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Zebrafish: Model for Organ Toxicity Studies

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Introduction

Animal research is important for drug development as it focuses; On understanding the biology & genetics of living organisms, to understand disease etiology & progression, to identify potential biochemical pathways (targets for drugs) to mitigate diseases and for the advance understanding of illnesses such as cancer, AIDS, chronic pain &many other medical conditions. Animal testing focuses on understanding four facets: Pharmacokinetics, pharmacodynamics, efficacy & safety or toxicity. Using animals in research is extremely beneficial as they are biologically similar to humans leading to a better result. Because laboratory animals have a short life cycle, they can be studied their entire lives which is crucial for a better understanding of how disease processes work.

Commonly used animal in research is Rat, Mice, Guinea pig, Hamster, Rabbit, Dog, Monkey and Zebra fish. From all this the zebrafish as a model organism was 1st time pioneered at the University of Oregon, U.S.A. by George Streisinger in 1972 (Khan & Alhewairini, 2018).

Scientific Classification of zebrafish

Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Cypriniformes
Family: Cyprinidae
Genus: *Danio*
Species: *Danio rerio*

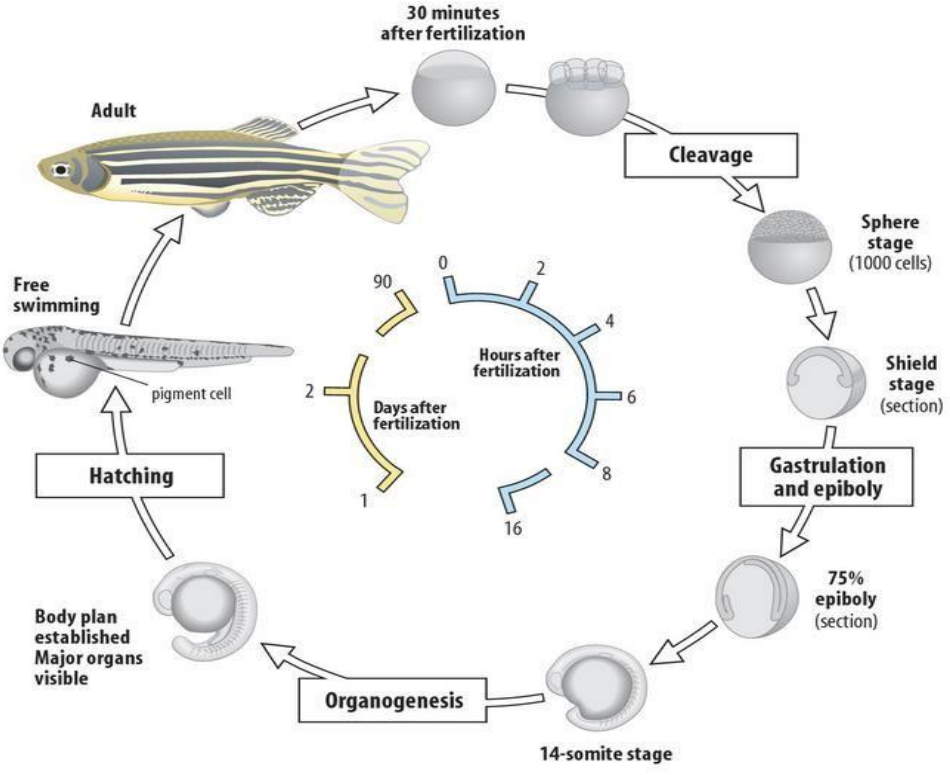
Basic characteristic

There are five uniforms, pigmented, horizontal stripes on the side of the body which are similar to the zebra's stripes and extend to the end of the caudal fin, so it is given name as zebrafish. Its shape is fusiform and laterally compressed, with its mouth directed upwards. Zebrafish are omnivores, consuming larval and adult insects, as well as small crustaceansand other zooplankton, but also partaking of algae, plant material and assorted detritus. (Arunachalam *et al*., 2013). Approximate 200–300 eggs / week by single pair of male-female at one mating. Generation time for *Danio rerio* is three to four months. (Nikam *et al.*, 2020).

Fish are poikilothermic. The temperature of housing water in different facilities is typically kept in a range between 24-29 ℃ (28.5 ℃). The "standard" light cycle in most zebrafish laboratories is 14 hr L:10 hr D. The intensity of light should be as uniform as possible across tanks and intensities should be adjusted to between 54 and 334 lux at the front of the tank (Alestrom *et al*., 2020).

Developmental life cycle of Zebrafish

Advantages of zebrafish as experimental model



Small in size (approx. adult 4-5 cm & larvae 3 mm) therefore less housing space, small amount of feed & water is required which leads low cost of maintenance. High fecundity (200-300 egg/week by single pair of male-female at one mating). External fertilization and development: The eggs are fertilized and undergo development outside the mother's body. This allows examining and manipulating the embryos from the moment they are fertilized. Transparency of embryo or larvae for several days postfertilization: Allow direct observation of morphological development & abnormality. Rapid embryonic development: Zebrafish develop from fertilized eggs to resembling tiny fish in 24 hours.

This same period of development for mice takes up to 21 days. High-throughput screenings: Experiment is done on zebrafish embryo or larvae in 96 wellplate which allow screening of multiple samples at a time.

