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Evaluation of the effect on cardiac & circulatory system of zebrafish (*Danio rerio*) embryo by applying amino acids i.e. arginine & cysteine

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Abstract

Cardiac disorders have become the foremost cause of morbidity and mortality in recent decades. *Danio***(Brachydanio)** *rerio* is a tiny vertebrate model comes under the family- *Cyprinidae*, which is easily obtainable and provides affordable cost of maintaining. Evaluation on cardiac consequences as well as various cardiac developments along with circulatory system is a composite method including detailed analytical observation of morphological study of the embryos of ***Danio rerio***. Due to the optical transparency of thisvertebrate embryo the morphological observation of cardiac system can be distinctly supervised under light microscope. In this research work we consider some amino acids such as Arginine and Cysteine as the selective materials of the experiment. Arginine performs a major function in nutrition and metabolism which can be used to prevent and treat some disorders related to cardiovascular system. L-cysteine plays a remarkable role in control of cardiovascular system which is more significant comparing with other amino acids. Zebrafish, the robust vertebrate oviparous model, has also been considered as an impressive animal model to analyze different aspects of alcohol influence on embryonic development. After treating those selective molecules, toxicity effects of cardiac and circulatory systems can be remarked including some morphological deformities of this oviparous species with exhibiting a distinct effect of cardiac toxicity.

Keywords

Prokinetic effect, Oviparous, Cardiac toxicity, Soaking Method,Deformities.

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1. INTRODUCTION:

Danio rerio is appearing as a prognostic vertebrate model for in-vivo assessment of drug efficacy, safety and toxicity study. [5, 6, 8, 9, 10] Zebrafish is the most advantageous animal model because of their resemblance with morphological as well as cellular basis of tissue and organs to other vertebrates including human beings. [2, 3] Evaluation on cardiac effects as well as cardiac development along with circulatory system is a composite method including distinct observation of morphological study of the embryos of *Danio rerio*. This exvivo model has become an apparent robust exemplanary to unravel the development of circulatory system based on genetic, molecular and cellular mechanism. According to the study of Stainier and Fishman in 1993; the development of heart at the embryonic stage of this vertebrate model is very prominent and can be easily scored. [11] In 1996 Weinstein and Fishman and in 1997 Fishman et al. proposed that the molecular mechanism of cardiac patterning of this oviparous model is almost identical to the more complex patterning of higher vertebrates cardiovascular system. [12] As zebrafish is a rapidly developed small vertebrate model, after 24 hours of post fertilization (hpf) the fertilized eggs of this oviparous exemplanary has grown up into an embryo. After that the embryos may initiate the commencements of various organs which include the formation of heart tube with contraction followed by blood circulation.[12] Studies of assessment on cardiac effects using the zebrafish embryos will become an exigent work for the purpose of large-scale screening of the active cardiovascular substances, mainly for the new drugs [12]the effects on cardiac system along with blood circulation of zebrafish embryos by using the experimental drug such as L-Arginine and L-Cysteinecan be distinguished and the efficacy and the safetyassessment of these drugs can be observed properly.

2. MATERIALS & METHODS:

2.1. Handling of Zebrafish: Adult zebrafish were housed properly by maintaining the light-dark cycle as the ratio of 14:10 along with maintaining the proper temperature i.e. 25±2°c. They fed live brine shrimp twice daily and dry flakes once a day. For mating purpose we generally used adult female and male zebrafish in the ratio of 3:2 and allowed them for spawning method by maintaining the pH (6.9-7.2) and temperature of fresh water using thermostat (55W) for production of a large number of healthy embryos and the embryos were maintained consciously. [11]

2.2.Method of Observing the Cardiac Development of Embryos under Light Microscope:

Danio rerio is a rapidly developing vertebrate oviparous model organism which is genetically and phenotypically resemblance with the human model. After getting a huge number of healthy embryos of this oviparous species, we were able to observe the various developmental stages of the species distinctly under the light microscope after a certain hours of post fertilization (hpf) and also identify various developmental structures of the cardiac system as well as the overall circulation of the body. Gastrulation begins at 6 hpf (hour post fertilization) and embryogenesis can be completed about 96 hpf. As per the microscopical observation the normal heart beat of a healthy zebrafish embryo is approximately 120-180 beats per minutes. Development of the cardiac system and the various parts of the cardiac system can be distinctly visible under light microscope. **(Fig. 1)**

