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ZEBRAFISH AS A POTENTIAL MODEL IN DRUG DISCOVERY

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ABSTRACT

The use of zebrafish (Daniorerio) has gained importance in technical research in the past years and is rising very rapidly. Initially, it was considered as a popular model for vertebrate development as zebrafish have many advantages over rodent which allows large scale drug screening. The zebrafish genome sequence is 70% similar to humans and the genes causing disease in zebrafish is seen in human as well. Zebrafish has attracted the research field area in pharmacology, toxicology, drug screening, target identification, target validation, drug discovery, qualitative structure-activity relationships study, and structure-activity. With the understanding of technologies for manipulating zebrafish increases, it is believed to play a key role in accelerating the emergence of precision medicine. This paper reviews on using zebrafish as a potential tool in drug discovery and to make zebrafish as a prominent model in drug discovery and research development.

INTRODUCTION

Daniorerio, commonly known as zebrafish is considered as an important vertebrate model to study various diseases. They grow rapidly producing 100 to 200 offspring per week with a single pair of adults. The transparency of embryos is an advantage to conduct experiments at an early stage which can survive in 100µl of fluid, reducing the maintenance costs to less than 1/1,000th of the cost of similar study in mice. At the molecular and cellular level, zebrafish is remarkably similar to humans with 71% of human proteins and 81% of disease-causing human proteins ortholog. An advantage in conducting research at embryonic stages in lower vertebrates is in the strive to reduce, replace and refine.

Zebrafish In Cancer Research: Cancer is major disease causing death worldwide at present. There will be an increase of 18.1 million new cancer cases and 9.6 million deaths from cancer according to WHO[1].Zebrafish due to its small size, heavy brood and rapid maturation time, has gained importance in the cancer research field that complements what can be achieved in mice and cell culture system. In this model, a wide range of tests can be performed, from target detection, target validation or toxicological studies to tumor generation to perform the appropriate in vivo efficacy tests[2,3].A broader range of phenotypes can be tested in zebrafish so it has an advantage against cell-based study [4]. There are various approaches to human cancer in zebrafish, such as development of mutatant and transgenic lines and tumor cell transplantation . Embryos are most widely used when the study's main purpose is to visualize a concrete tumor process

because their bodies are transparent and allow observation of microscopy. Additionally, cancer develops faster in embryos, showing tumor formation in two days after induction.Therefore, they could be employed in projects that demand rapidities, such as imaging cancer processes or screening campaigns. In contrast, adults suggest a more accurate in vivo model as all their organs and immune systems are developed; however, it takes 10-14 to 1 month to establish cancer[5].

MUTANT LINES: Initiation processes of tumor cannot be observed, and so approaches for manipulating zebrafish genome and to mimic cancer initiation and progression are necessary to save time and make it operable. The introduction of a mutation into the genome of zebrafish can be done through many ways such as chemical mutagenesis, irradiation mutagenesis, insertional mutagenesis, and can be transposon-based or viral-based vector mutagenesis. A researcher found the development of the various type of cancer by using carcinogenic compounds such as dimethylbenzanthracene (DMBA)[6], diethylnitrosamine (DEN)[7], N-nitrosodimethylamine (NDMA)[8], N-ethyl-N-nitrosourea (ENU)[9], and N-methyl N1nitro-N-nitrosoguanidine (MNNG)[10]. Genetic mapping, sequencing analysis, and phenotype validation are methods to identify the genes that harbor genetic mutations[11]. The invertebrate model system such as yeast, and Drosophila are also used to show a successful strategy to discover novel genes that function in pathways affected by cancer[12]. Mizgireuv and colleagues stated, zebrafish resulted in various types of hepatocellular carcinomas, hepatoblastomas, hepatoma, cholangiocarcinoma, and pancreatic carcinoma when exposed to DEN [13]. Cholangiolartumors and hepatocellular tumors were observed when zebrafish were exposed to NDMA for 2 months[8]. Liver and tumorigenesis were reported in zebrafish exposed to ENU and MNNG[9,14].