A high degree of conservation exists between the human and zebrafish genomes (approximately 75% similarity). Genetic manipulation: Zebrafish embryos can be easily manipulated, including making alterations to their genomes. This is often an effective way of identifying new genes or discovering novel functions of known genes (Chakraborty *et al.*, 2009; Nikam *et al.*, 2020).

Drug Discovery & Development process

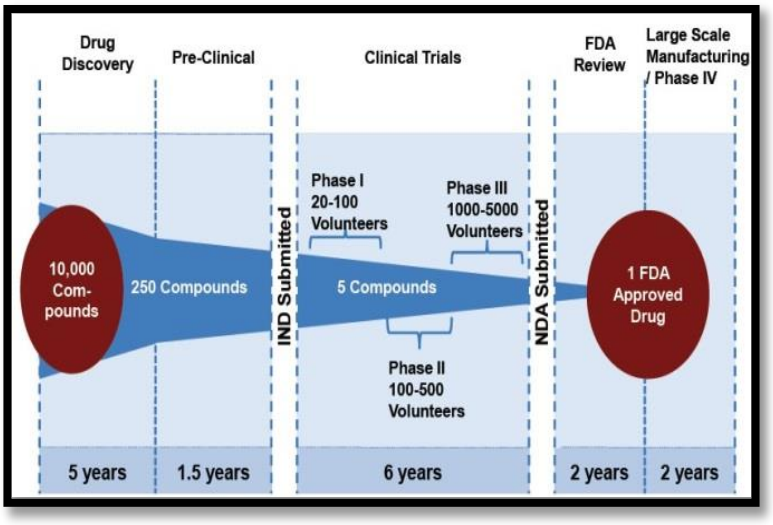


Figure 1: Drug Discovery & Development process

Before coming to the market all of drugs were passed from this drug discovery & development process. At pre-clinical stage, chemical compound tested on experimental animal.At these stages usage of zebra fish can minimize the chances of toxicity to human clinical trials.After coming to the market numbers of drugs are withdrawal due to their organ specific toxicity. Example include; Trovafloxacin (Hepatotoxicity, 1999), Nimesulide (Hepatotoxicity, 1999), Grepafloxacin (Cardiotoxicity, 1999), Cisapride, (Cardiac arrhythmias, 2000), Troglitazone (Hepatotoxicity, 2000), Cerivastatin (Rhabdomyolysis, 2001), Rofecoxib (Cardiotoxicity, 2004) and Valdecoxib (Cardiovascular adverse effect, 2005).

Organ Toxicity Studies

Initially, zebrafish have been used for toxicity studies of environmental pollutants, & agrochemical compounds; however, in the last 20 years, the pharmaceutical industry witnessed

increasing use of zebrafish whole-organism screening model for toxicity studies (Nikam *et al*., 2020). As a vertebrate animal, zebrafish have a high degree of geneticconservation and their morphological & molecular basis of tissue and organ development is either identical or similar to other vertebrates (Chen & Fishman, 1996). Zebrafish is increasingly used as an *in vivo* model system for evaluation of efficacy and safety testing of novel drug and many other studies have confirmed that mammalian and zebrafish toxicity profiles are strikingly similar (Hill *et al*., 2012). Recently, Zebrafish is very popular to study organ toxicity like Cardiotoxicity, Hepatotoxicity, Nephrotoxicity, Neurotoxicity and Reproductive toxicity.

Cardiotoxicity

Drug-induced cardiotoxicity has been a leading reason for drug withdrawals, and limitsdrug efficacy and its clinical use. As in all vertebrates, the heart is the first organ to function inthe zebrafish & its fully formed by 2dpf, compared with 12dpf in the mouse and 35dpf in the human embryo. Although physiological differences are evident between zebrafish and mammalian heart, the zebrafish has become a good option to study heart development and heartregeneration (Poss *et al*., 2002). Most importantly, zebrafish can survive in the presence of major vascular defects for several days, unlike many large animals (Rocke *et al*., 2009).Unlikemammalian heart, zebrafish heart consists of two chambers, the atrium and ventricle. In zebrafish heart, the deoxygenated blood enters in sinus venous and then flows through single two-chamber heart. The heart contraction in zebrafish starts at 24 hpf (Nikam *et al*., 2020).

Heart rate measurement is quite easy in zebrafish, making it an attractive screening tool for assessing cardiovascular risk after treatment (Musso *et al*., 2014). Zebrafish can regenerate heart muscle which help identification of the barriers to efficient cardiac regeneration in mammals and enable the design of novel therapeutic strategies for improved regeneration of the infarcted mammalian heart (Chablais & Jazwinska, 2012).

