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Simple Analysis and Scoring of Zebrafish Larvae Cardiac Performance in Teratogenicity Study Using Light Microscopy and Imagej Software

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ABSTRACT

***Background*:** Zebrafish is one of the suitable model organisms for teratogenicity study. One of the organs assessed during the study is the heart. There has been an established scoring system for the cardiac morphology, but not cardiac performance. Furthermore, the assessment of cardiac function in zebrafish often use expensive instruments and complicated methods.

***Materials and Methods*:** Cardiac performance scoring method and system was proposed for zebrafish larvae aged 5 dpf. Observation was done using light microscope and the video of the observation was taken using smartphone or camera. The videos were then converted and rendered to be compatible with the ImageJ program using the measure function and time series analyzer plugin. The assessed parameters were the heart rate and rhythm, strength of heart contraction, and synchronicity between atrium and ventricle.

***Results*:** The scoring system was formulated based on the assessments towards healthy control 5 dpf zebrafish larvae and references. The score for cardiac performance ranged from 3 – 6, with score 3 representing arrhythmic, weak, and asynchronous contractions, while score 6 representing rhythmic, strong, and synchronous contraction. The scoring system was also valid to point out the difference in cardiac performance between healthy control zebrafish larvae and larvae that previously exposed to lithium as teratogenic agent ($p = 0.000$).

***Conclusions*:** The scoring methods and system developed in this study allowed for a non – expensive and simple assessment of cardiac performance in zebrafish larvae aged 5 dpf, especially for a teratogenicity study.

KEYWORDS: Cardiac performance, ImageJ, Scoring, Teratogenicity, Zebrafish

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I. INTRODUCTION

Zebrafish is an ideal model organism for research because they have several characteristics. First, zebrafish can reproduce throughout the year, so that the embryos needed for research can always be available. Second, fertilization and development of zebrafish embryos occur outside the mother's body, thus observation and treatment can be carried out during the embryonic development period. Third, one female zebrafish can produce up to hundreds of eggs in one breeding process, allowing a statistically significant number of embryos to be studied. Fourth, zebrafish embryos and larvae are transparent, so the cells and organs in the process of development can be observed using a microscope. Fifth, zebrafish embryos develop quickly, so the development of cells and structures in the embryo can be observed in real-time [I]. Zebrafish can be used as animal model for various types of study, one of them is a teratogenicity study. In a teratogenicity study, zebrafish embryos whether in a whole or dechorionated state are exposed to teratogenic agents, and the effects of the exposure is analysed on 5 days post fertilization

(5 dpf) when the organs in zebrafish larvae are fully formed and functional [II].One of the organs analysed in zebrafish teratogenicity study is the heart. Analysis towards the response of zebrafish heart after teratogenic agent exposure includes the cardiac morphology and performance or function. There are a few reasons on why the zebrafish heart is a suitable model for cardiac teratogenicity assay. The most compelling reason is that zebrafish cardiovascular system is homologous with that of a human's [III]. Moreover, zebrafish heart has a similar mechanism in the pumping function, including contraction and relaxation, with the human heart [IV]. Thus, the results of the cardiac teratogenicity study using zebrafish embryos as animal models have a high probability to be applied to humans, and can be used as a reference in formulating guidelines for the use of suspected teratogenic drugs by pregnant women.Previously, cardiac morphology scoring assessment for teratogenicity assay using stereo microscopy has been described [II]. However, there are still no established cardiac performance scoring assessment, and most of the observation methods towards zebrafish heart function are done with the aid of advanced instruments, such as electrocardiography, echocardiography, and even fluorescent labelling [5,6]. In this study, we formulated a scoring system for assessing zebrafish larvae cardiac performance based on the heart rhythm, contraction strength, and synchronicity, observed using the instrument of light microscopy and analysed by ImageJ program. We also validated the scoring system using lithium exposure as a teratogenic agent.