TRANSGENIC LINES: Induction of transgenic lines to zebrafish is created by microinjecting exogenous DNA into one-cell-stage zebrafish embryos[15]. The investigation of gene functions in zebrafish has been developed with a number of reverse genetic tools such as morpholinos, TILLING (Targeting Induced Local Lesion In Genomes), ZFNs (Zinc Finger Nuclease) CRISPR- Cas system (Clustered Regularly Interspaced Short Palindromic Repeat) and TALENs (Transcription Activator-like Effector Nucleases)[16,17].Zebrafish knockdown p53 by morpholino oligonucleotide resulted in apoptosis due to DNA damage[18].Zebrafish PTEN (ptena and ptenb) development has been identified by two approaches such as morpholino knockdown and germline ENU mutation identified by TILLING. The loss of either ptena and ptenb result in an increased AKT pathway in zebrafish development embryos[18].

TRANSPLANTATION OF TUMOR CELL IN ZEBRAFISH: Transplantation of tumor cell in zebrafish is an ideal method to understand the processes of tumor cell extravasation, migration, angiogenesis, and metastasis[19,20,21].The immune rejection of inoculated tumor cells is one of the major transplant disadvantages. It and colleagues reported, a approach to evade that process in the mouse model is the use of NOD/SCID mouse, which has alterations ofmultiple immunology, such as the immunosuppression of T, B, and natural killer cells[22]. Zebrafish embryos are considered most preferable in transplantation assay[23] as the embryos have not completely developed their innate and adaptive immune system until 21 days of life[24]. At this point, immature T and B cells reach the thymus, finalizing the immune maturation process[25]. The development stages of zebrafish are to be considered for transplantation of tumor cell as adult zebrafishinvolve immune system ablation to evade engraftment rejection.Traver and colleagues proved that sublethal radiation (20–25 Gy) as one strategy to produce immune ablation and 90% survival[26].Hematopoiesis is subsequently resumed 12 days after irradiation and the marrow is completely restored 20 days after irradiation, killing embedded cells (15).Gy of gamma-irradiation can ablate T cells in embryos 6 dpf to 1 month old[27]. Chemical treatment with dexamethasone is another strategy for immunosuppression allowing solid tumor transplantation[28]. This method is not considered as lines are difficult to maintain and is associated with other diseases [29]. Zhang and collegues have developed a novel strategy for

tumor cell transplantation without immunosuppression.This method involves transplanting irradiated human tumoor cells into an embryo of zebra fish and retransplanting non-irradiated cells into the same zebrafish three months later[30].

TERATOGENICITY: Zebrafish has been proven as a developmental model to study chemically induced teratogenicity.Zebrafish has advantages over another animal model such as inexpensive species, easy to breed and produce large progeny. The morphological changes in organ systems and structure can be detected due to the embryo transparency and rapid embryonic development, thus providing an actual alternative model to test the teratogenic and embryotoxic potential of chemicals[39]. The zebrafish genome is 1700 million base pairs in length, which is about half the size of the human genome. Most human genes have homologs to zebrafish and the functional domain of the protein such as ATP binding domain of kinases are almost 100% identical between homologous genes, although the similarity over the entire protein is about 60%[3]. Various approaches such as genetic and molecular biology due to developmental pathways conserved between zebrafish and humans[40], it is used to study mechanisms of teratogenicity of chemicals.Exposure of ketamine after 256-cell development to zebrafishembryos resulted in bone and cartilage malformations which is considered as the most susceptible phase. Concentration-dependent mortality and malformations such as lordosis, kyphosis, and microcephaly were observed at 256- cell stage[41].Anticancer drugs such as Sunitinib, quinine and cisplatin showed moderate toxicity towards zebrafish embryos at a dose of 20µM[42]. Teratogenicity was also performed in another animal model such as chick where Shauna and colleagues performed teratogenicity in chick embryos and confirmed that angiogenesis inhibitors, regardless of the molecular target, are teratogenic when exposed to chicken embryos[43]. Teratology was tested in zebrafishembryos with compounds such as retinoic acid, lithium hydroxide, ochratoxin A, 6 aminonicotinamide, sodium arsenate, and ethanol. All compounds except sodium arsenate were teratogenic in zebrafish embryos[44]. The teratogenic potential of seven AEDs carbamazepine (CBZ), ethosuximide (ETX), valproic acid (VPN), lamotrigine (LMT), lacosamide (LCM), levetiracetam (LVT), and topiramate (TPM)) was tested in the zebrafish[45]. The above methods proved that zebrafish can be used as a tool to study teratogenicity.