Fang *et al.*, 2016 assessed *in vivo* cardiotoxicity induced by methamphetamine, ketamine, and methadone in zebrafish embryos. Zebrafish embryos were exposed to methamphetamine (METH) & methadone solutions of 100, 200, 300, 500, and 1,000 mg/L,

ketamine (KT) solutions of 250, 500, 1,000 and 2,000 mg/L continuously. Cardiotoxicities were recorded at 12 h and 24 h posttreatment using specific endpoints, i.e., pericardial edema and heart rate.They observed severe pericardial edema due to methamphetamine at 1000 mg/Lat 12 & 24 h post treatment (Figure 2). Doses of methamphetamine up to 500 mg/L did not have any

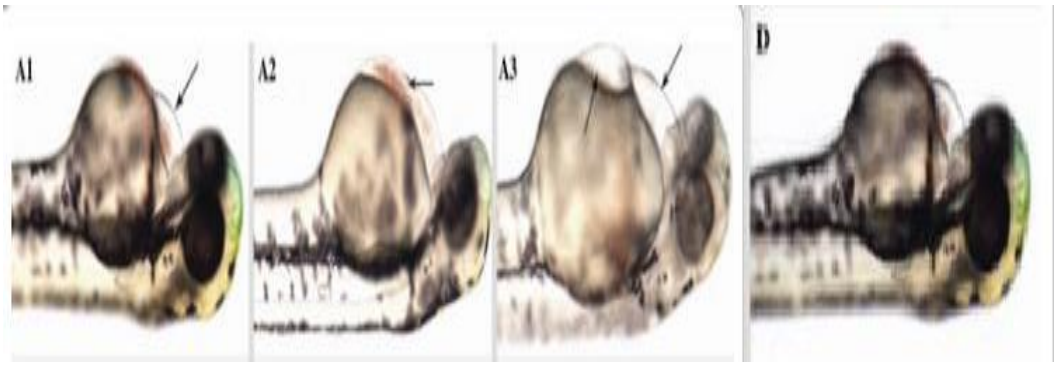


Figure 2: Pericardial edema due to methamphetamine (METH). A1. 500 mg/L METH at 24 hpost treatment, A2. 1000 mg/L at 12 h post treatment, A3. 1000 mg/L at 24 h post treatment, D. Control group

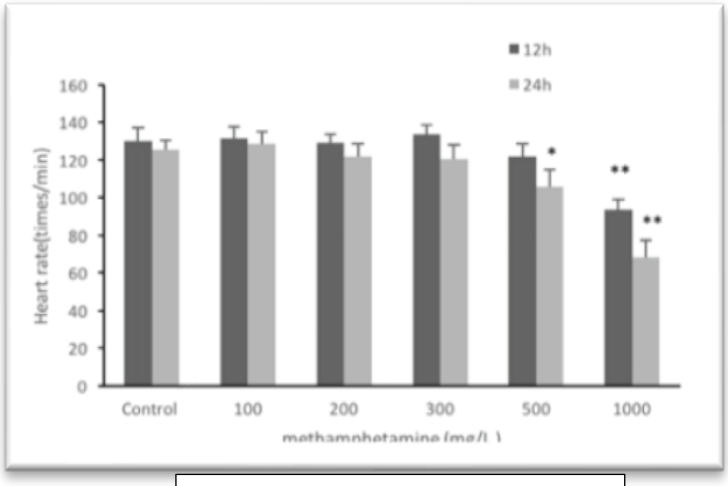


Figure 2.1: Effect of METH on the heart rate.

significant effect at 12 h post-treatment but dose of 1,000 mg/L significantly reducedthe heart rate at 12 h and 24 h posttreatment (Figure 2.1). Ketamine at 2000 mg/L at 12 h & 24h post treatment showed severe pericardial edema (Figure 3) & up to 500 mg/L on heart rate caused no significant difference at 12 h posttreatment. By contrast, the heart rate was significantly reduced compared with the control group at 24 h posttreatment ($p<0.01$). Doses of 1,000 mg/L and above significantly reduced the heart rate at 12 h and 24 h posttreatment (Figure 3.1). Methadone of 300 mg/L at 24 h post treatment showed severe pericardial edema (Figure 4) & Doses of 200 mg/L and above significantly decreased the heart rate compared with the control group, which exhibited an acute and strong cardiotoxicity whereas all

zebrafishembryos at 500 and 1,000 mg/L died at 12 h post-methadone treatment (Figure 4.1). The study revealed that methamphetamine, ketamine and methadone have potential cardiotoxicity, particularly of methadone. Therefore, more attention must be focused on the risk of clinical application of methadone. Moreover, the zebrafish larva was shown to be a good model for the investigationson drug induced cardiotoxicity.

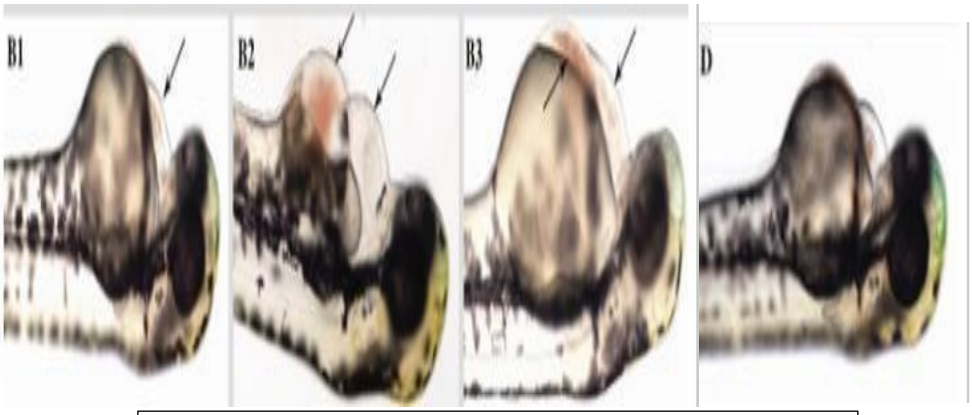


Figure 3: Pericardial edema due to Ketamine (KT). B1. 500 mg/L at 24 h posttreatment, B2. 2000 mg/L 12 h post treatment, B3. 2000 mg/L 24 h post treatment, D. Control group

