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Research Article

Mangosteen (*Garcinia mangostana* L.) Peel Decoction Effect on Embryological Development of Wader Pari Fish *Rasbora lateristriata* (Bleeker, 1854)

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ABSTRACT
Mangosteen (*Garcinia mangostana* L.) is a tropical fruit that has become a sought-after commodity by enthusiasts from various countries, including Indonesia. The active components found in mangosteen peel primarily consist of active xanthone compounds, such as mangostenol, mangostin, mangostino A, mangostino B, tvophylin B, trapezifolixanthone, alpha mangostin, beta mangostin, garcinon B, mangostano, as well as flavonoids epicatechin and gartanin. These compounds exhibit a range of beneficial properties, including anti-inflammatory, antibacterial, antifungal, antihistamine, antidiabetic, anticancer, and more. Consequently, there is significant potential in developing mangosteen peel extract as a valuable ingredient in herbal medicine. However, there is currently no available data on the effects of exposure to mangosteen peel decoction on fish animal models. Therefore, it is essential to investigate the impact of mangosteen peel decoction on wader pari fish *(Rasbora lateristriata)* embryos. In this study, wader pari embryos were subjected to various concentrations of mangosteen peel decoction (0.5, 1, 5, and 25 µg/mL). The effects on egg hatchability, survival rate (SR), heart rate frequency, and heart morphology of the larvae were meticulously examined using a Leica microscope. The data obtained were subjected to statistical analysis using one-way ANOVA. The findings demonstrated that exposure to mangosteen peel decoction resulted in lower hatching rates and embryonic survival, alongside an increased heart rate frequency. Additionally, the exposed embryos displayed cardiac edema and cardiac bending, particularly at the concentration of 25 µg/mL. In conclusion, the exposure of wader pari fish embryos to mangosteen peel decoction at the concentrations of 25 µg/mL and higher significantly affected the hatching rate, survival rate, and heart rate of *R. lateristriata* fish larvae.

INTRODUCTION
Natural treatment using herbal medicine is widely practiced in Indonesia. Herbal medicines consist of active ingredients derived from various parts of plants, which are processed into forms such as powders, pills, and liquids. The production of herbal remedies strictly avoids the use of synthetic chemicals, which is believed to contribute to the minimal side effects associated with herbal medicine (Pane et al. 2021).

The mangosteen fruit originates from a tropical evergreen plant and is extensively distributed in countries like Malaysia, Indonesia, India, Thailand, Singapore, and Sri Lanka. Mangosteen is known for its array of beneficial properties, including anti-inflammatory, antibacterial, antifungal, antihistamine, antidiabetic, anticancer effects, among others (Kalick et al. 2023). Its active content primarily comprises xanthone compounds like mangostenol, mangostin, mangostino A, mangostino B, tvophylin B, trapezifolixanthone, alpha mangostin, beta mangostin, garcinon B, mangostano, as well as flavonoids such as epicatechin and gartanin (Rohman et al. 2019). Previous research has demonstrated significant potential of mangosteen fruit peel extract for use in herbal medicine formulations (Suttirak & Manurakchinakorn 2014).

Phytochemical screening tests conducted on a 95% ethanol extract of mangosteen (*Garcinia mangostana* L.) peel revealed the presence of flavonoid compounds, saponins, alkaloids, triterpenoids, tannins, and polyphenols. However, herbal medicines cannot be directly applied to humans without undergoing prior screening tests. The World Health Organization (WHO) (2005) stipulateds that substances used for medicinal purposes must undergo preclinical and clinical trials. This is consistent with the Regulations of the Minister of Health of the Republic of Indonesia Number 760/Menkes/Per/IX/1992, which mandated efficacy and safety examinations of plant ingredients before their application. Therefore, the utilisation of mangosteen peel for herbal purposes necessitates a toxicity test (Mustapa et al. 2018).