TOXICITY: Zebrafishis presented as a potent vertebrate invivo model for the testing of drug toxicity and efficacy to acknowledge the new generation of drugs. According to the US government, toxicity testing for rodents and rabbits has been the standard for evaluating acute toxicity since the 1950s. The process is however expensive and time-consuming, which led to a backlog in chemical testing.[40,46]. Because of these restrictions, the need for use of other substitute animal models has increased. Zebrafish are used to evaluate varioustoxicity studies such as cardiotoxicity, neurotoxicity, nephrotoxicity, genotoxicity, and hepatotoxicity.

Cardiotoxicity: Although zebrafish (Daniorerio) and mammalian heart have some physiological differences, it is used to study heart regeneration and heart development[49,50]. Zebrafish is a vertebrate species whose genome has been sequenced[51], produces large progeny which are transparent for visualization of the drug effect on the heart[26,52]. Geno-cardiotoxicity can be evaluated by using zebrafish as a model organism as it has a cost-effective benefit[53].The heart of zebrafish is two-chambered and the electrical properties are similar to humans such as heart rate and action potential[54,55]. Drug-induced cardiotoxicity has been successfully tested to study the effect of drugs in zebrafish[56,57]. Zebrafishcardiotoxicity test describes the potential toxicity of drugs to the human cardiovascular system concluding in vivo studies as an essential step in drug development and toxicity studies[47]. Dibutyl phthalate (DBP) caused morphological alteration of heart development in zebrafish embryos, such as pericardial edema and cardiac structural deformities, characterized by elongated, thin and string-like heart.[58]. Doxorubicin effectfor cardio toxicity study inzebrafish has been evaluated and stated high doxorubicin doses showed lethal effects whereas low doxorubicin doses resulted in sub-lethal effects, malformations, and changes of heart rate[59]. High concentration of *Sutherlandiafrutescens*extracts cause bleeding

and pericardial cyst formation to the zebrafish embryo culture and chronic teratogen toxicities, pericardial edema, yolk sac swelling, and other abnormal developmental characteristics, were reported[60].

Neurotoxicity: Zebra fish has been used as a model organism to evaluate neurotoxicity by several chemical candidates. Zebrafish models were used to assess the toxic effect of different xenobiotics on specific cell types in the nervous systems, like dopaminergic neurons or the mechanosensory system[61,62]. Neurodegeneration was mainly caused when nanoparticles reached the brain leading to cause changes in the activity of the Central Nervous System[63,64].Using zebrafish embryo, nanoparticle neurotoxicity was determined to study the radioprotective effect of dendrofullerene nanoparticle (DF-1), which resulted in dose-limiting toxicity level[65]. Zebrafish embryos were used to study the effects of the drug on dopaminergic neurons by using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and sodium benzoate, both the drugs were reported to decrease the expression of slc6a3, a membrane transport protein involved in dopamine reuptake that is a specific marker of dopaminergic neurons[66,67]. Neurotoxicity effect was evaluated by TiO2 nanoparticle in zebrafishmodel, expressions of different genes such as BDNF C-fos and C-jun was activated by TiO2 nanoparticle.

Nephrotoxicity: Zebrafish has also been used to study the nephrotoxicity of various compounds.There was an increase in proinflammatory genes and the formation of cystic glomerular and tubular lesions along with reduced kidney functionality when zebrafishfish embryos were exposed to two mycotoxins, citrinin, and patulin[68].Renal failure and kidney malformationswas observed when zebrafish was exposed to aristolochicacid, a medicinal plant extract[69]. It was reported that treatment with acetaminophen[70,71] and sodium benzoate[72]caused nephrotoxicity that resulted in malformed kidneys and defective pronephric tubes. Similarly, Exposure ofmicrocystin-LR reported nephrotoxicity where apoptosis was triggered in female zebrafish and oxidative phosphorylation pathway was seen to be affected and the renal tubes showed eosinophilic casts[73].