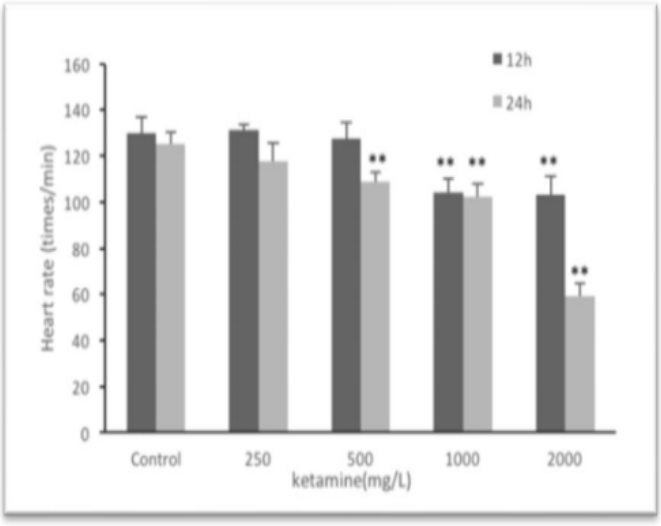


Figure 3.1: Effect of KT on the heart rate

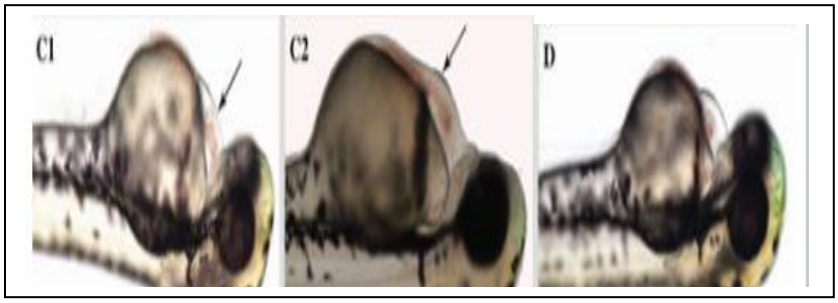


Figure 4: Pericardial edema due to Methadone.C1. 100 mg/L at 24 h post treatment, C2. 300 mg/L 24 h post treatment, D. Control group

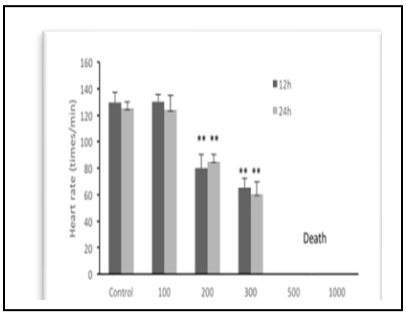


Figure 4.1: Effect of methadone on the heart rate.

Han *et al.*, 2014 evaluated cardiotoxicity of anthracycline drug in zebrafish embryo which were exposed to the doxorubicin & doxorubicin-liposome solutions at 10, 20, 50 and 100 µM in petri dishes (with 20–30 embryos per dish) at 28 °C during the exposure stages, which were designed in three stages: 6-72, 24-72 and 48-72 hpf. The end points, i.e.,heart rate were observed at 72 hpf. The normal control group was treated with embryo water. Doxorubicin reduced heart rate in dose dependent manner during exposure stages but at 50 µM cause death of zebra fish during 6-72 hpf (figure 5.1). Doxorubicin-liposome has no effect on heart rate during 24-72 & 48-72 hpf but at 50 µM also cause death of zebra fish during 6-72 hpf (figure 5.1). These results suggestthat the cardiotoxicity might be dependent on the drug concentration & exposure time. Dosageforms can improve the safety of doxorubicin. For example, encapsulated doxorubicin in liposomes reduced cardiotoxicity.Zebrafish embryos proven to promising model for evaluatingdrug-induced cardiotoxicity.

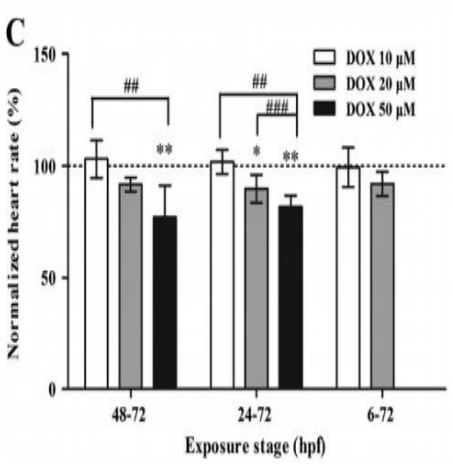


Figure 5.1: Effect of Doxorubicin exposure on heart rates of zebrafish embryos

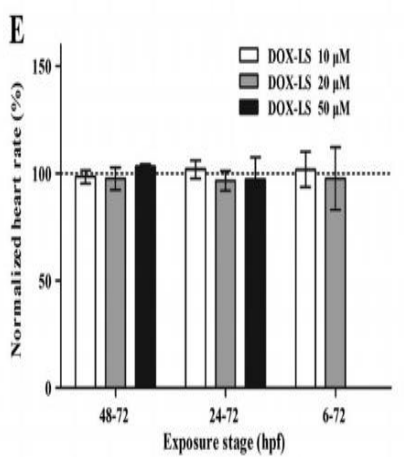


Figure 5.2: Effect of Doxorubicin-liposome exposure on heart rates of zebrafish embryos

