyperplasia are the major characteristic of obesity. Multiple adipose tissue depots are observed in zebrafish and their development has been

characterized. As zebrafish grow appearance and accumulation of neutral lipid droplets appear in visceral adipocytes. Same as mammalian white adipose tissue (WAT), multiple small lipid droplets are present in early-stage zebrafish and single large lipid droplet present in adult zebrafish adipocytes [49]. Like mammals, zebrafish store lipids in visceral, intramuscular and subcutaneous adipocyte depots [50], this helps to understand the regulation and distribution in body. In Comparison of mammals, the high degree of protection in distribution and formation of adipose tissue in the zebrafish makes it a promising model to study the obesity [47].

3.1.1 Methods to Quantitate Adiposity in Zebrafish

Quantitative determination of adiposity is important to assess the degree of obesity-related metabolic mess. Human's adiposity can be measured by body mass index (BMI) and quantitative computed tomography (CT) but application of same methods are more difficult in zebrafish. Instead of this methods, for determination of lipids in adult zebrafish sections and fixed zebrafish larvae some common lipophilic dyes are used for visualizing lipids in histological sections and cultured cells majorly used visualizing agents are Oil red O, Nile red, and Sudan black B[46,51]. It is more easy to take live-imaging, video and fluorescence based screens have been developed in zebrafish larvae as they have optical transparency for screening the study of lipid metabolism and digestive physiology. Nile red is particularly use for live imaging and quantification of intracellular neutral lipid droplets [52] also for purification of adipocyte tissues [48, 49]. Most of the newly developed methods use pretty much accurate methodology of quantifying zebrafish body fat mass using MR images (MRI) and EchoMRI [53]. The body fat mass of eight adult male zebrafish was measured using the two methods and the two techniques showed high correlation. Overall, all these methods provide not only accurate measurements of adiposity but also work as means for longitudinal monitoring [47]. 3D micro-CT is also available for zebrafish and allows determination of total adipocyte tissue and different fat depots [54].

3.1.2 Diets induce obese (DOI)

A common approach is to induce obesity by excess fat intake. Overfeeding larva starting onset of feeding at 5 dpf can leads to obese adult zebrafish. To quantifying obesity progression indicators like Lipid droplets saturation in the blood stream and whole-larval triacylglycerol level can be used. High fat diet leads to zebrafish adiposity and it can be induced in both adult and larval zebrafish by using high fat diet mostly chicken egg yolk solution, although some heavy creams are also used. In this method, adult zebrafish (3 to 4 months of age) were fed with 60mg or 5mg of freshly hatched Artemia per day for 8 weeks (150 calories vs. 20 calories). The over fed zebrafish leads to increased BMI, hypertriglyceridemia and hepatosteatosis when compared to the normally fed zebrafish. Over-nutrition treatment gives same response to both male and female. In this method, adult zebrafish (3 to 4 months of age) were fed with 60mg or 5mg of freshly hatched Artemia per day for 8 weeks (150 calories vs. 20 calories). Instead of Artemia, other methods like providing high-fat zebrafish diets in combination with 20% corn oil/lard and overfeeding, a commercial tropical fish flakes (Tetramin) and 20% crude vegetable oil combination to zebrafish for 256 days starts cardiovascular overload [47, 48, 55]. When compared the metabolic phenotype of obesity induced by extra feeding of a normal fat diet (NFD; Artemia cysts, 22% fat) to that by high fat diet (HFD; egg yolk powder, 59% fat) no doubt both leads increase adiposity but zebrafish with NFD-induced obesity are metabolically healthy than HFD-induced obesity. HFD-induced fish are metabolically unhealthy and also shows fatty liver, glucose intolerance, and increase of visceral fatin body [53].

3.2 Zebrafish as diabetic model

The basic cellular architecture and morphogenesis of zebrafish pancreas are similar to mammalian pancreas [56]. Same as mammals, zebrafish have pancreas consist of exocrine and endocrine compartments which are connected to the digestive tract through a ductal system. Zebrafish's consist of a central core of insulin-producing β-cells surrounded by glucagon-producing δ-cells (somatostatin producing cell), α-cells and ε-cells (ghrelin producing cell) in pancreatic islets, the primary islet can be observed as early as 1 dpf [57]. The zebrafish are highly homologous to mammals in signalling pathway and mechanism of its endocrine pancreas development. Lots of genes have been identified which influence β-cell progress in zebrafish[58]. The similarities of the pancreas structure and glucose homeostasis system make zebrafish useful to identify novel target model in pancreas related diseases such as diabetes in humans.

3.2.1 Induced model for diabetes in zebrafish

There is several ways to induce diabetes in zebrafish as given below.