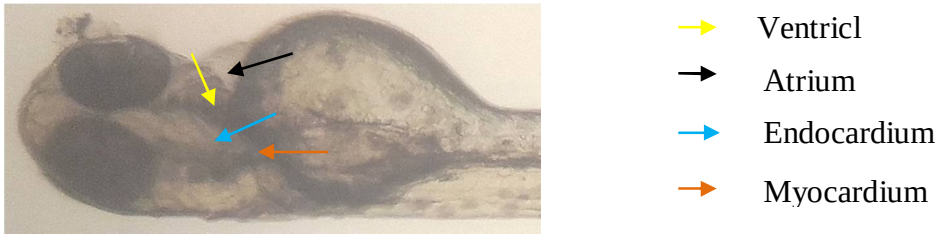

Fig.1: Lateral view of heart chambers and layers of 4-day old Zebrafish larvae

2.3.Materials &Method:

- The fundamental experimental drug molecules used in this experiment were L-Arginine and L-Cysteine. This experimental study was experimented at the year of 2017 and we get an analytical as well as experimental conclusion of this experiment at the mid month of the year 2019.
- **Atenolol:** Atenolol was purchased from Sigma- Aldrich (India). To standardize the values of the effect on cardiac system of this embryo we used a standard drug such as Atenolol which is categorized as a β blocker. It is not only an established drug for the treatment of first-line therapy of hypertension but also used in the treatment of several others cardiovascular problems such as coronary artery disease, heart failure, angina pectoris, congenital heart disease, hypertrophic cardiomyopathy, and trachyarrythmias. [7,11] Under light microscope we observe and analyzed the cardiac effects along with blood circulation by using the standard drug Atenolol.
- **L-Arginine:** Arginine was purchased from Sigma- Aldrich (India). For last few years, L-Arginine can be used to treat or prevent some cardiovascular disorders which are the major cause of death. Dietary Arginine supplementation exhibits a novel purpose of nutrition in case of prevention and treatment of cardiovascular diseases such as- coronary and peripheral arterial disease, ischemic heart disease and heart failure.[4]

- **L-Cysteine:** Cysteine was purchased from Sigma- Aldrich (India). Due to propensity of activating the autonomic nervous system by the triggering effect of releasing vasopressin, this amino acid exhibits its effects on circulatory system. L-Cysteine plays a remarkable role in the control of cardiovascular system. Generally, L-Cysteine persuades trachycardia in a significant way accompanying with bradycardia after its administration.[1]

2.4.Experimental Methods-

2.4.1. Determine the LC_{50} Value of Atenolol (STANDARD DRUG)& the test drugs such as- L-Arginine and L-Cysteine-

- At first the LC_{50} value of the standard drug Atenolol was determined by taking 20 embryos at a random manner treating them with different concentrations (0.025 gm/L, 0.05 gm/L, 0.07gm/L, 0.10gm/L, 0.12gm/L, 0.15gm/L, 0.20gm/L, 0.23gm/L, 0.26gm/L) and represent the LC_{50}graphical presentation**(Fig. 2)**of Atenolol with the above mentioned concentrations.
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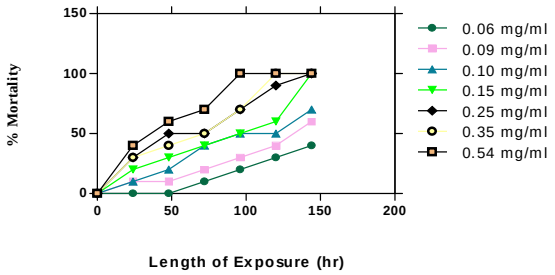

Fig.2: Curve of % Mortality of Atenolol at different concentrations.

- Another most important selected experimental drug was L-Arginine. We have determined the LC_{50} value of the test drug Arginine same as standard drug with different concentrations (0.05mg/ml, 0.06mg/ml, 0.09mg/ml, 0.1mg/ml, 0.15mg/ml, 0.25mg/ml, 0.35mg/ml, and 0.54mg/ml). With those concentrations the LC_{50}graphical presentation has been presented at **Fig. 3**

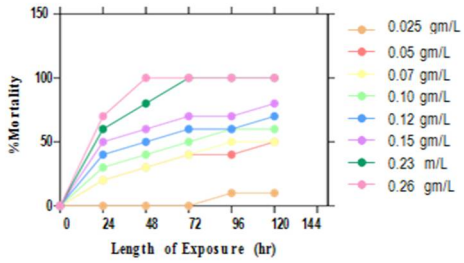

Fig.3: Curve of % Mortality of L-Arginine at different concentrations.