Hepatotoxicity

In vitro cytotoxicity assays have less than 25% sensitivity for the detection of hepatotoxins, whereas *in vivo* assessment of organ toxicity frequently involves full histopathological analysis in rodents or higher order species such as macaque monkeys that arevery laborious, costly and time-consuming (O'Brien *et al.,* 2006). General defensive mechanisms against xenobiotic chemicals such as enzyme induction and oxidative stress in zebrafish are equivalent to those in mammals. Mammalian drug metabolizing enzymes including CYP450 3A family are present in the zebrafish liver (McGrath & Li, 2008). When exposed to a hepatotoxicant, changes to liver morphology can be evaluated visually due to transparent zebrafish larvae (Hill *et al.,* 2012).

He *et al.*, 2013 assessed hepatotoxicity of 6 mammalian hepatotoxic drugs (acetaminophen [APAP], aspirin, tetracycline, sodium valproate, cyclophosphamide and erythromycin) and 2 non-hepatotoxic compounds (sucrose and biotin) in larval zebrafish. Specific phenotypic endpoints of hepatotoxicity: liver degeneration and yolk sac retention wereexamined. Thirty larval zebrafish (72 hpf) were distributed into 6-well plates for a treatment period of 48 h until 120 hpf. Larval zebrafish treated with 0.1% DMSO were used as vehicle controls. After treatment, Larval were subject to visual observation and image acquisition under a stereomicroscope. To determine MNLC and LC10 of drugs, zebrafish larvae were treated with 5 initial concentrations (0.1, 1, 10, 100 and 1000 μM) of test drugs and mortality was recorded every 24 h. If a LC10 could not be found from the initial tests, additional concentrations within the range of 0.001 μM to 10000 μM were tested. Vehicle-treated (a) andnon-hepatotoxic compounds biotin (b) & sucrose (c) treated group show clear healthy

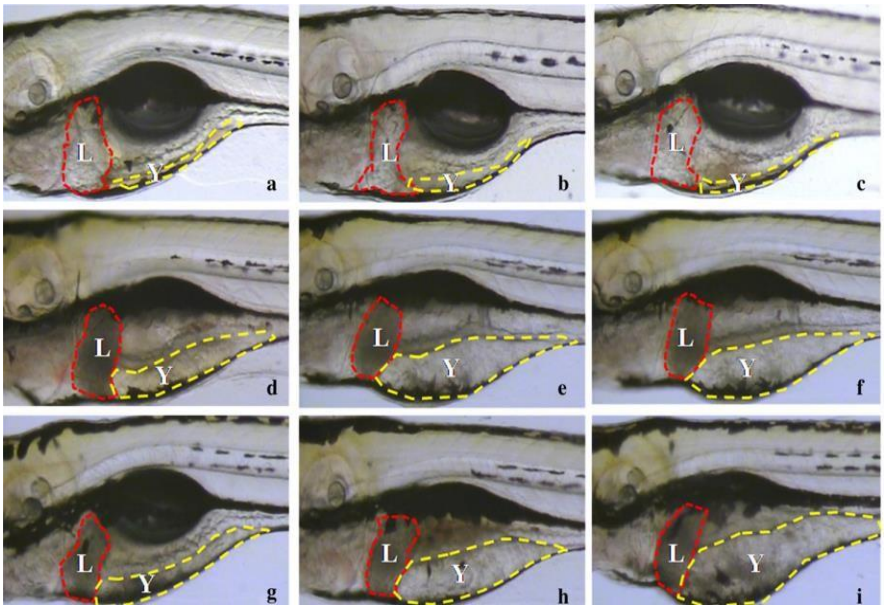


Figure 6: Effect of hepatotoxic and non-hepatotoxic compounds on liver & yolk sacof larval zebrafish

liver &no retention of yolk sac whereas larval zebrafish treated with mammalian hepatotoxic (d: APAP; e: aspirin; f: tetracycline HCl; g: valproate sodium; h: cyclophosphamide; i: erythromycin) show dark, brown degenerative liver & retention of yolk sac (figure 6). In control group liver showed normal tissue and cell structure and shape, and tight cell contact, and the liver was filled with well-delineated polygonal cells with well-preserved cytoplasm and prominent nucleus whereas liver from zebrafish treated with APAP showed loose cell-to-cell contact and the cells were dissociated and irregular in shape with vacuoles (figure 6.1).They concluded that zebrafish can be used to eliminate potentially unsafe hepatotoxic compoundsrapidly in the early stages of drug development.