3.2.1.1 Pancreatectomy

For this method, direct physical removal of pancreas from adult fish is required under the microscope in transgenic zebrafish with islet specific expression of green fluorescence protein. This method is used only for type 1 diabetes mellitus (T1DM) [69]. However this method seems much tidies and technically difficult and is not commonly used in zebrafish.

3.2.1.2 Chemical-induced diabetes

Chemical-induced method is preferred for rodents and also in zebrafish for inducing diabetes. Streptozotocin (STZ) injection by Intra-peritoneal route shows effect on b-cell ablation in juvenile zebrafish and leads to increased fasting blood glucose and decreased insulin levels. An average 6 doses of STZ within 1 month induce stable hyperglycaemia and diabetic complications including nephropathy, retinopathy, and impaired fin regeneration. Alloxan exposure through incubation or intra-peritoneal injection pusses T1DM in larval and adult fish, it causes b-cell necrosis, decreased neuromas number [59-62].

3.2.1.3 Incubation in glucose solution

This method is used for type 2 diabetes mellitus (T2DM) in juvenile zebrafish by directly immersing zebrafish in glucose solution. Due to its convenience this method is mostly preferred for T2DM. Introducing zebrafish into concentrations of 0 and 2% glucose water every other day or chronic exposure to 2% glucose solution respectively for 28-30 days and 14 days. Which results in diabetic

phenotypes such as elevated blood glucose levels, diabetic retinopathy and impaired response to exogenous insulin [63, 64]. Zebrafish with age of 4-11 months acclimate to glucose exposure much better than zebrafish with age 1-3 years, but persistence hyperglycaemia can also be achieved in 4-11 months old zebrafish by gradually rises glucose level [65].

3.2.1.4 Over nutrition

Over nutrition results insulin resistance, elevated fasting blood glucose, and impaired glucose tolerance in mammal's similar result observed in zebrafish overfeeding to zebrafish with a commercial food diet [66].

3.3 Zebrafish for atherosclerosis

The formation of athermanous plaques within arteries leads to the development of atherosclerosis. Atherosclerosis is closely linked with high plasma lipid levels and ensuing vascular inflammation.

In zebrafish, a high cholesterol diet (HCD) can induces the extreme hyperlipidaemia same as mammals, with the added benefit that the temporal course of pathogenic events can be observed in zebrafish, *in vivo* via the use of fluorescent reporters and live imaging. For example, *fli1: EGFP* transgenic zebrafish on HCD diet with a fluorescent lipid tracer has allowed researchers to visualise lipid accumulation in the vascular wall [67]. However, the recruitment of myeloid cells in the vasculature can be monitored by using the transgenic line *lyz: Ds Red* labels macrophages and granulocytes. Ezetimibe is an anti-dyslipidaemia drug, a synthetic compound which used to treat hyper-cholesterolaemia in humans which decreasing the absorption of cholestrol by intestine result in reduce cholesterol level, same compound used in zebrafish. Which make zebrafish promising model for anti-dyslipidaemia drug [68].

3.4 Zebrafish for fatty liver disease

Excess accumulation of lipids in the liver causes a various disorders that encompass inflammation, fibrosis and cancer. All this conditions are collectively known as non-alcoholic fatty liver disease (NAFLD). Very less information is available on what triggers and drives the progression of NAFLD. zebrafish have advantage that within 2 dpf the differentiation of hepatocytes and cholangiocytes and the formation of the liver primordium [69].

3.4.1 Dietary fish models

Zebrafish DIO exhibit increased BMI, hepatic steatosis and hypertriglyceridemia. In both DIO-zebrafish and obese mammals, it is observed that dysregulation of genes such as apoH, interleukin-6 (IL-6) involved in the blood coagulation pathway and genes such as SREBP1, PPARα/γ, NR1H3 and leptin involved in lipid metabolism [48, 70].

3.4.2 Chemically treated fish models

Zebrafish treated with Thioacetamide-treated HCP transgenic fish indicate phenotype like Steatohepatitis, cirrhosis, HCC [71] thioacetamide-treated fish shows Steatohepatitis [72].

3.4.3 Genetically mutant animal

For NAFLD mutant zebrafish could be a useful model to study its pathophysiology. Mutants like *ahcy* zebrafish focusing new potential therapeutic options for patient with AHCY deficiency and reverted hepatic steatosis and liver degeneration because of morpholino knockdown of tumour necrosis factor-α [73].

4. Cancer study

To study human cancers zebrafish has now a day's emerging as an invaluable model with a different advantage of it arise from the evolutionary conservation of genetic pathways implicated in cancer that are shared between fish and humans, that's why zebrafish is unique tool for underlining cellular processes and modelling human diseases. In last decade, well-established transgenic methodologies and the identification of lines with gene-specific mutations is helpful tool for developing zebrafish for human cancer study. Human hematologic malignancies, melanoma, rhabdomyo sarcoma, and other solid tumours studies are included in these models. Cancer reveals a high similarity in cancer-associated gene and its expression between zebrafish and humans [74].

