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ZEBRAFISH: A SMART MODEL FOR PRECLINICAL STUDY

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Abstract: Zebrafish are utilized in many fields and as a clever model for preclinical research. Because compared to other animal models, it is more convenient. This review paper primarily focuses on the benefits, anatomy, and application of the model in various disease conditions. Zebrafish is more convenient to use in study because of their anatomy, which is quite similar to that of humans. Its advantages over other animals are also greater. Zebrafish are utilized as smart models in a variety of disease conditions to study the situation in detail. The application of models of zebrafish in variety of illness conditions, such as cardiovascular disease, mental disorders, obesity, and larval styphaloccous infections, is the main topic of this review paper.

Introduction:

A rapidly growing and highly significant model system for researching the developmental biology of vertebrates is the zebrafish, Danio rerio. Early in the 1980s, zebrafish were employed as a modern experimental model organism. and since then, a number of innovative techniques have been created for seeing and modifying their early developmental stages."Given the high genomic and molecular similarities between zebrafish and other vertebrates including humans, many of the important discoveries in zebrafish development are applicable to humans."

FIG.1: Zebrafish

Zebrafish and mammalian anatomy are comparable in that both species have similar hematopoietic tissue, peripheral blood, and other organs. Nowadays, zebrafish are frequently employed as model animals in scientific investigation because of their resemblance to mammals.

In the field of organ regeneration biology, the zebrafish, or Danio rerio, is a relatively recent animal model. The zebrafish possesses grown in significance for scientific study since 1960. It is an intelligent model for researching human genetics and disease because of its many properties.[Matthew B. 2008]

In 1972, George Streisinger at University of Oregon pioneered his research utilizing zebrafish. Due to its exquisite embryonic development, not only has the zebrafish become a distinctive model in fundamental

research, because of its sophisticated genetic and developmental technology produced worldwide. Zebrafish that are adults possess the ability to renew several organs like their kidney, retina, spinal cord, heart, telencephalon, and all of their fins. The cryprinidae family, which has over 200 species, includes zebrafish. Its larval stage is transperant, and its adults range in length from 2.5 to 4 cm.

Where you can find zebrafish?

The zebrafish is a native of south Asia and is found in Nepal and Bhutan. The South Himalyas, which stretch from the state of Arunachal Pradesh in northeast India to the Suttej river basin near the border between Pakistan and India, serve as the northern boundary.

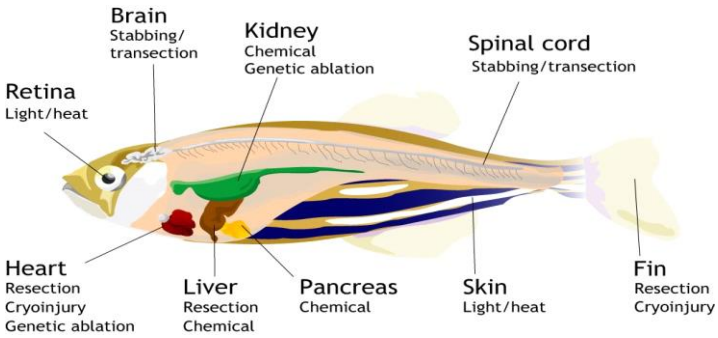

FIG.2: Antomy of Zebrafish:

1. **The anatomy of hematopoietic tissue:** Many types of blood and bone marrow cells develop from these types of tissues. Zebrafish have hematopoietic tissue, just like mammals do. They can be found in the renal interstitium and Stroma of the spleen, which is the extravascular intertubular area of the parenchyma. Hematopoiesis occurs mostly in the posterior kidneys and interstitium of adult zebrafish. Hematopoietic stem cells are found in the reticuloendothelial tissues.