Suwignyo (2014) reported that mangosteen peel caused pericardial and brain abnormalities in zebrafish embryos, which could lead to lethality. *Rasbora lateristriata* is an Indonesian native fish that can adapt to and resist changes in extreme environmental conditions (Retnoaji et al. 2016). This freshwater fish is commonly used as a bioindicator of environmental pollution (Zakeyudin et al. 2012). Wader fish are prolific animals with the potential to be used as animal models in research (Zahro et al. 2022) because wader pari fish genetically exhibit faster embryo development than Danio rerio (Raharjeng & Retnoaji 2021). Thus, this study aimed to determine the toxic effects of mangosteen peel extract at various concentrations on wader pari fish embryos.

MATERIALS AND METHODS

Materials

The materials were wader pari fish from the Faculty of Biology wader pari cultivation, mangosteen (*Garcinia mangostana* L.) peel decoction, egg water media (1,000 mL water, 1.5 mL salt water, and 1–2 drops methylene blue), and distilled water (aquadest-Genera Labora).

Methods

The fish embryos were treated with the ingredients at blastula stage (Raharjeng et al. 2022). The following are the experiment methods, which consist of fish reproduction and egg collection, range finding test, egg treatment, egg hatchability rate, larval survivability, heart rate, heart morphology examination, and data analysis.

Fish Reproduction and Egg Collection

Female and male fish with mature gonads were selected and maintained in standard condition and feed with fish commercial food as well as controlled environmental conditions of 14 = 10 photoperiod cycle (Pratama et al. 2021), 28-29°C of temperature, and dissolved oxygen levels around 6-8 ppm. Eggs were obtained by spawning male and female fish in a spawning aquarium, with a ratio of two males and one female. Eggs were collected at around 07:00 am, cleaned and maintained on egg water for further treatment at 24-72 hpf.

LC_{50}-48h Test of Wader Pari's Egg Fish in High Concentration

The LC_{50}-48-hour preliminary toxicity test were conducted to determine the lethal concentration (LC_{50}) of mangosteen peel decoction based on a 48-hour mortality effects study that caused the death of 50% of eggs with the concentrations of 0; 250; 500 and 750 µg/mL. following Widiastuti et al. (2018).

Egg Treatment with Mangosteen Peel Decoction Exposure

Mangosteen peel at the weight of 0.5, 1, 5, and 25 mg was dissolved and boiled at 90°C for 30 minutes in 500 mL distilled water. A total of 120 pre-gastrulation embryonic stage embryos (3.5–4 hpf) were chosen for the treatment, for the reason of, it's an initial stage for organogenesis to perform; 10 wader eggs were placed at each well plate containing egg water and mangosteen peel decoction at the concentrations of 0.5, 1, 5, and 25 µg/mL, respectively. Exposure of mangosteen peel decoction was conducted for the period of 72 hours, with three replications for each treatment, following Raharjeng & Retnoaji (2021).

Water Quality Monitoring for Hatchability Rate Wader Pari Fish

Water properties were similar on control and treatment, with overall water quality parameters during experiment period were in optimal quality for the fish embryo to develop and hatch, as presented in Table 1.

Table 1. The water parameters values measured during the experiment period.

Parameter	1st 24 hours value	
	control	0.5, 1, 5, and 25 µg/mL
pH	7.5	8
Temperature (°C)	28	28
DO (ppm)	8	8

The optimal water quality assures that only treatment factors contribute to the experiment result. The optimum temperature for egg hatching and larval development in *Rasbora lateristriata* is at the range of 27-30 °C and the pH ranges from 7-8 and the good DO range for hatching eggs is around 7 to 8 (Abida et al. 2021)

Egg Hatchability Rate of Wader Pari Fish (*Rasbora lateristriata*)

Egg hatchability is an indicator of good quality egg with normal cleavage and development processes (Zahro et al. 2021). The eggs hatchability rate was measured by the number of egg hatch at the period of 24-72 hours after the treatment started. The number of hatched eggs were recorded in the concentrations of 0.5, 1, 5, and 25 µg/mL, respectively.

Heart Rate and Heart Morphology of Wader Pari Fish (*Rasbora lateristriata*)

Embryonic heart rate was measured at 48 hpf stage. Larvae were randomly picked and placed in a petri dish at the position of latero-lateral and were observed by using a Leica light microscope. the heart rate was counted for one minute period.