Hepatotoxicity: Several methods have been established to study the effect of hepatotoxicity in zebrafish and other higher animals as well. The pharmaceutical main concern for toxicity was found tobe hepatotoxicity.Zebrafish liver organogenesis begins at 3 days post fertilization and is fully functional by 5 days post-fertilization[74]. Zebrafishhave a wide range of cytochrome P450 enzymes that allow metabolic reactions including hydroxylation, oxidation, conjugation, demethylation, and de-ethylation[75]. Goldstone and colleagues characterized a total of 94 *CYP* genes in the zebrafish genome and reported that these genes fitted into 18 *CYP* gene families which are also present in humans and other mammals based on homologous amino-acid sequences[76]. It was also suggested that zebrafish have an analogous metabolic system which is similar to human CYP2C8/9 as hydroxylated ibuprofen was detected in exposed ibuprofen embryos[77].Compounds like amiodarone, simvastatin, tetracycline or valproic acid which were found to induce steatosis in the liver showed similar effects in zebrafish[78,79]. Exposure of gold nanoparticles[80], mercury[81], arsenic[82] and methyl parathion, a pesticide[83]in zebrafish embryos resulted in hepatotoxicity. Hepatotoxicity is derived from metabolic processes so zebrafish are useful to study drug-induced liver injury to evaluate parameters such as apoptosis, liver opacity or size. Oxidative stress and apoptosis in the liver were associated when exposed to silver nanoparticles[84].

Genotoxicity: In toxicology and drug development, assessing genotoxicity is an important component. The tests carried out to determine genotoxicity include in vitro and in vivo micronucleus assay, Ames test, Comet assay, and chromosomal aberration tests. Zebrafish emerged as an alternative method to evaluate genotoxicity. Other animals models such as rats and medaka fish[85] were also used to study genotoxicity of drugs through comet assay, micronucleus test, and gene profiling techniques. The comet assay was used to evaluate the presence of micronuclei in gonad, liver, or an alkylating agent methyl methanesulfonate when exposed to adult zebrafish for 2 weeks[86]. Thus, zebrafish was measured as an efficient vertebrate model to study genotoxicity through comet assay and the

micronucleus test. Exposure of xenoandrogens and xenoestrogen confirmed DNA damaged of zebrafish by erythrocytic nuclear abnormality assay[87].

EPILEPSY: Zebrafishwas considered as a desirable model for epilepsy as it has a complex nervous system capable of sophisticated behaviors and susceptible to seizures. During the years, zebrafish was considered as an alternative model to other experimental animals such as rodents to study the molecular mechanisms resulting in deficitandthescreeningofpotentialtherapeuticco mpounds[88]. Pentylenetetrazole (PTZ) was induced in zebrafish embryos of 6-7dpf to induce an epileptic seizure , and resulted in full body convulsion followed by a brief loss of posture[89]. Pilocarpine, a muscarinic acetylcholine receptor agonist injected in rats showed cognitive and memory deficits which were commonly found in temporal lobe epilepsy patients[90]. Zebrafish larvae were used as a model to understand the relationship between carboxypeptidase A6 (CPA6) and seizures by morpholinoknockdown of *cpa6* mRNA which resulted in resistance to the effect of seizure-inducing drugs pentylenetetrazole and pilocarpine on swimming behaviors. After 1-day pilocarpine treatment, like *cpa6* knockdown,led to a reduced sensitivity to pentylenetetrazole[91]. Kainate administered to adult zebrafish resulted in seizures of various stages depending on dose-dependent which was similar to seizures seen in rodents[92]. The above statement concluded that zebrafish produces seizures similar to rodents and aids in the field to investigate the role of new compounds in drug discovery.

DIABETES: Diabetes mellitus is a chronic disease that results in the health problem leading to reduced life expectancy. Diabetes mellitus can be associated with a complication such as retinopathy, nephropathy, neuropathy, impaired wound healing, heart disease, and stroke.Rendering to the American Diabetes Association, 9.4% of the population has diabetes. Diabetes is classified as Type 1 and Type 11. Animal models for both classes have been established to study the role of diabetes.

Type 1 Diabetes: Type 1 diabetes is an autoimmune disease that leads to decreased insulin production due to the destruction of the pancreatic beta cells in the islets of Langerhans. Animal models are available to study metabolic diseases where rodents are chemically induced with streptozotocin (STZ) or alloxan due to their structural similarity to glucose[93]. The induced diabetes models resulted in endogenous beta cells destruction with little insulin production. The changes in P450 isozymeswhich can be regarded as toxic was also noted in the induced diabetes model[45].. Similarly, zebrafish as a model for the study of type1 diabetes was induced with streptozotocin which was associated with known human secondary complications. In a hyperglycemic environment, zebrafish exhibited impaired limb regeneration and endogenous pancreatic beta cells regenerationwith a duration of 2 weeks which was reverted back to normal glycemia after drug removal. In the acute diabetic state, limb regeneration remains impaired where complications were observed which can be susceptible to metabolic memory[94].