2. **Peripheral blood;**
   a) **Erythrocytes**:- Zebrafish erythrocytes are nucleated and elliptical, just like those of birds and Reptiles. Zebrafish erythrocytes are thought to play a role in blood rheology, metabolic control, and oxygen delivery in addition to other functions. The primary means of transporting oxygen and, to a lesser extent, carbon dioxide are erythrocytes.

   b) **Leukocytes**:- As with other mammals, zebrafish rely heavily on their leukocytes to protect them against foreign objects and infectious diseases. There are two different kinds of granulocytes: eosinophilic and neutrophilic granulocytes. In the zebrafish, monocytes make up roughly 5 to 15% of the circulating leukocyte population, while lymphocytes make up roughly 71 to 92 percent.

   c) **Heart**:- The heart of a zebrafish is situated ventral to the esophagus and prior to the main body cavity. The sinus venous is filled with deoxygenated venous blood. A thin teabecuale causes the atrium's lumen to become a loose meshwork. Compared to the atrium, the ventricle's wall is far thicker. Blood is distributed by the ventral aorta from the zebrafish heart. Blood enters the ventricle through the atrioventricular valve when the atrium contracts and the ventricle dilates.The walls of the ventricle and atrium are substantially thicker. Muscle is composed of two films: a spongy inner surface with many trabeculae and a compact outer coat. The ventricular-bulbar valve opens to pump blood into the onion-shaped bulbus arteriosus at a relatively high pressure produced by the ventricle contracting. There are some smooth muscle fibers and fibro-elastic tissue in the thick wall of the bulbus arteriosus. Its suppleness allows it to spread out significantly, which lowers the ventricular pulse pressure. Blood is transported from the heart to the gills by the ventral aorta's afferent bronchial arteries.

d) **Kidney**: - Zebrafish kidneys are located in the retro peritoneum, directly ventral to the spinal column. Vertebrate nephron composition is conserved, and zebrafish renal regeneration is associated with two different pathogenic reactions. Its head and trunk regions are separate. Similar to a mammalian kidney, it has a kidney with glomerulus, proximal, distal, and collecting ducts. There are hematopoietic cells in the renal interstitium. However, regular hematoxylin and eosin (H&E) staining makes it difficult to identify the distal tubules from the proximal tubules. There are hematopoietic cells in the renal interstitium. [Aswin L.Menke 2011]

TAXONOMY OF ZEBRAFISH:

TABLE-1: Taxonomy of zebrafish

FAMILY	CRYPRINIDAE
SPECIES	DANIO RERIO
KINGDOM	ANIMALIA
CLASS	ACTINOPTRGIII

LIFE CYCLE OF ZEBRAFISH EXPERIMENTAL MODEL:

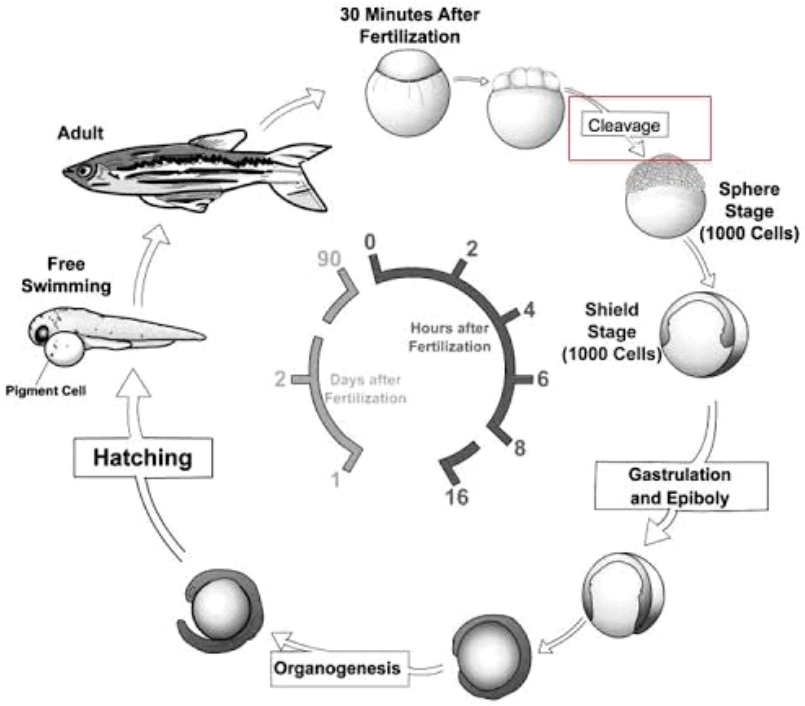

FIG.3: LIFE CYCLE OF ZEBRAFISH [Peralta M, Mart V, González-Rosa J, 2006 & Haack T, Abdelilah-Seyfried S. 2016]