II. MATERIALS AND METHODS

A. Animals

The larvae of zebrafish (Danio rerio) aged 5 days post fertilization (dpf) or 120 hours post fertilization (hpf) were used in this study. The zebrafish embryos that would develop into larvae were kept since the age of 1 hpf in embryonic medium solution (0.05 g/L CaCl2, 0.03 g/L KCL, 1 g/L NaCl, 0.163 g/L MgSO4, and 0,5 mg/L methylene blue in Hydrobatt water) inside 6 multi–well plates with the ambient temperature of 27 ± 1°C.The zebrafish embryos were obtained from the fertilisation between wild-type male and female in a ratio of 1:1. The wild-type zebrafish were reared at the Hydrology Laboratory, Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang, Indonesia. All experimental work was approved by the Health Research Ethics Commission of Brawijaya University with the ethical approval number 272/EC/KEPK – S2/09/2023.

B. Observation and Analysis of Zebrafish Cardiac Performance

The processes involved in the observation and analysis of zebrafish cardiac performance are light microscopy observation, video recording, video conversion, and video analysis. The observation was conducted using light microscope Olympus CX21FS1 (Tokyo, Japan) at 100x magnification focused on the zebrafish heart. The video was recorded for at least 15 seconds [VII] using tripod-mounted Samsung Galaxy A31 with the video camera specification of 48MP, 1080p, 30fps (Suwon, South Korea).The resulting .mp4 videos were converted into Audio Video Interleave (AVI) format using the aid of FormatFactory (Free Time. Available online at http://formatfactory.org/). After conversion, the AVI videos were rendered using VirtualDub software (Available online at http://www.virtualdub.org/) with the help of X264vfw software (Available online at https://sourceforge.net/projects/x264vfw/files/x264vfw64) so VirtualDub can recognize the AVI video files.The converted and rendered videos were then analysed using ImageJ software (NIH and LOCI. Available online at https://imagej.net/ij/download/src/) with time series analyzer plugin (Available online at https://imagej.net/ij/plugins/time-series.html). The analysis performed including the heart rate and rhythm, width of the ventricles during ventricular systole and diastole yielding the strength of contraction, and the synchronicity of contraction between the atrium and ventricle. The steps of the observation and analysis of zebrafish cardiac performance were described in figure 1.

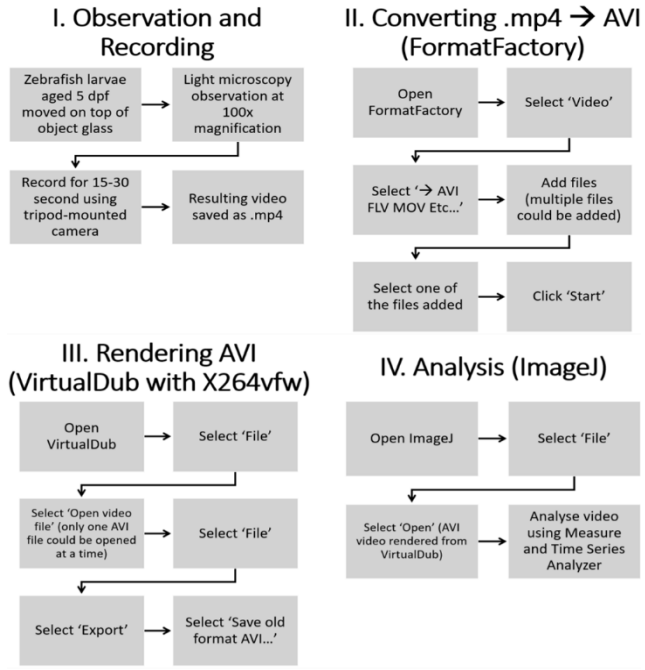

Figure 1. Observation and Analysis Flowchart of Zebrafish Cardiac Performance

There were four main steps involved in zebrafish cardiac performance analysis, which are the initial microscopic observation and recording, the conversion of recorded videos into AVI format, the rendering of the AVI videos, and finally analysis using ImageJ. All the steps were done **in** the schemed order.