Type 2 Diabetes: Type 2 diabetes is a chronic disease categorized by insulin resistance.T2DM is mainly associated with obesity and more than 90% of people with T2DM are overweight or obese. The zebrafish (Daniorerio) can be considered as an established model organism for the study of molecular and metabolic diseases. Diet-induced obesity(DIO) model in zebrafish can be obtained by overfeeding of artemia at 5dpf, an advantageous over rodent models since diet can only be manipulated after weaning, which is at least 3 weeks after birth.Increased BMI, hypertriglyceridemia, and hepatosteatosiswere reported inoverfed zebrafishwhen compared to zebrafish which was fed normally[95]. In addition, a comparative transcriptome analysis of visceral adipose tissue in zebrafish, mouse, ats and humans revealed that zebrafish lipid metabolism networks are similar to those in mammals[96].Another method for T2DM model in zebrafish can be performed by immersing zebrafish embryos or adult zebrafish in alternating concentrations of 0 and 2% glucose solution for a 28-30days or exposure for 14 days with 2% glucose solution showed diabetic phenotypes similar to mice such as elevated blood glucose levels and impaired response to

exogenous insulin[97]. Thus, zebrafish can be considered as an established model organism for the study of metabolic diseases.

NEUROPHARMACOLOGY: Zebrafish is gaining its importance in the field of neuropharmacology as they display neuropathological and behavioralphenotypes relatable to man. With the increase in age, neurological disease is increasing and there is a much need for effective therapies to treat neurological diseases. Invivo models are available which does not quantify the approaches to treat the disease. Zebrafish came into the picture as the zebrafish genome organization and the genetic pathways controlling signal transduction and development are highly conserved between zebrafish and man[103].The available resources justify zebrafish as an excellent model for Neuropharmacology.

ALZHEIMER'S DISEASE (AD): Alzheimer disease is the main cause of dementia in the human population and is characterized mainly by impairment of speech and motor ability, delusion, depression, hallucination and aggressive behavior[104]. It is noted that in the cerebral cortex, there is massive neuronal loss and impaired synaptic processes. Pharmacological models of zebrafish can be studied into three main domain which includes cholinergic neurotoxins, glutamatergic neurotoxins and GABAnergic neurotoxin[105]. Scopolamine, a cholinergic muscarinic receptor antagonist was induced in zebrafish to demonstrate the activity of decreased cholinergic system which resulted in learning deficits showing similar occurrence on mammals.Further, the scopolamine-induced learning deficit was prevented by quercetin and rutin in zebrafish[106,107,60]. AD results in neuronal damage or death when glutamate receptors are overactivated known as excitotoxicity[108]. Zebrafishcan be used as a model to study excitotoxicity by inhibiting seizures using a specific glutamate receptor.Domoic acid was microinjected in fertilized eggs of zebrafish and showed results which reduced the hatching rate and uncontrolled pectoral fin motions and tonic-clonic like convulsion[109].Zebrafish were exposed to pentylenetetrazole (PTZ) to study GABAnergic neurotoxins which resulted in a number of behavioral changes leading to clonus-like convulsion[110]. In adult zebrafish, PTZ showed effects in the acquisition and maintenance of passive avoidance response[51].