- After that we have determine theLC_{50}value of the selected drug L-Cysteine. The selected different concentrations are- 0.04gm/ml, 0.045gm/ml, 0.05gm/ml, 0.056gm/ml, 0.06gm/ml,

0.08gm/ml, 0.1gm/ml, 0.15gm/ml, 0.2gm/ml and 0.25gm/ml. With those concentrations the LC_{50}graphical presentation has been shown at **Fig. 4.**

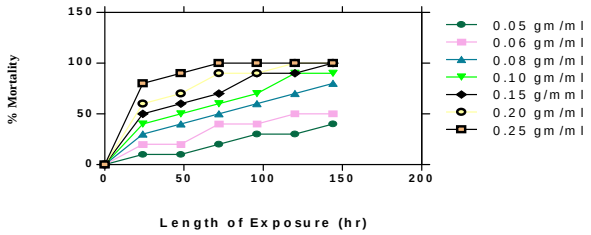

Fig.4: Curve of % Mortality of L-Cysteine at different concentrations.

The mortality was recorded at the end of the each treatment. Dead embryos were identified as the absence of heartbeat distinctly under microscope. By using probit scale analysis we have identified the LC_{50} value of these test drugs.

3. Observation-

3.1.Study the Effect of Test Drugs on Heart & Circulatory Systems of Zebrafish Model-

- As per the solubility study of all the test drugs; we have prepared the stock solutions of L-Arginine and L-Cysteine. From these stock solutions we have prepared five different concentrations of each drug. For each concentration we consider six numbers of larvae of equal period of hpf (hours post fertilization). The embryos were treated with the test drug molecule as per the selected concentrations at various time periods such as 30min, 1hr, 2hr, 3hr, 4hr and 24hr.Test drugs were delivered into *Danio rerio* by direct soaking.
- Drug effects on zebrafish embryos can be observed by image-based morphometric analysis by using light microscope. The heart beats and circulation throughout the body after soakingthe test drugs after certain periods of time can be observed clearly and toxic effects on heart and circulatory system was identified. Count the normal heart beats of the embryos before treating them with the test drug and also counts the heart beats after treating test drugs and a drastic change of heart beat was observed at repeated period of times. Some morphological deformities also can be observed under the light microscope after treating at high concentration of the test drugs.

4. Result & Discussion

As a result, from the above experiment, the toxicity effects of the test drug molecules such as L-Arginine and L-Cysteine at a specific dose on the cardiac and circulatory system of the experimental model of zebrafish has been clearly obtained and also the 50% Lethal Concentration (LC_{50}) value has been obtained and alsorepresented at the graphical presentationof

the **Fig. 2, 3, 4**. From the experiment the safe dose and also the toxic dose of the test drugs have been identified.

- During the LC_{50} study of thetest drug L-Arginine we have found that at the concentration of 0.35mg/ml and 0.54mg/ml the maximum numbers of embryos and larva of *Daniorerio* becomes dead with the length of exposure of 5 days to 7 days of the LC_{50} study.
- During the LC_{50} study of thetest drug L-Cysteine we demonstrated that at the concentration of 0.15gm/ml the body portion of the larvae of zebrafish becomes bended and at the concentration of 0.2gm/ml and 0.25gm/ml the maximum numbers of the healthy embryos of this oviparous model become dead.
- From the statistical observation we may demonstrate that as per the continuous usage of the standard drug Atenolol the normal heart rate of the zebrafish larvae has been decreasing with increasing the concentration of the drug which is represented graphically.
- From the statistical representation of the heart rate of the amino acids Arginine and Cysteine we demonstrate that, at high concentration of Arginine using in regular basis may decrease the heart rate comparing with the control (before drug treated) up to 4 hours from the time of drug treating and after 24 hours the heart rate has been increased but it was also at low rate comparing with the control heart rates. In case of control the normal heart rate of zebrafish larvae was found to be 140 beats/minutes. After the drug treated at the concentration of 0.025 mg/ml and 0.030 mg/ml the heart rate becomes 96 beats/minutes at each mentioned concentration.
- From the statistical representation we also demonstrate that after treating the test drug Cysteine it may drastically increase the heart rate from the control heart rate upto 4 hours of drug treatment and then at 24 hours the heart rate of this vertebrate model has been decreased. In case of control the normal heart rate before drug treated was 120 beats/minutes. After that at time of exposure of 4 hours of drug given the heart rate become 132 beats/min atthe concentrations of 0.060 gm/l and 138 beats/minutes at the concentration of 0.100 gm/l respectively.