C. Exposure to Teratogenic Agent

Lithium Chloride (LiCl) (Sigma-Aldrich, 310468-100G, Jakarta, Indonesia) was used as a teratogenic agent in a concentration of 30mg/L, according to the previous study

[VIII]. LiCl in a powder form was diluted in Hydrobatt water to make a 10g/L LiCl stock solution, then the stock solution was diluted in embryonic medium solution for exposure towards the zebrafish embryos starting at the age of 1 hpf until 72 hpf, or during the length of the embryonic period.

D. Statistical Analysis

The data obtained was analysed using IBM SPSS version 26 (Illinois, USA). To represent the results, Mean ± Standard Error of the Mean (SEM) was used. Since the resulting scores were in an ordinal scale, the cardiac performance score between the two groups were compared using Kruskal-Wallis' test. P values < 0.05 indicated significant differences.

III.RESULTS

A. Heart Rate and Rhythm

In the scoring method proposed in this study, the heart rate and rhythm were scored as 'rhythmic' and 'arrhythmic'. Arrhythmia itself is defined as the abnormal rhythm of the contraction of the heart, and widely classified into bradyarrhythmia, where the heart rate is slower than the normal range, and tachyarrhythmia, where the heart rate is faster than the normal range [IX]. The normal range of zebrafish larvae heart rate at the age of 5 dpf is 140 – 180 beats per minute (bpm) [X]. Using this reference, heart rate less than 140 bpm is considered bradycardia, and heart rate more than 180 bpm is considered tachycardia. Both bradycardia and tachycardia were classified as 'arrhythmic' in the scoring system proposed in this study.

Bradyarrhythmia can also manifest as missed beats or contractions, as seen in second degree atrioventricular (AV) block [IX]. In this study, missed beats were identified as pauses between the heart contractions. From the heart rate of 140 – 180 bpm, it could be inferred that the time between each contraction is normally around 300 – 420 milliseconds (ms). Thus, if there are pauses longer than that time in between contractions, whether regular or irregular, the heart rhythm was classified as 'arrhythmic'.

This study analysed the videos for heart rate and rhythm from ten control zebrafish larvae aged 5 dpf without any treatments, and the results are shown in table I.

Table I. Measurement of Heart Rate and Rhythm in Control Zebrafish Larvae

Sample No.	Heart Rate (bpm)	Interval between Contractions (ms)	Pauses between Contractions
1	157	382.17	No
2	154	389.61	No
3	152	394.74	No
4	152	394.74	No
5	176	340.91	No
6	150	400	No
7	150	400	No
8	160	375	No
9	163	368.1	No
10	189	317.46	No
Mean	160.3 ± 3.6	376.27 ± 5.24	
Reference Range	140 – 180	300 – 420	

Heart rate and rhythm were measured in 10 healthy control zebrafish larvae. The mean of heart rate and interval between contraction were in the range of the normal references from the previous study[X]. Thus, the numbers in that reference were used as the basis of the scoring system.

From table I, it could be inferred that in control zebrafish larvae with rhythmic heart contraction, the mean heart rate was 160.3 ± 3.6 bpm with the range of 150 – 189 bpm, the mean interval between contractions was 376.27 ± 5.24 ms with the range of 317.46 – 394.74 ms, and there were no pauses in between contraction longer than that. Thus, those assessments were within the normal boundaries according to the references, and the above references were used as the basis for classifying a zebrafish heart contraction as 'rhythmic', while the zebrafish heart showing a slower or faster heart rate, longer interval, or irregular pauses between contractions, were classified as 'arrhythmic'.

B. Strength of Heart Contraction

Similar to the scoring system of heart rate and rhythm explained above, this study proposed the two-point score of 'strong' or 'weak' for the assessment of the zebrafish heart contraction. The strength of contractility was measured using the difference in the width of the ventricle during ventricular systole and ventricular diastole. According to a previous study in control zebrafish without treatment, the width of the ventricle during ventricular systole is 80±5% of the width of the ventricle during ventricular diastole [VII]. Thus, higher than 85% ventricular width ratio during systole compared to diastole shows a smaller difference in size, meaning that the ventricle does not contract adequately to function as a pump, therefore referred to a 'weak' contractility.