BENIFITS OF ZEBRAFISH MODEL: [Giorgia Beffagna 2019]

I. Compared to mice, it requires less maintenance.
II. It may generate hundreds of offspring.
III. It grows quickly on the outside.
IV. Its reproductive cycle is brief.
V. Zebrafish embryos are almost transparent, making it simple for scientists to study how internal structures develop.
VI. Its genome has been completely and extremely well sequenced.
VII. The zebrafish genome contains a genuine ortholog for over 70% of human genes.
VIII. The zebrafish shares the majority of human organs and tissues as a vertebrate.
IX. Zebrafish are unusual in that they can heal heart muscle.
X. It's a Cheap, quick and simple upkeep and reproduction.

XI. Minimal dimensions and optical clarity.
XII. Quick development.
XIII. High-throughput drug screening.
XIV. Gene manipulation is accessible and simple.
XV. Optical transparency of Embryo and larvae.
XVI. Excellent model for developmental toxicology studies.

MODIFICATION IN ZEBRAFISH RESEARCH AS PER YEAR:

TABLE-2: MODIFICATION IN ZEBRAFISH RESEARCH

YEAR	DEVELOPMENT
1981	Zebrafish introduced as a vertebrate
1998	Zebrafish intrnational stock and disease resource center
2001	Anatomica charactrization of the zebrafish aminergic circuitry
2004	First drug screen in zebrafish and parkinson's disease
2005	Huntington's disease
2011	Head from a zebrafish based on drug screnning entered clinical trials
2013	Zebrafish genome sequenced. Zebrafish bheavioural catalogue and Relt syndrome.

ZEBRAFISH: ANIMAL MODEL IN DIFFERENT DISEASE CONDITION:

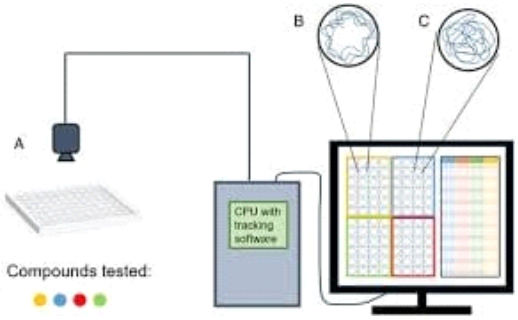

FIG.4: Video Tracking System [Kalogirou S 2014 & Kikuchi K 2011]

CARDIVASCULAR DISEASE:

Cardiovascular disease (CVS) is another term for conditions that affect the heart or blood arteries. Heart conditions include coronary artery disease, irregular heartbeat, heart failure, and rheumatic heart disease. 2016 saw about 17.6 million deaths from CVS. The proper analysis of the heart to understand the mechanisms underlying the aetiology of cardiovascular illness using animals as the model that fulfills the lifetime requirement for heart regeneration.

HEART REGENERATION IN ZEBRAFISH:

Zebrafish possess the ability to renew numerous organs and tissues, including the tissues of the Retina , Brain , cardiac muscle , spinal cord and fines. Similar to mice, zebrafish can repair their hearts, however this capacity disappears on the seventh day after birth. The remarkable ability of zebrafish to regenerate their hearts following ventricular damage is due to the blood stops the flow of blood at the injury site by donning clothing. When an injury occurs occurs, platelets form a plug and it contains surface-located thrombin receptor that binds tothe thrombin molecule transforms into fibrinogen , since fibrin has the ability to form

solution into fibrin lengthy insoluble protien strand that envelops platelets . Fibrin stabilizing factor facilitates further contraction and hardening which is cardiac muscles , it requries about two to three months for total regeneration of the heart .