4.1 Cancer model establishment in zebrafish

Neoplasia was hardly developed in wild zebrafish. Using a combination of various chemical treatments, genetic technology and tumour cell xenotransplantation, the vast majority of human tumours can be modelled in zebrafish [75]. Oncogenesis can be induced commonly by several carcinogenic compounds and carcinogenic chemical treatment, which are leads in canceration in a number of organs of fish; examples are given in table no. 1.

There are so many genetic tools which are developed for the study of gene function in zebrafish. For transient knockdown of the gene expression, embryos are injected by Morpholinos at 1-4 stage of cell division [76]. In 2005 the first human xenotransplant assays in zebrafish was done by injecting 1-100 melanoma cells into embryos after 3.5- 4.5 houre post fertilization, the migration in the developing larvae was clearly observed. Microinjection of glioma stem cells into the yolk sac region of embryo in 2 dpf embryos resulted in an observable invasion in the embryos via the vessels. Hepatocellular carcinoma (HCC) was also modelled for the identification of the curative effect of anti-cancer molecules. Several other types of tumour, such as lung cancer, pancreatic cancer ovarian carcinomas, breast cancer, prostate cancer, retinoblastoma, and leukemia, have also been transplanted in zebrafish [74].

5. Tuberculosis study

Now days, the means for preventing, diagnosing, and treating TB are not much satisfactory. There is lacks of good animal study for latency reactivation and dormancy of the TB which is one of the main reasons for the weak progress in TB. Although sophisticated *in vitro* and *in silico* methods are suitable for TB research and constantly being developed but they can't reproduce the complete vertebrate immune system and its interplay with pathogens and vaccines. To study the complex pathophysiology of mycobacterial infection traditional animal models such as mice, rabbits, guinea pigs, and non-human primates are can now replace by newly emerging zebrafish models [90].

M. tuberculosis is the pathogen responsible for TB infection in humans but does not seem to cause disease in cold-blooded animals that's why an alternative pathogen is used. Number of mycobacterial pathogens is capable for causing infection in zebrafish, *M. marinum* is the most interesting

candidate between them [91]. *M. marinum* is the causative agent of a tuberculosis-like disease in cold blooded animals [92], and it shows close genetic similarity to *M. tuberculosis. M. marinum* have good host range and capabilities to survive in the environment. Orthologous coding sequences share an average amino acid identity of 85% [91].

5.1 *Mycobacterium marinum* model for Tuberculosis study

This model is based on close similarity between *M. marinum* and *M. tuberculosis*. *M. Marinum* shows generation of macrophages aggregates and granulomas same as the *M. tuberculosis* infections in humans, in both adult and larvae zebrafish [90]. *M. marinum* is occasionally infects humans and generally spread via contaminated water, but for humans infection is usually limited to the skin commonly known as fish tank granuloma [93]. Thus, *M. Marinum* is safer to work with than *M. tuberculosis* [94, 95].

5.2 Infection to adult Zebrafish

Human TB has key feature a chronic infection with necrotic granulomas, intra-peritoneal injection with *M. marinum* shows same infection in adult zabrafish witch are most commonly found in the pancreas, adipose tissue, liver, spleen, and gonads. The first granulomas can be found already in the first weeks of post-infection. Even the first signs of necrosis, consisting of cytoplasmic and nuclear debris, are present at this time. Although, a latent, chronic, or active mycobacteria disease based on the *M. marinum* strain used and its infection dose [96-99]. For stable number of granulomas over time low infection dose is required, while high infection dose leads to a more progressive and active disease [96]. During a chronic disease course, bacterial growth mimics curves of various other animal models of TB and growth for the first 21-28 days and reaching a plateau when adaptive immunity develops [97-98].

5.3 Infection to zebrafish embryos

Pathology of TB in zebrafish embryos usually studied only for 5 to 6 days because of practical/ethical reasons. Within this short time frame, early granuloma formation can be studied by real time imaging. This helps to pasteurised initial steps in mycobacterial pathogenesis in the reference of innate immunity. *M. marinum* have endothelial and epithelial barriers of macrophages after infection which phagocytized it and form infectious clusters in deeper tissue within 4 day [100-103]. In these clusters mycobacteria genes are activated that are known to be particularly activated in mature granulomas in adults, this confirm that such a infectious clusters actually corresponds to granulomas[104]. This means that innate immune determinants are sufficient to drive *M. marinum* granuloma formation/ initiation[102, 103, 105].

6. Conclusions

Zebrafish have a lot of advantages including its size, maintenance, cost efficacy and genetic tactility, despite the presence of few limitations. Zebrafish will serve a good model for various metabolic and brain disease, cancer and indecisions disease such as tuberculosis.