Heart morphology observations were conducted on 72 hpf larvae. The larvae of each treatment were placed in a petri dish and photographed for ventral and lateral embryonic side. The cardiac morphology, the atrium and ventricles position and looping, the presence barrier in the atrioventricular canal (AVC), as well as the distance between the sinus venosus (SV) and the bulbous arteriosus (BA) were observed. The SV-BA distance was measured with Image-J software following Pratama et al. (2022).

Data Analysis

Quantitative data such as percent of eggs hatchability, survival rates, and heart rate, as well as, the mean differences of SV-BA lengths between treatments, were statistical analyzes with one way ANOVA to determine the significance between treatments ($p < 0.05$). Cumulative data on wader fish mortality in the LC_{50} test was analyzed using probit analysis to determine the 48-hour LC_{50} value, with SPSS probit statistical analysis.

RESULTS AND DISCUSSION

Preliminary Test (Range Finding Test) of Wader Pari's Eggs at High Concentrations

The results of the range finding test conducted at concentrations of 250, 500, and 750 μg/mL revealed lethality in all the embryos within 48 hours, with none of the embryos successfully hatching. This contrasted sharply with the control group treated with 0 μg/mL, which exhibited an 85% hatching rate and a 67.5% embryo survival rate. The results suggest that concentrations of 250 μg/mL and higher were lethal to embryos prior to the hatching period. Therefore, a lower concentration was selected for the sublethal treatment, with concentrations as follows: 0.5, 1, 5, and 25 μg/mL.

LC_{50} Toxicity Test 48 Hours of Wader Pari's Egg at Chosen Concentration

The results indicate that the mortality rate of fish embryos over the 48-hour period was not significantly different across all treatment groups. Lethality of the embryos was evident when the eggs exhibited cloudiness and a change in color, followed by drifting and sinking to the treatment media's bottom. The LC_{50}-48hours value, representing the median lethal concentration, was determined through probit regression analysis, yielding a result of 24.305 μg/mL.

However, when examining the effects of mangosteen peel decoction, it is notable that the highest concentration of 25 μg/mL demonstrated a higher average larval mortality rate compared to the other concentrations (refer to Figure 1).

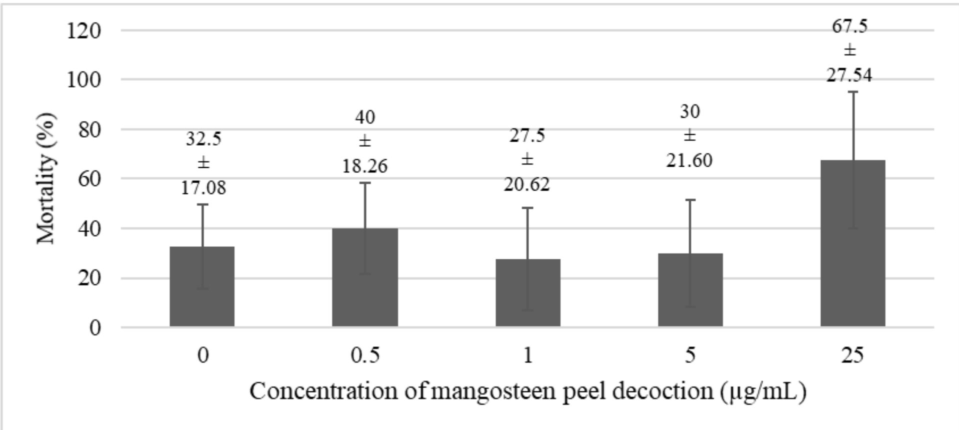

Figure 1. Embryo mortality percentage on each treatment, showed significant difference in treatment concentrations of 0, 1, and 5 μg/mL compared to 25 μg/mL ($p < 0.05$).

The mortality percentage of embryos in the control and all treatments showed a high mortality rate of 67.5% at the highest concentration of mangosteen peel decoction, which was 25 μg/mL, and the lowest percentage of 27.5% at a concentration of 1 μg/mL of mangosteen peel decoction, respectively. The statistical analysis ($p < 0.05$) revealed a significant difference in treatment concentrations of 0, 1, and 5 μg/mL compared to 25 μg/mL.