Cryoinjury is an additional method of causing muscle damage that facilitates precise image analysis using the Olympus microscope. Cryoinjury is the term used to describe the damage that a temperature reduction causes to cells and tissues. In the cryoinjury approach, the fish is first rendered comatose in freshwater using tricaine solution (0.032%wt/vol). After that, the fish is placed on foam so that a microscope can view it, with particular attention paid to the area of the heart between the operculum and the heart as well as the fin's base. The fish's body is then held in place with forceps, and a small ventricular cuspidal flap is made with the aid of micro dissection scissors. The ventricle's apex and ventral section must be visible. The extra water should be removed by blotting the area with tissue, and then the area should be rapidly frozen by applying a precooked cryoprobe to the ventricular surface. [Langheinrich U, Vacuum G 2003]

The fish must stick to the ventricular surface after being submerged in freshwater and having water blasted through a pipette close to the gills. The heartbeat should also gradually slow down for the prescribed amount of time. Eight to ten minutes after the gill movement starts, the fish should be swimming. After the break, the zebrafish is dissected once more by creating an incision to reach the heart, which is then cleaned with FDB sol and fixed with 4% (wt/vol) so that it can be photographed and its regeneration observed. [Kroehne V, Freudenreich D 2011]

CANCER:

Stanton et al. used zebrafish in cancer research for the first time in the 1960s to investigate the effects of substances known to cause cancer.15 Zebrafish seldom develop tumors during their lives; just 10% of fish will have spontaneous neoplasia. Conversely, fish that are exposed to carcinogens such MNNG (N-methyl-N-nitro-N-nitrosoguanidine), DENA (diethylnitrosamine), and DMBA (7.12-dimethylbenz(a) anthracene) will acquire cancer. from Bernardos RL to Bourque C 2011 Furthermore, using tumor transplantation experiments to investigate the aggressiveness of various cancers, zebrafish have shown to be an excellent model.[Taylor AM 2009] They have proven to be resilient and come with the bonus of having a high fecundity of fish, which produces many fish, both donor and receiver. Not only has this model been used to study a number of cancer types, including leukemia, [Jing L & Teittinen KJ 2012] melanoma, endocrine, and liver cancerMore importantly, however, is that tumor cell motility, metastasis, angiogenesis, and the impact of putative therapeutic targets can all be addressed by xenotransplanting human tumor cells into zebrafish embryos (xenografts). Together with the readily available forward and reverse genetic tools and non-invasive in vivo imaging techniques, these qualities have made zebrafish an excellent vertebrate model for studying cancer.

Zebrafish have emerged as a potentially useful model to study the progression of melanoma. It is well known that mutations that activate the RAS/RAF/MEK/ERK signaling cascade are often present in this type of tumor, with changes in the NRAS and BRAF genes being the most common. [Ghosh P 2009] Transgenic fish lines were created in order to learn more about the part these genes play in the disease's progression. Although the NRAS-mutant fish had serious issues with pigment patterning, they did not grow melanoma at the same rate as the BRAF-mutant fish.. Huge agglomerates growing melanocytes that histologically resembled human nevi appeared in the BRAFmutant fish. Notably, When p53 mutant lines were mated with the two transgenic fish lines, the melanoma phenotype developed far more quickly, underscoring the significance of p53 activity in melanoma tumor suppression. Studies conducted on these fishes revealed that this melanoma model involves epigenetic regulation.Overall, our findings indicate that the physical, genetic, and epigenetically similar zebrafish melanomas to human disease suggest the viability of zebrafish as a melanoma model. .The cancer type known as acute lymphoblastic leukemia (ALL), which has a very uniform immunophenotype and shape, significant genetic variation that can result in different therapeutic responses, was another type of cancer that was discovered to have a suitable model in zebrafish. The B-lymphocyte-related TEL-AML1 fusion is one of the primary causes of ALL. All cell lineages of transgenic fish with this mutation gave rise to lymphoblastic leukemia, which resembled children CD10+ pre-B ALL in

phenotype.By producing a transgenic line for the oncogene Myc, Sabaawy HE 2006 T-cell ALL modeling in zebrafish was accomplished. Zebrafish also used to study the other processes underlying ALL, such as Notch1-induced T-ALL.