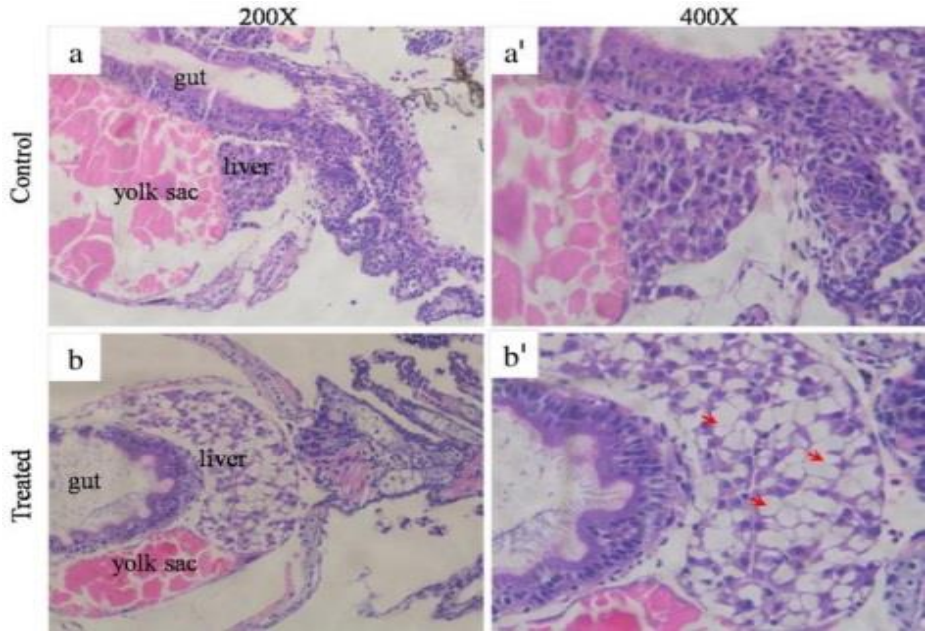


Figure 6.1: Liver histopathology of zebrafish from control group & treated with acetaminophen (APAP)

Neurotoxicity

In zebrafish, brain development occurs within 3 days post-fertilization (Howe *et al.,* 2013). Development processes and mechanisms of the central nervous system of zebrafish andother vertebrates are well-conserved (d'Amora & Giordani, 2018). Similarity between these species also includes the development of the blood brain barrier (BBB) (Eliceiri *et al.,* 2011). The counterparts of many brain subdivisions found in the developing mammalian brain are morphologically identifiable in the developing zebrafish (Wullimann, 2009). Effects of different chemicals on brain development can be evaluated by different neurotoxicity endpoints including gene expression patterns, neural morphogenesis and neurobehavioral profiling (Chueh *et al.,* 2017).

Shi *et al.,* 2011 evaluated developmental neurotoxicity of cypermethrin which is a

pyrethroid insecticide, widely used throughout the world in agriculture, forestry, horticulture and also in veterinary field as insecticide. Zebrafish embryos (4hpf) were exposed to cypermethrin solutions with concentrations of 0 (control), 25, 50, 100, 200 and 400 µg/ L in a6-well plate with 20 embryo per well for 96 h. The observation of zebrafish development wasdirectly in the 6-well plate using a stereomicroscope. They found morphological abnormalitiesin zebrafish embryo at 25 µg/ L cypermethrin treated group at 24 hr (figure 7). This study demonstrates that neurodevelopmental toxicity of cypermethrin in embryo-larval stages of zebrafish indicated by increased malformations. They concluded that zebrafish can serve as an ideal model for studying developmental neurotoxicity of environmental contaminants.

Figure 7. Control group (left) & Cypermethrin treated group (right)

Nephrotoxicity

Kidney being one of the target organs for drug-induced toxicity since a large amount of blood filtered through it which are largely responsible for the elimination of various drugs and their metabolites.Therefore, it is important to efficiently select drug candidates with a low risk of drug induced kidney injury at the preclinical toxicological screening stage.The nephrons of the zebrafish kidney, in both the embryo and adult, display ahigh degree of conservation with mammals (McCampbell *et al.*, 2014).In contrast to mammals,adult fish able to produce new nephrons throughout their lives in response to growth requirements or injury (Kamei *et al.*, 2015). Zebrafish pronephros and mesonephroshave a segmental organization similar to that of mammals with glomeruli and tubules that contain proximal and distal segments (Kroeger and Wingert 2014). Nephrons are formed by 24hpf and begin to filter blood by approximately 48 hpf. Rapid formation and function of the embryonic kidney facilitates experimental analysis (Gerlach & Wingert, 2013).

Kamei *et al*., 2015 evaluated kidney injury in adult zebrafish by gentamicin. Adult zebrafish (6-12 months old) are injected by intraperitoneal injection of gentamicin at 40, 60, 80 mg/kg dose whereas control group with PBS. Place the injected fish in dark surface container individually at

28.5 °C overnight. After one day post injury, gentamicin injury can be confirmed visually by the observation of renal epithelial casts in the water by allowing the water in the tank to settle, pipetting the sediment out and observing under a microscope. Theyobserved no renal epithelial casts in the water at 40 mg/kg (Image F) whereas presence of medium & large cast at 60 mg/kg & 80 mg/kg of gentamicin doses (Image G & H), respectively(figure 8) and also the percentage of injured fish with casts is increased at 60 mg/kg and 80 mg/kg (figure 8.1). The presence of casts in tank water through urine is an easy, noninvasive and early indicator of acute kidney injury. Zebra fish allows the researcher to detect kidney injury by utilizing a noninvasive visual screening.