Exposure to high concentrations of mangosteen peel decoction caused lethality in fish embryos, possibly due to cellular damage or physiological abnormalities during the early stages of embryonic cleavage and development. Cell death is one of the cellular responses to disturbances occurring in embryos (Miller & Zachary 2017).

Fish Egg Hatchability Rate of Wader Pari Fish (*Rasbora lateristriata)*

The hatching rate of wader pari fish eggs, treated with various concentrations of mangosteen peel decoction, is presented below (Figure 2). The hatching rates for treatments at 0, 0.5, 1, 5, and 25 μg/mL ranged from 52.5 to 85%. The lowest hatching percentage was observed at a concentration of 25 μg/mL (52.5%), while the highest survival rate of 85% was recorded in both the control and the 1 μg/mL concentration treatments. Statistical analysis ($p < 0.05$) revealed significant differences in the average egg hatching rates between treatments.

Egg hatchability could be used as an indicator to determine the normal process of fish development and the potential influence of external factors. Fish eggs are known to possess an acellular envelope composed of multiple layers of materials, including a protein called chorion, which functions as a protective layer for embryos (Rothenbucher et al. 2019). However, the results (Figure 2) demonstrate a decrease in hatchability at higher concentration treatments. Certain substances are believed to modify the structure of the chorion, resulting in shrinkage and damage to the protective coating. This, in turn, leads to disruptions in embryonic development and ultimately embryonic lethality (Pelka et al. 2017).

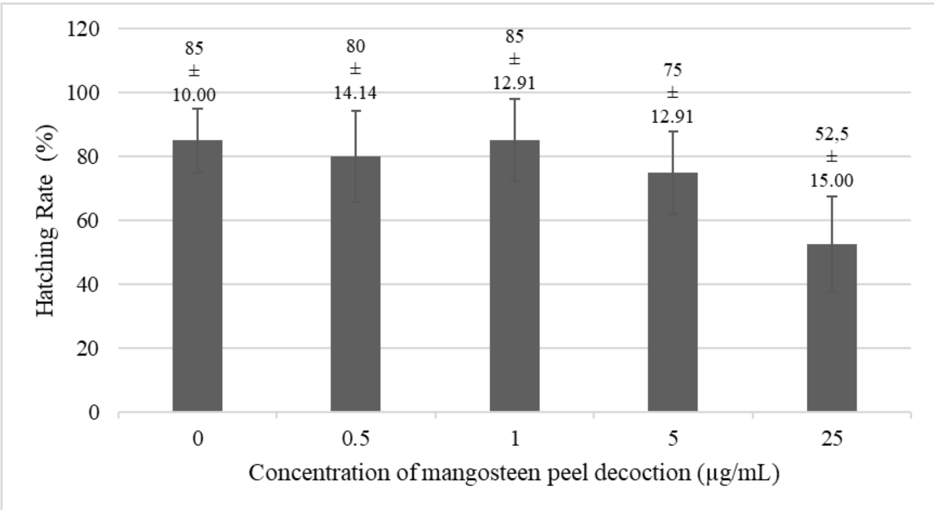

Figure 2. The percentage of egg hatching rate in the control group and all treatment exposure at concentrations of 0.5, 1, 5, and 25 μg/mL showed significant differences between treatments ($p < 0.05$).

Survival Rates of the Fish Larvae Wader Pari (*Rasbora lateristriata*)

The treatment during the pre-gastrulation egg stage resulted in a decreased larvae survival rate. The control and treatments with 0.5, 1, and 5 μg/mL mangosteen peel decoction exhibited similar survival rates of 67.5, 60, 72.5, and 70%, respectively. In contrast, the mangosteen peel decoction at a concentration of 25 μg/mL showed a lower survival rate of 32.5% (Figure 3). Statistical analysis ($p < 0.05$) revealed no significant differences in survival rates among the fish larvae treatments.