MODEL OF ZEBRAFISH FOR TYPE 2 DIABETIES MELLITUS:

Diabetes Mellitus Type 2 is characterized by insufficient insulin production and preserved β-cell mass. Ninety percent of the patients have type 2 diabetes. The primary reason is an anomaly in the β-cell's glucose receptor, which causes them to react to elevated glucose levels and it also reduces the sensitivity of peripheral tissue towards the insulin. When doing experiments, overfeeding animal's glucose for about three months can result in diabetes in the animals. Various tests can be used to monitor changes in the blood glucose content.

TECHNIQUES:

This tolerance test of type two is carried out.

A) Test for intraperitoneal glucose tolerance, or IPGTT
B) Test for oral glucose tolerance, or OGTT.

Internal evaluation of glucose tolerance In this experiment, fish were submerged in ice water for around five minutes to induce slumber. Intraperitoneally, 0.5 mg/g of fish weight was administered, and the fish was allowed to recover at intervals of 30, 90, and 180 days. Blood is obtained using the appropriate tool, and blood glucose levels are recorded at 30, 90, and 180-minute intervals. For performing oral glucose tolerance test the first anesthesia was given to the fish. Following that, 1.25 mg of glucose per g of fish weight is administered orally using a micropipette with a tiny tip. After that, the fish is given 30, 90, or 180 minutes to get well. A blood sample is obtained and the blood glucose level is monitored during that period.

MENTAL DISORDER:

A person suffering from a mental illness, commonly referred to as a psychiatric illness, had severe distress due to abnormal thinking or behavioral patterns. Classifying medical disease problems follows an international standard called The World Health Organization (WHO) maintains The International Classification of Diseases (ICD).The primary psychological resource issued by the American Psychiatric Association the Diagnostic and Statistical Mnual Of Mental Disorder lists are addressed in more than 450 definations of mental disorder. In terms of social behavioral testing concerning mental diseases, zebrafish are a good choice because they are gregarious animals that display schooling and shoaling patterns. A new gene family resembling chemokines called samdori has been linked to mental illnesses in zebrafish, according to research conducted by Kim and colleagues using mutagenesis screening. Among the five members of the sam family, sam2 is linked to autism spectrum disorder and intellectual disability because it expresses itself especially in the brain's habenular nuclei. Mouse and zebrafish Sam2 deletion mice showed aberrant emotional responses, such as anxiety and terror, which are connected to disorders associated to anxiety and/or autism.[Choi, J. H. 2018 Kim, S. 2017]

Furthermore,Syndrome known as Armfield X-linked intellectual disability (XLID) was found to be caused by FAM50A, according to whole-exome sequencing. The term "XLID" describes mental illnesses that include intellectual disabilities and are clearly linked to X-linked recessive heredity. There are about 100 genes that have been linked to XLID syndrome. Males are more likely than females to be impacted, XLID accounts for around 16% of all cases of intellectual disability [Stevenson, R. E. & Schwartz, C 2009]. .By using rescue studies in a zebrafish fam50aknockout model, human FAM50A missense mutations were functionally validated [Lee, Y. R. 2020]. Recently, a spliceosomopathy linked to faulty mRNA processing during development was identified with the use of the Zebrafish Disease Model as Armfield XLID syndrome.

THE ZEBRAFISH EMBRYO TOXICITY & TETRAGENICITY:

Teratogenicity is the malformation that proves lethal when a medicine is given to pregnant women; nevertheless, it has recently been discovered in zebrafish as an organism for genetic study and the development of vertebrates.Zebrafish are advantageous in that they enable for immediate evaluation of teratogenic activity by stereomicroscopy, thanks to their great advantage in the outside growth of the embryo. The drug's teratogenicity and embryotoxicity at the observed dose. [Jopling C & Kalogirou S 2014]

METHOD:

The egg or embryo is taken out of the zebrafish breading stage and filtered through a soft mesh screen before being put in a sterile embryo medium. The undeveloped egg or embryo is eliminated once the embryo that is capable of life has assessed it. The egg should be incubated for two to three hours at 28.5ºC, remove the chorion (soft eggshell), examine the sample under a stereomicroscope, and choose one embryo. This embryo is then placed into a single multiwall tissue well, containing a medication for testing at different concentrations and a vehicle, and the plate is heated at 28.5ºC for five days,with the help of a microscope, embryo's development can be observed at various stages; the larvae begin to move on the third or fourth day. following doing the endpoint analysis, which takes place on days two, three, and four following fertilization, the larvae are put to death by submersion in a 1.2% sodium hypochlorite (bleach) solution for a duration of four to seven days. [Kikuchi K & Holdway J 2011] The term "teratogenicity" describes a defect in the fetus's physiological growth. The zebrafish model is the most effective model in teratology. FIG 4 demonstrates the application of automatic video tracking for the simultaneous assessment of many attributes in zebrafish larvae. Panel (A) shows a 96-well holding plate that is used to provide zebrafish larvae various chemicals. An above camera records fish behavior, while tracking software processes the photos. Panel (B) illustrating how a swim trace works.The juvenile zebrafish in this instance are wall-hugging, staying close to the walls. The swim pattern is shown in reverse in panel (C). Here, the larvae of zebrafish aggressively explore their environment, particularly the middle of the tank.

GENETIC KIDNEY DISEASE IN ZEBRAFISH:

All components and functions of the kidney can be impacted by genetic renal disorders. Mutations in around 150 genes have been found to date to cause hereditary kidney illnesses in humans, including changes in kidney development and particular glomerular and tubular diseases [Eckardt KU, Coresh J] Based on where the species is located on the evolutionary tree, complex process of nephrogenesis in vertebrates results in the progressive creation of up to three kidneys: the pronephros, mesonephros, and metanephros . While the shape and the basic function of reneal unit and the nephrons are similar to the vertebrates, the complexity of each succeeding kidney develops. [Desgrange A, Cereghini S. 2015]

The fertilization in the zebrafish devleoped in a few days by the major vertebrate organ systems . By 48 hpf (hours after fertilization), the zebrafish pronephros is working and performing blood filtration and osmoregulation [Drummond IA.2000] It has a merged glomerulus at the midline and is composed of two nephrons The pronephric duct, the proximal and distal tubules of the pronephric, comprise the tubular system, which is analogous to the human kidney in terms of spatiotemporal gene-expression patterns and segment-specific conserved morphological and physiological characteristics [Anzenberger U, Bit-Avragim 2006] Similar to the metanephric glomerulus of higher vertebrates, the zebrafish glomerules is made up of femestrated endotheial cells and that form a functionong glomerular filteration barrier and podocytes with extended interdiitating foot processes.

For both normal and abnormal kidney development, zebrafish are currently mostly used as genetic models, for the research of human cystic kidney illnesses connected with ciliopathy, and for hereditary glomerulopathies, such as podocytopathies.These include polycystic kidney illnesses and disorders of the nephronophthisis/medullary cystic kidney disease complex, which encompasses more complex ciliopathies such as Joubert Syndrome, Meckel-Gruber Syndrome, and Bardet-Biedl Syndrome.

ZEBRAFISH LARVAE MODEL OF STAPHALOCOCCUS AUREUS INFECTION:

In vertebrate biology the zebrafish is highly valuable model organism having applications in immunology development biology,toxicity,and pharmaceutical drug discovery. Its numerous benefits have led to its widespread use. Within its jawed body, the zebrafish evolved two adaptive and an innate immune system. [Kasahara, M.; Suzuki T.;2004] .Since the innate immune system serves as the sole defense against infection in zebrafish larvae during earlier developmental stages, it is possible to investigate innate defense mechanisms apart from adaptive immunity. After fertilization, the adaptive immune system grows in four to six weeks. [Lam, S.H.; Chua, H.L. 2004]; Innate immune responses in zebrafish consist of a highly developed complement system [Zhang, S.; Cui, P. 2014 receptors for pattern recognition, such as NOD-like receptors (NLRs) and Toll-like receptors (TLRs) [Li et al. (2017); Cao et al. (2017); Jin et al]. .and innate immune cells like macrophages and granulocytes . When the zebrafish genome was compared with relation to the human genome, significant commonality was found despite the evolutionary gap; in fact, zebrafish orthologues exist for more than 70% of human genes. Zebrafish larvae offer a variety of injection locations. Microinjection into the Duct of Cuvier, the caudal vein/blood island, and the yolk sac circulation valley can cause a systemic bacterial infection, while being injected into the fourth hindbrain ventricle or the otic vesicle, two examples of bodily cavities, can cause a localized bacterial infection. The research question at hand may also determine which route of infection is best. A systemic route of infection provides a suitable model for bacteremia; the hindbrain can be used to study CNS infection; the pericardial cavity offers a viable treatment for pericarditis; and other compartments, such as the muscle and tail fin, allow for studies on immune cell chemotaxis.