PARKINSON'S DISEASE: Parkinson's Disease (PD) is considered as the second most common human neurodegenerative disease after Alzheimer[111]. It is characterized by loss of dopaminergic neurons and frequent formation of Lewy bodies which results in activity with resting tremor, muscular rigidity, bradykinesia and postural imbalance[111]. PD is associated with six genes such as α-Synuclein, Parkin, PINK1, DJ-1, LRRK2, and UCHL-1[112]. The exposure of MPTP in humans lead to a loss of dopaminergic neurons and parkinsonism[113]. Pharmacological model available for PD in zebrafish was demonstrated using 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)[114,112].MPTP was exposed in all developmental stages of zebrafish such as embryo, larvae, and adult. In the treated embryos, a loss of TH was marked whereby, loss of dopaminergic neurons, decreased the level of dopamine, norepinephrine, and serotonin, and impairment in motility was noticed in zebrafish larvae[115,116,117].The treated adult zebrafish showed decreased locomotor activity associated with bradykinesia, decrease swimming velocity and dyskinesia, erratic swimming pattern with no reduction in the dopaminergic cells[116,118]. In rodents, 6-Hydroxydopamine was used to induce dopaminergic lesions[119]whereas, decreased level of dopamine and norepinephrine level was observed when adult zebrafish was injected intramuscularly with 6-OHDA[118].Zebrafish larvae also showed decrease expression level of TH, reduce locomotor activity and anxiogenic behavior[120]. Thus, the availability of various models of PD In zebrafish will aid in screening novel compounds in drug discovery.

Autism Spectrum Disorder: Autism Spectrum Disorder is a neuro developmental disorder characterized by impaired social communication, motor, and cognitive deficits. It is a polygenic disorder and has a high heritability rate(90%)[121]. Rodents were designed to study ASD relatable symptoms such as the social deficit, behavioral preservation and cognitive deficit[121].

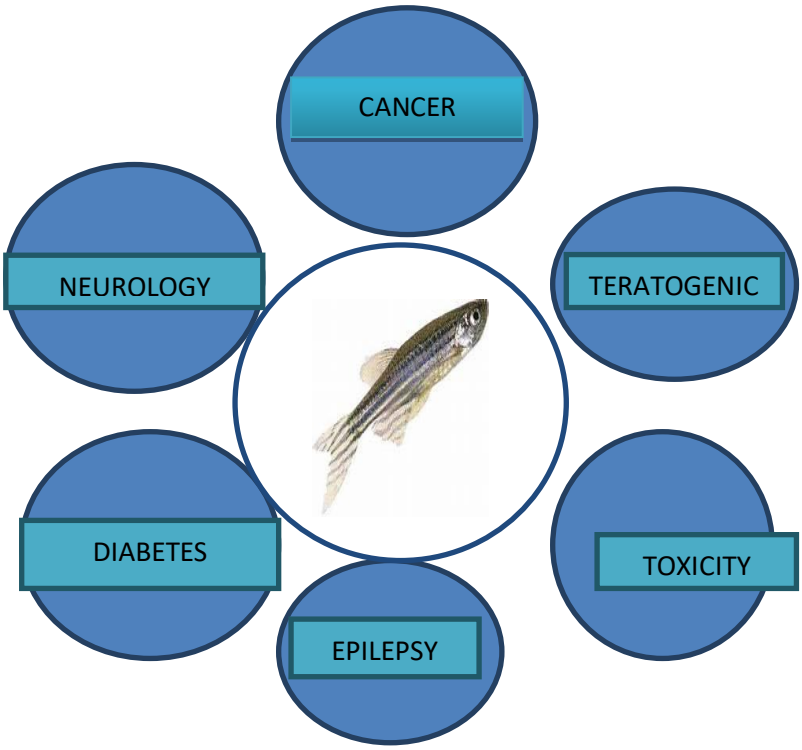

Figure 01: Zebrafish as a model organism in various diseases

Table 1: Zebrafish cancer models.

Chemical treatment	N-ethyl-N-nitrosourea (ENU) N-methyl N1 nitro-N-nitrosoguanidine (MNNG) Diethylnitrosamine (DEN) N-nitrosodimethylamine (NDMA) Dimethylbenzanthracene (DMBA)	Liver, testicular Liver, intestine and testicular Liver, bile duct and pancreas Liver and pancreas Liver, bile duct and intestine	31,9 32,10 7 8 6
Reverse genetics	P53 apc pten	Malignant peripheral nerve sheath tumor (MPNST) Colon, intestine and liver Hemangiosarcoma	33 34 35,36
Forward genetics	Bmyb Ribosomal protein gene	Malignant peripheral nerve sheath tumor (MPNST) Malignant peripheral nerve sheath tumor (MPNST)	37 38
Xenotransplantation	Transplant tumor cells in zebrafish	Melanoma, Glioma, Hepatoma, Lung cancer, Pancreatic cancer, 0varian carcinomas, Breast cancer, Prostate cancer, Retinoblastoma, Leukemia	16

Table 2: Zebrafish as a model to study toxicity.