This study analysed the videos for heart contraction strength from ten control zebrafish larvae aged 5 dpf without any treatments. The detailed results are shown in table II.

Table II. Measurement of Ventricular Width Ratio during Ventricular Systole:Diastole in Control Zebrafish Larvae

Sample No.	Ventricular Width (pixels)		Ratio of Ventricular Width during Systole:Diastole (%)
	Ventricular Systole	Ventricular Diastole	
1	7.616	8.975	84.86
2	4.955	5.935	83.49
3	10.609	12.966	81.82

4	13.017	15.366	84.71
5	17.339	22.987	75.43
6	7.075	8.839	80.04
7	10.308	12.369	83.34
8	6.083	7.126	85.36
9	10.462	12.238	85.49
10	9.545	11.338	84.19
Mean	11.814 ± 4.864	9.701 ± 3.621	82.87 ± 1.76
Reference Range	N/A	N/A	75 – 85

Ventricular width during ventricular systole and diastole were measured and ratioed in 10 healthy control zebrafish larvae. The mean of the ventricular width ratio during systole:diastole was in the range of the normal references from the previous study[VII]. Thus, the numbers in that reference were used as the basis of the scoring system.

From table II, it could be summarized that in control zebrafish larvae with strong heart contraction, the mean ratio of ventricular width during systole:diastole was 82.87 ± 1.76 % with the range of 75.43% - 85.49%. Therefore, those assessments were within the normal boundaries according to the reference, and the above reference was used as the basis for classifying a zebrafish heart contraction as 'strong', while the zebrafish heart showing a higher ratio of ventricular width during systole:diastole were classified as having a 'weak' contraction.

C. Atrium and Ventricle Synchronicity

The last criterion in the scoring system was also determined in a two-point score similar to the previous two, with the classification of 'synchronous' and 'asynchronous'. The synchronicity in atrial and ventricular contraction is important for maintaining haemodynamic, as seen in cases of atrio-ventricular (AV) dissociation, which can manifest as hemodynamic instability [XI]. In this study, the synchronicity between the atrium and ventricle was assessed based on the elapsed time between atrial and ventricular contraction in a single cycle, which in electrocardiography (ECG) known as PR interval. Since the ECG of zebrafish is comparable a human, we used three references to determine the normal PR interval in zebrafish larvae aged 5 dpf. The first step was to determine the normal PR and QT intervals in humans, which are 120 – 200 ms and 360 – 440 ms respectively [XII]. Afterwards, we converted that number into the PR interval in adult zebrafish with the heart rate of 100 bpm and the known QT interval of 230 ms, yielding the PR interval of 77 ms [XIII]. The last step was determining the PR interval in zebrafish larvae aged 5 dpf with the heart rate of 140 – 180 bpm [X], which after the calculation was determined as 110 – 140 ms. Thus, if the time elapsed between the atrial and ventricular contraction in a single cycle was more than 140 ms, it was ruled as 'asynchronous' contraction between the atrium and ventricles.

This study analysed the videos for the synchronicity between the atrium and ventricles from ten control zebrafish larvae aged 5 dpf without any treatments. The detailed results are shown in table III

.

Table III. **Measurement of Atrium and Ventricle Synchronicity in Control Zebrafish Larvae**

Sample No.	Interval between Atrial and Ventricular Contraction in A Single Cycle (ms)
1	130
2	110
3	140
4	110
5	110
6	110
7	140
8	140
9	120
10	140
Mean	125 ± 3.79
Reference Range	110 – 140

Interval between atrial and ventricular contraction in a single cycle was measured in 10 healthy control zebrafish larvae. The mean of the interval was in the range of the normal references from the previous studies[10,12,13]. Thus, the numbers synthetized from those references were used as the basis of the scoring system.