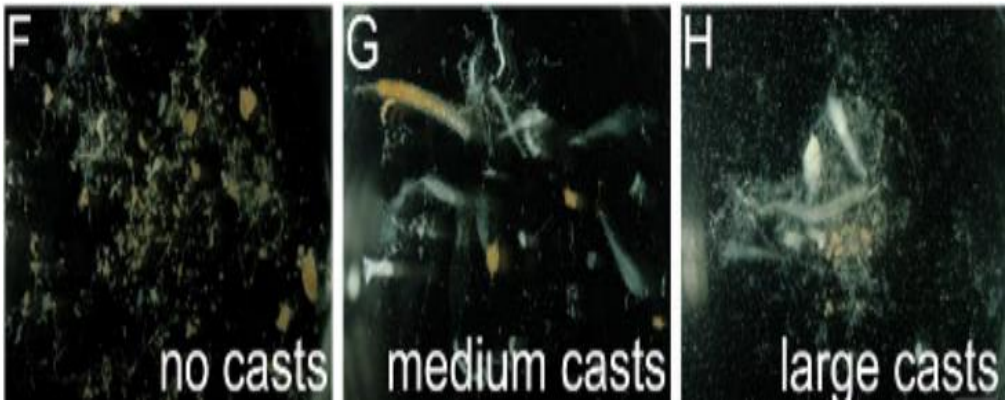


Figure 8: renal epithelial cast in the water

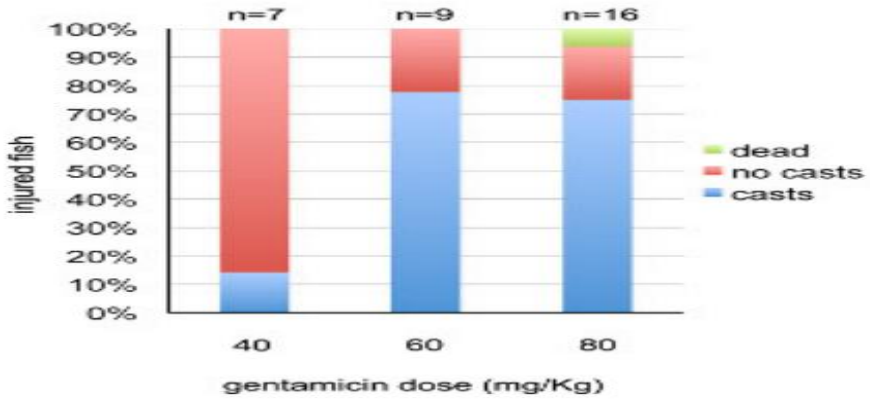


Figure 8.1: The percentage of injured fish with casts

Reproductive Toxicity

Zebrafish achieve reproductive maturity within 3 to 6 months after fertilization. Zebrafishwhich are oviparous can produce around 200 to 300 eggs per week, thus permitting large-scaleexperimental analysis.

Male zebrafish display similar anatomy of germ cell organs to that in humans. Male zebrafish have paired testes with tubule organizations. Within each tubule, the walls are lined by sertoli cells and they function mainly to support testes morphogenesis and spermatogenesis while leydig cells detected in the interstitial spaces act as primary testosteroneproducer (Siegfried and Nusslein-Volhard, 2008). As like vertebrates, ovaries are the site of development and production of female gametes in zebrafish (Gerlach, 2006 ; DeFalco and Capel, 2009).Similar to mammals, zebrafish follicle contains an oocyte surrounded by zone radiata along with a follicular layer made up of inner granulosa cells and outer thecal cells layer(Clelland and Peng, 2009).

Conclusion

Laboratory animals are used in biomedical research to advance scientific understanding,as model to study disease, to develop potential forms of treatment and to protect the safety of people, animals and environment. Small in size, high fecundity, external fertilization, transparency of embryo, high degree of conservation between the human and zebrafishgenomes and easy genetic manipulation makes zebra fish as alternative to currently used rodents & non rodents model organisms. Zebrafish is increasingly used as an in vivo model system for the evaluation of novel drug for efficacy and safety testing, and many other studieshave confirmed that mammalian and zebrafish toxicity profiles are strikingly similar.

Mammalian drug metabolizing enzymes including cytochrome P450 3A family are present inthe zebrafish liver. Zebrafish larva was shown to be a good model for the investigations on drug induced cardiotoxicity. Zebrafish can be used to eliminate potentially unsafe hepatotoxic compounds rapidly in the early stages of drug development. The counterparts of many brain subdivisions found in the developing mammalian brain are morphologically identifiable in the developing zebrafish also includes the development of the blood brain barrier. Zebrafish can serve as an ideal model for studying developmental neurotoxicity of environmental contaminants. The nephrons of the zebrafish kidney display a high degree of similarity with mammals & also adult zebrafish able to neonephrogenesis throughout their lives in response togrowth requirements or injury. Zebrafish can produce more than 200 eggs per week, thus permitting large-scale experimental analysis.

References

Alestrom, P., D'Angelo, L., Midtlyng, P. J., Schorderet, D. F., Schulte-Merker, S., Sohm, F., & Warner, S. (2020). Zebrafish: Housing and husbandry recommendations. *Laboratory animals*, 54(3), 213-224.