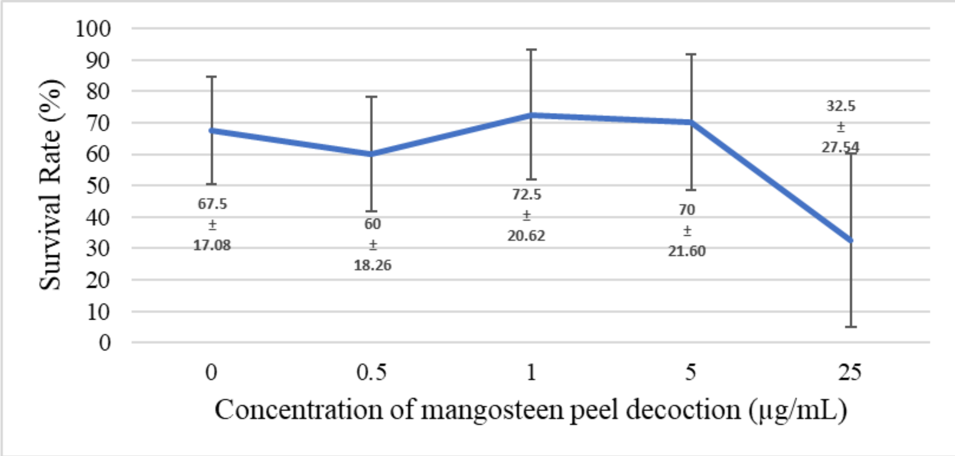

Figure 3. The percentage of survival rate of treated fish larvae showed no significant differences between treatments ($p < 0.05$).

The larvae survival rate in all treatments displayed a tendency of high survivability at the initial stage, followed by a consistent reduction as the treatment period progressed. The survival rate represents the fish's ability to withstand exposure during the research period. A total of 120 embryos were exposed during the experiment. Figure 3. illustrates the number of larvae that survived the treatment period from 24 to 72 hpf. The control and treatments with 0.5, 1, and 5 µg/mL of mangosteen peel decoction exhibited survival rates of 67.5, 60, 72.5, and 70% respectively. On the other hand, the mangosteen peel decoction at a concentration of 25 µg/mL exhibited a survival rate of 32.5%. These findings suggest that higher concentrations of mangosteen peel decoction (25 µg/mL or more) led to a reduction in larvae development and survivability.

Mangosteen peel contains tannins, which can potentially inhibit cellular metabolic activities and lead to metabolic abnormalities. These abnormalities could result in fish fatality and an inability to survive. Additionally, it contains compounds that can physiologically hinder sterol carrier protein-2 within the larvae's body, which is crucial for lipid metabolism. This disruption can lead to impaired integrity and disability. This situation triggers lipid degradation and disrupts the fish's body metabolism, ultimately causing death (Masduki 1996).

Heart Rate of the Fish Larvae Wader Pari (*Rasbora lateristriata*)

The heart rate of fish larvae was measured at 48 hpf (Wan-Mohtar et al. 2021) to examine the physiological responses of the larvae to the treatment (Figure 4). Control larvae showed a heart rate of 165.5 beats/minute, while higher heart rates were observed in the treatment group, ranging from 166.5 to186 beats/minute.

Exposure of fish larvae to mangosteen peel decoction significantly affected the average heart rate of the larvae between treatments ($p < 0.05$). It has been reported that heart physiological function is closely related to heart rate and blood flow (Männer et al. 2010). Abnormalities in the heart muscle could lead to a decrease in heart rate and reduced blood flow, potentially resulting in heart failure. Zhu et al. (2007) stated that the normal heart rate for a 48 hpf fish embryo ranges from 165 to 186 beats/minute. Therefore, the results showed a heart rate of 186 beats/minute in embryos at 48 hpf when exposed to a treatment concentration of 25 µg/mL, which is considered higher than the normal rate. This indicates that mangosteen peel decoction exposure could alter heart physiological activity.