Name	Activity	Dose	Effect/ toxicity observed	Reference
Doxorubicin	Anticancer drug	30.3mg/l	Teratogen, kidney, cardiovascular, liver	[47]
Dexamethasone	Corticosteroid	324mg/l	Liver, gastrointestinal, kidney	[47]
Cyclosporine A	Immunosuppressive drug	69mg/l	Teratogen, kidney, cardiovascular, liver	[47]
Caffeine	Methylxanthine drug	108.4mg/l	Behavioral: muscle contraction or spasticity, change in motor	[47]
Methotrexate	Anticancer drug	454mg/l	Teratogen, gastrointestinal, liver, kidney	[47]
Fluorouracil	Anticancer drug	3.3mg/l	Liver, kidney	[47]
Amifostine	Radioprotector	4mM	Swim bladder	[48]

Table 3: Glucose induced metabolic regulations and complications observed in zebrafish:

Sl.no	Hyperglycaemic induction	Age	Organs and defects	References
1.	Incubate embryos (6 hpf) with 0.5 % glucose for 24 h	Embryo (6–72 hpf)	Heart: Cardiac development and expression of cardiac markers tbx5, tbx20, has2 altered, loop defect.	[98]
2.	Incubate embryos with 0/1/2/3% glucose for 24/48/72h	Embryo (72, 96, 120 hpf	Increased cortisol level.	[99]
3.	In adults: transdermally 25 % glucose; Larvae: Incubation with 0.7% glucose at 96 hpf for 48 h	Adult and larvae (144 hpf)	PEPCK expression downregulated.	[100]
4.	Inject Streptozotocin (50 mg/kg) ip or directly into caudal fin	Adult	Kidney: Thick glomerular basement membrane. Retina: Thin photoreceptor layer Caudal fin: Impaired limb generation.	[101]
5.	Incubation with oscillation for every 24 h change between 2 % and 0 % glucose solution	Adult	Retina: Reduced inner plexiform and inner nuclear layer.	[102]
6.	Incubation with oscillation for every 24 h change between 2 % and 0 % glucose solution for 30 days	Adult	Retina: cone photoreceptor neurons disrupted, dilated and thickened blood vessels in central retina, enhanced VEGF expression	[102]

Ref: A model for understanding diabetes complications

Glutamatergic antagonists phencyclidine[122]and ketamine[123]were reported to evoke social deficit and resulted in circulatory behavior in rats. Amphetamine also disrupts the social deficit in rodents[124]. This provides evidence that rodent models are a valuable tool to study ASD pharmacological related symptoms. However, due to cost and time constraint, an alternate model for efficient and high throughput models for this study was necessary. Zebrafish and humans share high physiological and genetic homology[121]. A method to induce social deficit in zebrafish can be obtained by pharmacologically disrupting

their behavioral and cognitive function. Social behavior was tested in zebrafish by introducing two unfamiliar fish, duration and frequency of various social contact were assessed for social behavior[121]. Various behavioral stereotypes can be observed in zebrafish such as circling behavior, stereotypic thigmotaxic swimming near the walls[121]. Common effects are reported to be seen in zebrafish and rodent models such as circling behavior by glutamatergic antagonist Phencyclidine or Ketamine[125], various psychoactive drugs disrupt the shoaling in zebrafish and social behavior resembling ASD in rodents[126]. Multiple behavioral endpoints related to social and behavior in zebrafish can be studied by ethanol which reported to decrease shoal cohesion mildly and strongly affects polarization in zebrafish[127].

CONCLUSION:

With the immense use of zebrafish in various diseases, zebrafish has gained major importance in the field of drug discovery. The maintenance costs, simplicity and feasible work to develop models have added zebrafish in the tool of science. To facilitate evaluation of chemicals for DNT, the zebrafish vertebrate model system has emerged as a promising to facilitate evaluation of chemicals for DNT, the zebrafish vertebrate model system has emerged as a promising to facilitate evaluation of chemicals for DNT, the zebrafish vertebrate model system has emerged as a promising to facilitate evaluation of chemicals for DNT, the zebrafish vertebrate model system has emerged as a promising

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