From table III, it could be converged that in control zebrafish larvae with synchronous heart contraction, the mean interval

between atrium and ventricular contraction in a single cycle was 125 ± 3.79 ms with the range of 110 – 140 ms. Thus, those assessments were within the normal limits according to the reference, and the above reference was used as the basis for classifying a zebrafish heart contraction as 'synchronous', while the zebrafish heart showing a longer interval were classified as having an 'asynchronous' contraction.

D. Overall Scoring System

From the three criteria above, this study formulated the following scoring system for cardiac performance with the scale of 3 – 6, with the score of 3 showing the most malfunctioned heart, and the score of 6 representing the normal contractile function of the heart. The scoring system and criteria were summarized in table IV.

Table IV. Proposed Cardiac Performance Scoring System for 5 dpf Zebrafish Larvae

Criteria	Observation Results	Assessment	Score
Heart Rate and Rhythm	Heart rate = 140 – 180 bpm Time between each beat = 300 – 420 ms No observed pause between beats	Rhythmic	2
	Heart rate <140 or >180 bpm Time between each beat > 420 ms Observed pause(s) between beats	Arrhythmic	1
Strength of Heart Contraction	Ventricular width ratio during ventricular systole:diastole = 80±5%	Strong	2
	Ventricular width ratio during ventricular systole:diastole > 85%	Weak	1
Atrium and Ventricle Synchronicity	Interval between atrial and ventricular contraction in a single cycle = 110 – 140 ms	Synchronous	2
	Interval between atrial and ventricular contraction in a single cycle > 140 ms	Asynchronous	1
Total			3 - 6

The scoring system of zebrafish larvae cardiac performance was formulated based on heart rate and rhythm, strength of heart contraction, and atrium and ventricle synchronicity in a two – point score system for each criterion. The normal ranges of each criterion were determined using references that were validated by observing samples from healthy control zebrafish larvae.

E. Teratogenic Effect of Lithium on Zebrafish Larva Cardiac Performance

After establishing the scoring system, it was validated in a teratogenicity study using lithium as a teratogenic agent. According to previous studies both in zebrafish and humans, lithium is an agent that causes malformation and malfunction of the heart if exposed to an organism during the organogenesis process [14,15]. Lithium exposure during the embryonic period causes teratogenic effects in zebrafish heart because it affects the expression of nkx2.5, a gene playing a role as the general regulator of heart organogenesis both in myocardial formation and heart conduction system development [VIII].This study validated the cardiac performance scoring system by comparing the score between 10 control zebrafish larvae without any treatments and 10 zebrafish larvae exposed to 30mg/L of lithium chloride (LiCl) during the embryonic period. The graph representing the resulting score from the two groups was depicted in figure 2.

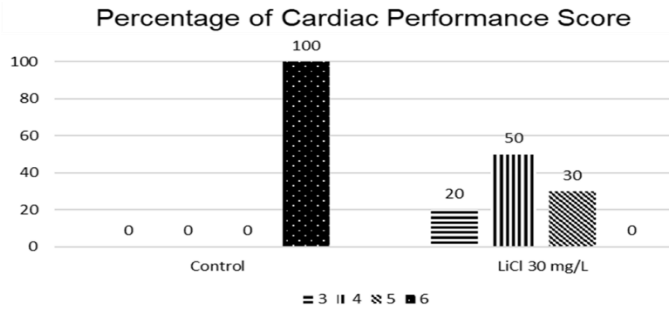

Figure 2. Cardiac Performance Score in 5 dpf Zebrafish Larvae

100% of control zebrafish showed the cardiac performance score of 6. None of zebrafish larvae previously exposed to 30 mg/L LiCl showed the score of 6, with the majority (50%) showed the score of 4, and the mean score in the lithium-exposed group was 4.1 ± 0.86.From figure 2, it could be inferred that the mean cardiac performance score in control zebrafish larvae was 6, while in the group exposed to 30 mg/L LiCl during the embryonic period, the mean score was 4.1 ± 0.86. The difference was statistically significant with the p value of 0.000 (< 0.05).