For memory and learning, a lot of behavioural models are available which will help researcher to screen new drug, to find mechanism of psychoactive drug and to use the drug in brain diseases. However, these models are much similar to mammalian behavioural model. Hyperthermia induced seizers are characterised by only one method which required more authentication. Same as in mammals, obesity can induce by fat diet and nutrition, and hereditary obesity can be studied in zebrafish by genetically modified models. As various cancer drugs have been discovered, they can be screened on zebrafish as it develops various type of cancers. *M. tuberculosis* is infective agent in humans and for aquatic animals; *M. Marinum* is infective and shows same infection like *M. tuberculosis.* Although zebrafish are not having lungs, they still serve as a successful animal model for TB. Zebrafish can be debated to be a better alternative for animal study for various diseases. As it is small animal yet giving good results and use full in broad area of diseased research work it can be preferred for pharmacological study.

7. Tables and Figures

Table 1: Some methods used and type of tumor indused.

Models	Treatment	Types of induced tumour	References
Chemical treatment	DMBA	hepatoma, cholangio carcinoma and intestinal cancer	77
	DEN	hepatoma, cholangio carcinoma and pancreatic carcinoma	78
	NDMA	hepatoma and cholangio carcinoma	79
	ENU	hepatoma and testicular cancer	80
	MNNG	hepatoma and testicular cancer	81
Genetic technology Knockout	P53	malignant peripheral nerve sheath tumour	82
	APC	colon adenoma	83
	NF1	gliomas and malignant peripheral nerve sheath tumour	84
	BRCA2,MYBL2,ESP11	testicular cancer	85
	CDS GENE,BMYB	epidermal cancer	81,86
	GSTT1	lymphoma	87
	VHL	hepatoma and intestinal cancer	88
	PTEN	T-cell acute lymphoblastic leukemia and hemangio sarcoma	89
Xenotransplantation	Transplant tumour cells in zebrafish	Melanoma, glioma, hepatoma, lung cancer, pancreatic cancer, ovarian carcinomas, breast cancer, prostate cancer, retinoblastoma, leukemia	74

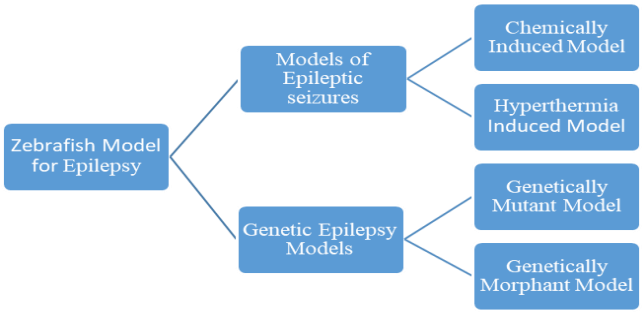

Fig 1: Classification of current zebrafish models of epilepsy and epileptic seizures.

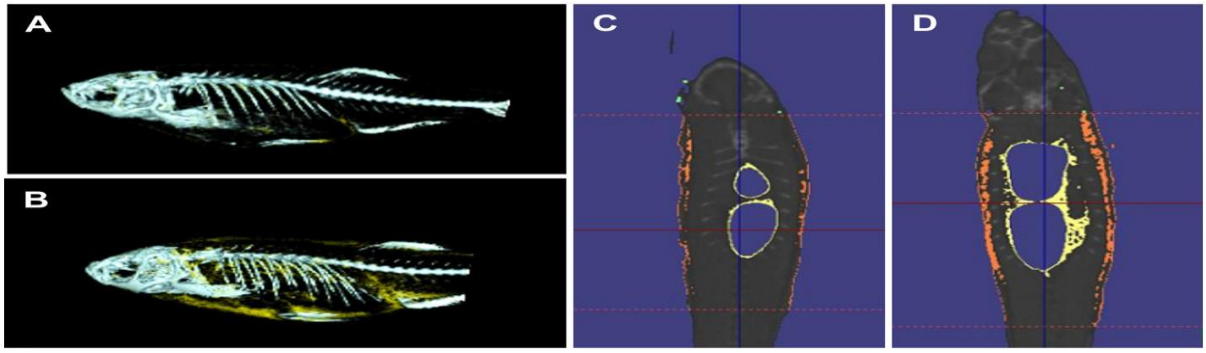

Fig 2: 3D micro-CT analysis in normal fed and diet-included obese zebrafish. (A) 3D-images of normal fed zebrafish. Grey colour indicates skeleton and yellow colour means adipocyte tissue. (B) 3D-images obese zebrafish. (C) Cross-sectional images of normal fed zebrafish. Yellow colour indicates visceral adipose tissue and orange colour indicates subcutaneous adipocyte tissue. (D) Cross-sectional images of obese zebrafish [47].

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