Arunachalam, M., Raja, M., Vijayakumar, C., Malaiammal, P., & Mayden, R. L. (2013). Natural history of zebrafish (Danio rerio) in India. *Zebrafish*, 10(1), 1-14.

Chablais, F., & Jazwinska, A. (2012). The regenerative capacity of the zebrafish heart is dependent on TGFβ signaling. *Development*, 139(11), 1921-1930.

Chakraborty, C., Hsu, C. H., Wen, Z. H., Lin, C. S., & Agoramoorthy, G. (2009). Zebrafish: acomplete animal model for in vivo drug discovery and development. *Current Drug Metabolism*, 10(2), 116-124.

Chen, J. N., & Fishman, M. C. (1996). Zebrafish tinman homolog demarcates the heart field and initiates myocardial differentiation. Development, 122(12), 3809-3816.

Clelland, E., & Peng, C. (2009). Endocrine/paracrine control of zebrafish ovariandevelopment. *Molecular and Cellular Endocrinology*, 312(1-2), 42-52.

d'Amora, M., & Giordani, S. (2018). The utility of zebrafish as a model for screening developmental neurotoxicity. *Frontiers in Neuroscience*, 12, 976-979.

Fang, J. P. M., Zhu, D., & Luo, C. (2016). An *in vivo* assessment: Cardiotoxicity induced by three kinds of addictive drugs (methamphetamine, ketamine, and methadone) in zebrafish embryos. *International Journal of Public Health and Safety, 1*(3), 1-4.

Gerlach, G. (2006). Pheromonal regulation of reproductive success in female zebrafish: femalesuppression and male enhancement. *Animal Behaviour*, 72(5), 1119-1124.

Gerlach, G. F., & Wingert, R. A. (2013). Kidney organogenesis in the zebrafish: insights into vertebrate nephrogenesis and regeneration. *Wiley Interdisciplinary Reviews: Developmental Biology*, 2(5), 559-585.

Han, Y., Zhang, J. P., Qian, J. Q., & Hu, C. Q. (2015). Cardiotoxicity evaluation of anthracyclines in zebrafish (Danio rerio). *Journal of Applied Toxicology*, 35(3), 241-252.

He, J. H., Guo, S. Y., Zhu, F., Zhu, J. J., Chen, Y. X., Huang, C. J., ... & Li, C. Q. (2013). A zebrafish phenotypic assay for assessing drug-induced hepatotoxicity. *Journal of Pharmacological and Toxicological Methods*, 67(1), 25-32.

Hill, A., Mesens, N., Steemans, M., Xu, J. J., & Aleo, M. D. (2012). Comparisons between *invitro* whole cell imaging and *in vivo* zebrafish-based approaches for identifying potential human hepatotoxicants earlier in pharmaceutical development. *Drug Metabolism Reviews*, 44(1), 127-140.

Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., ... & Teucke, M. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature*, 496(7446), 498-503.

Kamei, C. N., Liu, Y., & Drummond, I. A. (2015). Kidney regeneration in adult zebrafish by gentamicin induced injury. *Journal of Visualized Experiments*, 102, 1-6.

Khan, F. R., & Alhewairini, S. S. (2018). Zebrafish (Danio rerio) as a model organism. *CurrentTrends in Cancer Management*, 3-18.

McCampbell, K. K., Springer, K. N., & Wingert, R. A. (2014). Analysis of nephron composition and function in the adult zebrafish kidney. *Journal of Visualized Experiments*, 90, 1-17.

McGrath, P., & Li, C. Q. (2008). Zebrafish: a predictive model for assessing drug-induced toxicity. *Drug Discovery Today*, 13(9-10), 394-401.

Musso, G., Tasan, M., Mosimann, C., Beaver, J. E., Plovie, E., Carr, L. A.,& MacRae, C. A. (2014). Novel cardiovascular gene functions revealed via systematic phenotypeprediction in zebrafish. *Development*, 141(1), 224-235.

Nikam, V. S., Singh, D., Takawale, R., & Ghante, M. R. (2020). Zebrafish: An emerging whole-organism screening tool in safety pharmacology. *Indian Journal of Pharmacology*, 52(6), 505-522.

O'Brien, P. J., Irwin, W., Diaz, D., Howard-Cofield, E., Krejsa, C. M., Slaughter, M. R., ... &Hougham, C. (2006). High concordance of drug-induced human hepatotoxicity with in vitro cytotoxicity measured in a novel cell-based model using high content screening. *Archives of Toxicology*, 80(9), 580-604.

Poss, K. D., Wilson, L. G., & Keating, M. T. (2002). Heart regeneration in zebrafish. *Science*,298(5601), 2188-2190.

Rocke, J., Lees, J., Packham, I., & Chico, T. (2009). The zebrafish as a novel tool for cardiovascular drug discovery. *Recent Patents on Cardiovascular Drug Discovery*, 4(1),1-5.

Siegfried, K. R., & Nusslein-Volhard, C. (2008). Germ line control of female sex determinationin zebrafish. *Developmental Biology*, 324(2), 277-287.

Wullimann, M. F. (2009). Secondary neurogenesis and telencephalic organization in zebrafishand mice: a brief review. *Integrative Zoology*, 4(1), 123-133.