IV. DISCUSSION

All title and author details must be in single-column format Zebrafish is one of the animal models suitable for teratogenicity assay, due to their abundant yield of embryos reaching up to hundreds just from one breeding session, rapid development of embryo that produces hatched larvae in only 72 hours, and transparency of the embryo and larvae up to the age of 2 – 3 weeks [2,16]. Furthermore, the major organs and organ system in zebrafish are fully developed and functional as early as 96 – 120 hpf or 4 – 5 dpf [XVII], allowing a prompt evaluation of the teratogenic effects.One of the organs assessed in teratogenicity assay is the heart, among others [II]. The heart is most susceptible to teratogenic agents during the first phases of its development, which is on the second to seventh week in human gestation [XVIII] or the equivalent 5 – 13 hpf in zebrafish embryos [XIX]. There are two aspects of the heart that are analysed for teratogenicity study. The first one is the morphology of the heart, which scoring for 5 dpf zebrafish larvae has been established previously [II]. The second, no less important is the assessment of the function of the heart as a contractile or pumping organ. The cardiac performance is expressed as cardiac output, which is the volume of blood that is pumped by the heart to the systemic circulation per minute [XX]. Cardiac output is the function of heart rate and stroke volume [XXI]. This study proposed the scoring system of cardiac performance in 5 dpf zebrafish larvae based in factors that influence the cardiac output.The first component of cardiac output is the heart rate and rhythm. If the heart rate is too slow or what is known by bradyarrhythmia, it can cause cardiac output to be inadequate. Conversely, if the heart rate is too fast or called tachyarrhythmia, ventricular filling will be compromised due to the shorter length of atrial contraction, thus the blood pumped to the arteries are less, producing low arterial pressure and hypotension [XXII]. The heart rate and rhythm are regulated by the cardiac pacemaker and conduction system, where the heart rate is determined by the firing of impulses from the cardiac pacemaker, and the rhythm is dependent on the function of the conduction system to propagate the impulses. The main pacemaker of the heart is the sinoatrial (SA) node located at the atrium, which generates impulses that traverse through the cardiac conduction system. From the SA node, the impulses travel to atrioventricular (AV) node located between the atrium and the ventricle, then towards the ventricular conduction system [XXIII]. In human, the atrioventricular conduction system is comprised of His bundle in the membranous interventricular septum, right and left bundle branches that continue inferiorly through the interventricular system, then the Purkinje fibres that form networks in the papillary muscle of the ventricles [24,25]. Despite the zebrafish heart only having one atrium and one ventricle, zebrafish cardiac conduction system is homologous to that of a human's, thus zebrafish can be used as a model organism to study cardiac conduction and its result, namely the heart rate and rhythm [XXIII]. Thus, one of the criteria used in this study to assess cardiac performance was the heart rate and rhythm.The second component of cardiac output is the stroke volume, which is the volume of blood that is pumped out of the heart at each ventricular systole contraction. The stroke volume is further defined by the volume of the left ventricle at the end of diastole (end – diastolic volume / EDV) subtracted by the volume of the left ventricle during the end of systole (end - systolic volume / ESV). The strength of ventricular contraction is the main factor influencing the stroke volume, because the weaker the ventricular contraction, the less fraction of EDV is ejected from the ventricle, resulting in a lower stroke volume [XX]. Another factor influencing the stroke volume is the EDV itself, which is determined by the contractile function of the atrium and synchronicity between the atrium and ventricle. When the atrium and ventricle do not contract synchronously, which in this study was determined by the prolongation of the interval between atrium and ventricle contraction in a cycle, the EDV will be compromised, leading to lower volume of blood that can be pumped by the ventricle despite the normal ejection fraction of the ventricle [XXVI]. Therefore, the other two criteria for cardiac performance assessment in this study were contraction strength and synchronicity between the atrium and ventricles, which affects the stroke volume.The use of ImageJ for the analysis of cardiac performance parameters in this study was based on the consideration that ImageJ is an open-source software that can be used without previous knowledge in coding or script-writing [IV]. The software used for converting and rendering the videos to be compatible with ImageJ are also open-source software that can be used without former coding knowledge, namely