7. GRAPHICAL REPRESENTATION OF HEART RATE OF ZEBRAFISH MODEL BY USING SELECTED TEST DRUGS:

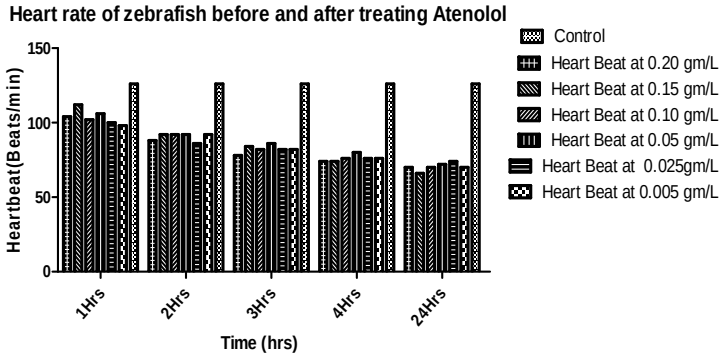

Fig 5: Observation of heart beats of developing zebrafish embryos (48 hpf) exposing various doses (selected concentrations are closely related to physiological concentration) of Atenolol (standard) after 1hr, 2hr, 3hr, 4hr and 24hr.

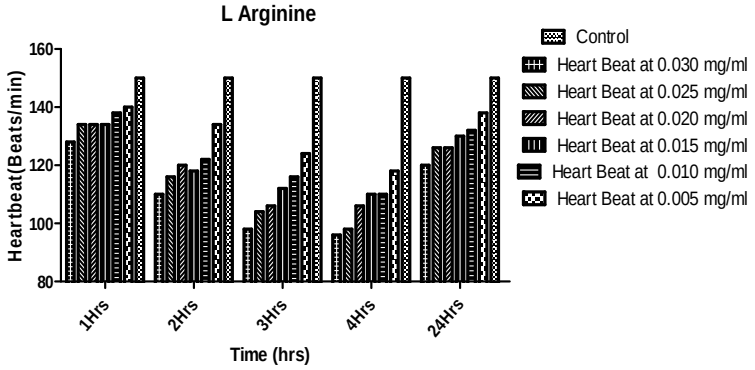

Fig.6: Observation of heart beats of developing zebrafish exposing various doses (selected concentrations are closely related to physiological concentration) of Arginine after 30mins, 1hr, 2hr, 3hr, 4hr and 24hr.

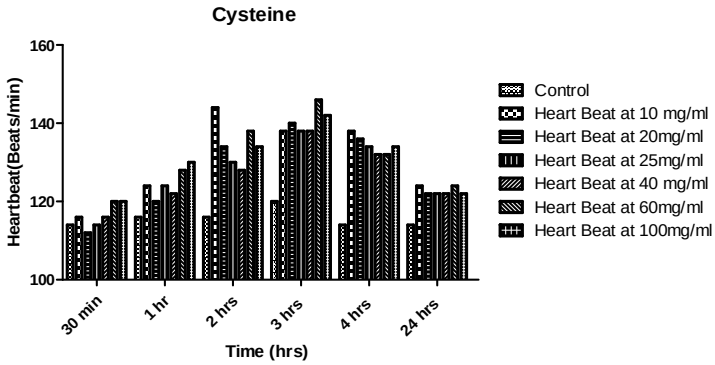

Fig.7: Observation of heart beats of developing zebrafish exposing various doses (selected concentrations are closely related to physiological concentration) of Cysteine after 30mins, 1hr, 2hr, 3hr, 4hr and 24hr.

7.1. Tabular Representation:

Sl No.	Test DrugGiven	Heart rate & Blood Circulation (Before drugtreated)	Heart rate &Blood Circulation (After drugtreated)
1.	L-Arginine	+++	+
2.	L-Cysteine	+++	++++

Table 1: Representation of Heart Rate and blood Circulation Before and after experiment

[+++ denotes the first (normal) heart rate and the blood circulation; ++ denotes the moderate hear rate and blood circulation; + denotes the very low heart rate and blood flow]

8. CONCLUSION:

Due to the transparency of the embryos of the *Danio rerio* the heart rate and the blood circulation in this model can be very feasible. This vertebrate exemplanary provides some definite advantages for the heart development study because of its availability in embryology and genetic approaches. Therefore, the cardiac and circulatory toxic effects of these test drugs such as L-Arginine and L-Cysteine has been demonstrated significantly. Summarization of the knowledge acquired from this research work is due to the resemblance of the genetic nature of *Danio rerio* with the human beings a wide range of informations can be found which helps to identify the mechanism of the test drugs on the cardiac and circulatory system. In this research work, the main focus is the cardiac developmental study of the zebrafish model and studies the cardiovascular effects of the test drugs on this species. As a future aspect of this research work, it can be predicted that in upcoming days, this field of research will progress in rapid manner due to the growing interest of the researchers in cardiac diseases and also due to the ease of availability of this oviparous vertebrate model.

As the amino acids are very much essential for our body therefore many people take those as a food supplements but the novelity of this research work is to study the drastic effects of cardiovascular system on *Danio rerio* by using these amino acids which are not yet found out.

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