ZEBRAFISH AS A MODEL ORGANISM FOR OBESITY CAUSED BY DIET:

By feeding adult zebrafish Artemia nauplii, [Oka et al. (2010)] initially developed the use of zebrafish in diet-induced obesity investigations. The fish in these trials acquired hypertriglyceridemia, hepatic steatosis, elevated body mass index, and deregulation of some lipid metabolism genes. [Chen et al. (2018)] provided high-cholesterol diets to zebrafish, which led to elevated body weight, elevated triglyceride levels, and hepatic fat accumulation. According to [Forn-Cuní et al. (2015)], overfeeding zebrafish with high levels of fat from various sources or cholesterol can also result in hyperglycemia, ectopic lipid buildup, increased body weight, enlarged adipose tissue, cardiovascular overload, and steatosis. Zebrafish were utilized by [Landgraf et al. (2017)] to compare the effects of overfeeding with regular and high-fat diets on the development of obesity.

They came to the conclusion that although the fish fed the standard fat diet became obese, both diets increased the amount of adipose tissue in the fish while maintaining their metabolic health. The other fish that were fed a diet heavy in fat weren't good. Along these lines, we also found in our study that zebrafish with hepatic steatosis were both adults and larvae fed a high-fat diet. Diet-induced in zebrafish obesity is often utilized to evaluate food types and the influence of dietary compounds on formulation, experimentation, or identification of various medications to treat or prevent obesity through modifications to fat metabolism. According to [Oka et al. 2010],the zebrafish model of diet-induced obesity that was overfed with artemia can be exploited to find potential pharmaceutical targets of human obesity as it shares pathophysiological pathways with obesity in mammals.Diet-induced obesity allows us to analyze the disease in the setting of systematic obesity since it matches the most common mechanism seen in individuals with this condition.

CHALLENGES AND OUTLOOKS:

A well-established model for researching heart development is the zebrafish.Examining the fuctional inputs and the physical cues that go along with them,like calcium or mechanical cues , more information in vivo will shortly follow due to development of a genetic tools and technological advancements. Ion channel dysregulation, including that caused by LTCC, can contribute to arrhythmias and ventricular hypertrophy, two underlying diseases that ultimately cause sudden cardiac death. Therefore, fresh routes for future therapeutics involving prevalent cardiac disorders, such as cardiac hypertrophy, heart failure, and

arrhythmias, will also be expedited by subsequent research where inputs have a major influence. Within the subject of zebrafish vacular and lymphatic investigations,there are still many unwansered concrens. In addition to VEGF and Wnt,the Hippo pathway is one of the signaling pathways in vacular development that is being researched more and more. [Nagasawa-Masuda and Terai, 2017]; Likewise, it appears that hemodynamic forces are essential for the development and differentiation of the cardiovascular system [Chen et al., 2012, 2017;Goetz et al., 2014] Our objective involves creating an interactive atlas of zebrafish vascular and lymphatic architecture through the use of contemporary technology like fluorescent markers with various colors and light sheet microscopy. It would be desirable for field researchers to incorporate perivascular cells, like mural cells, as well. It is anticipated that in the upcoming years, the utilization of mutant lines and reporter pathway lines with conditional modulations to find novel regulators will increase along with the application of single-cell sequencing technologies.

CONCLUSION:

The Zebrafish model is an intelligent and highly suitable model for usage in several research fields to investigate the toxicological effects of drugs. Because of its anatomy, it is more practical or sometimes referred to as a smart model. Zebrafish anatomy is more suitable for study because it resembles that of mammals. In addition to this a clever model, the zebrafish model is more applicable to several scientific fields than other animal models. In addition, it costs less than other animal models.

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