FormatFactory and VirtualDub.The validation of the scoring system proposed in this study used the teratogenic agent lithium, which has been proven to cause heart malformation and malfunction by changing the expression of nkx2.5, one of the transcription factors that plays a general regulatory role in heart organogenesis [VIII]. During the early stage of heart organogenesis, lithium exposure causes the downregulation of nkx2.5 expression [VIII], which causes a disruption in the development of the second heart field that will form the cardiac pacemaker and conduction system [XXVII]. This disruption potentially causes abnormalities in the cardiac performance. The results in figure 2 showed that the formulated scoring system in this study could be applied to assess the heart malfunction in zebrafish larvae previously exposed to lithium during the embryonic period.Some of the aspects that could be improved in this study were the use of better camera equipment to record the microscopic observation, and longer duration of observation might be needed to identify variations and irregularities that were not observed during the 15-second observation. The counting of heart rate and measurement of the ventricular dimensions were also done manually; thus, it could be improved by automatization to reduce the analysis time and increase the accuracy of the measurements.

V. CONCLUSIONS

In conclusion, the methods and scoring system developed in this study allowed for a non – expensive and simple assessment of cardiac performance in zebrafish larvae aged 5 dpf, especially for a teratogenicity study. This scoring system integrated the components of cardiac output, namely heart rate and rhythm, strength of contractility determining the ventricular ejection fraction, and atrium – ventricle synchronicity influencing the end diastolic volume, with the latter two parameters being the influencing factors of stroke volume. There are aspects that still can be improved, but for a simple assessment of cardiac performance, this scoring system was adequate in identifying the difference in cardiac performance between control zebrafish larvae and zebrafish larvae previously exposed to lithium as a teratogenic agent.

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REFERENCES

I. Eisen JS. History of Zebrafish Research. In: The Zebrafish in Biomedical Research. Elsevier; 2020. p. 3–14.
II. Panzica-Kelly JM, Zhang CX, Danberry TL, Flood A, DeLan JW, Brannen KC, et al. Morphological score assignment guidelines for the dechorionated zebrafish teratogenicity assay. Birth Defects Res B Dev Reprod Toxicol. 2010 Oct 10;89(5):382–95.
III. Teame T, Zhang Z, Ran C, Zhang H, Yang Y, Ding Q, et al. The use of zebrafish (Danio rerio) as biomedical models. Animal Frontiers. 2019 Jun 25;9(3):68–77.
IV. Sampurna BP, Audira G, Juniardi S, Lai YH, Hsiao C Der. A simple ImageJ-based method to measure cardiac rhythm in zebrafish embryos. Inventions. 2018 Jun 1;3(2).
V. Lee L, Genge CE, Cua M, Sheng X, Rayani K, Beg MF, et al. Functional assessment of cardiac responses of adult zebrafish (danio rerio) to acute and chronic temperature change using high-resolution echocardiography. PLoS One. 2016 Jan 5;11(1).
VI. Zhao Y, Yun M, Nguyen SA, Tran M, Nguyen TP. In Vivo Surface Electrocardiography for Adult Zebrafish [Internet]. 2019. Available from: https://www.jove.com/video/60011/
VII. Hoage T, Ding Y, Xu X. Quantifying cardiac functions in embryonic and adult zebrafish. Methods in Molecular Biology. 2012;843:11–20.
VIII. Rahmi KA. Lithium Exposure during Embryonic Stage Affects Heart and Eye Morphology in Zebrafish Larvae via Changes in Transcription Factor Expression . [Malang]: Brawijaya University; 2023.
IX. Desai D, Hajouli S. Arrhythmias. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing https://www.ncbi.nlm.nih.gov/books/NBK558923/. 2023.
X. Niedbalska-tarnowska J, Ochenkowska K, Migocka-patrzałek M, Dubińska-magiera M. Assessment of the Preventive Effect of L-carnitine on Post-statin Muscle Damage in a Zebrafish Model. Cells. 2022 Apr 1;11(8).
XI. Rahman M, Yandrapalli S. Atrioventricular Dissociation. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing https://www.ncbi.nlm.nih.gov/books/NBK563205/. 2023.
XII. Ernstmeyer K, Christman E. Interpret Basic ECG. In: Nursing Advanced Skills. Chippewa Valley Technical College: Eau Claire (WI); 2023.
XIII. Vornanen M, Hassinen M. Zebrafish heart as a model for human cardiac electrophysiology. Vol. 10, Channels. Taylor and Francis Inc.; 2016. p. 101–10.
XIV. Patorno E, Huybrechts KF, Bateman BT, Cohen JM, Desai RJ, Mogun H, et al. Lithium Use in Pregnancy and the Risk of Cardiac Malformations. New England Journal of Medicine. 2017 Jun 8;376(23):2245–54.
XV. Siebel AM, Vianna MR, Bonan CD. Pharmacological and toxicological effects of lithium in zebrafish. Vol. 5
XVI. ACS Chemical Neuroscience. American Chemical Society; 2014. p. 468–76.
XVII. Cho SJ, Park E, Baker A, Reid AY. Age Bias in Zebrafish Models of Epilepsy: What Can We Learn From Old Fish? Front Cell Dev Biol. 2020 Sep 10;8.

XVIII. Nikam V, Singh D, Takawale R, Ghante M. Zebrafish: An emerging whole-organism screening tool in safety pharmacology. Indian J Pharmacol. 2020;52(6):505.

XIX. Lynch TA, Abel DE. Teratogens and congenital heart disease. Journal of Diagnostic Medical Sonography. 2015 Sep 19;31(5):301–5.

XX. Kemmler CL, Riemslagh FW, Moran HR, Mosimann C. From stripes to a beating heart: Early cardiac development in zebrafish. Vol. 8, Journal of Cardiovascular Development and Disease. MDPI AG; 2021. p. 1–21.

XXI. Bruss Z, Raja A. Physiology, Stroke Volume. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing https://www.ncbi.nlm.nih.gov/books/NBK547686/. 2022.

XXII. Muir WW, Hamlin RL. Myocardial Contractility: Historical and Contemporary Considerations. Vol. 11, Frontiers in Physiology. Frontiers Media S.A.; 2020.

XXIII. Moulton K, Bhutta B, Mullin J. Evaluation of Suspected Cardiac Arrhythmia. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing https://www.ncbi.nlm.nih.gov/books/NBK585054/. 2023.

XXIV. Gauvrit S, Bossaer J, Lee J, Collins MM. Modeling Human Cardiac Arrhythmias: Insights from Zebrafish. Vol. 9, Journal of Cardiovascular Development and Disease. MDPI; 2022.

XXV. Padala SK, Cabrera J, Ellenbogen KA. Anatomy of the cardiac conduction system. Pacing and Clinical Electrophysiology. 2021 Jan 12;44(1):15–25.

XXVI. Miller C. Human Biology Chapter 14.3 Heart. Thompson River University Pressbooks; 2020.

XXVII. Husby MP, Soliman EZ, Goldberger JJ, Liu K, Lloyd-Jones D, Durazo-Arvizu R, et al. The association between the PR interval and left ventricular measurements in the multiethnic study of atherosclerosis. Cardiol Res Pract. 2015;2015.

XXVIII. Mandal A, Holowiecki A, Song YC, Waxman JS. Wnt signaling balances specification of the cardiac and pharyngeal muscle fields. Mech Dev. 2017 Feb;143:32–4