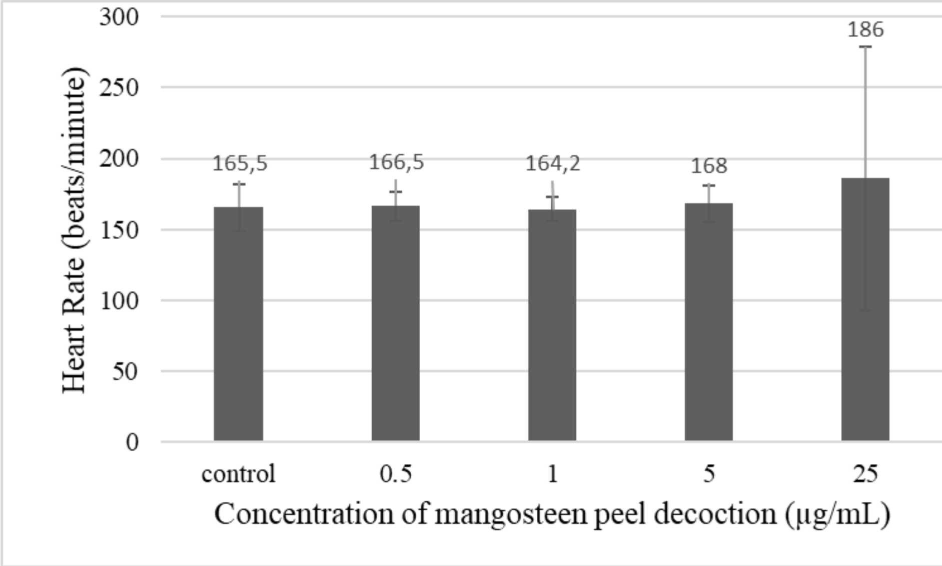

Figure 4. The average heart rate of treated fish larvae showed significant differences between treatments ($p < 0.05$).

Heart Morphology of the Fish Wader Pari (*Rasbora lateristriata*)

Heart Morphology of the Fish Wader Pari (*Rasbora lateristriata*) The observation of heart morphology (Figure 5) revealed the occurrence of pericardial edema, as well as the presence of indentations or obstructions in the atrioventricular canal (AVC) within the mangosteen treatment group. In contrast, the control group showed no signs of edema or indentations in the AVC.

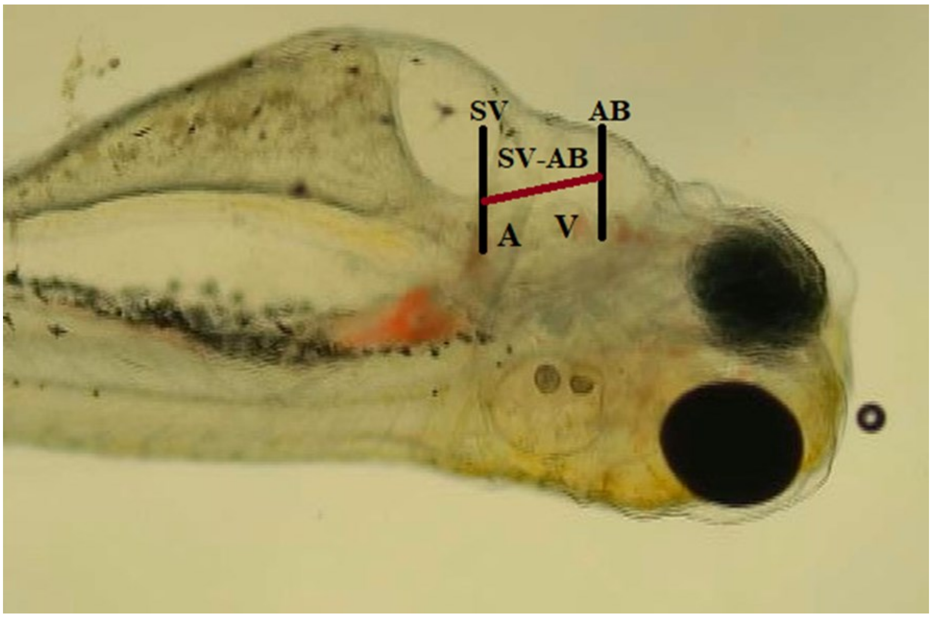

Figure 5. Measurement of sinus venosus and bulbous arteriosus distances of wader fish (*Rasbora lateristriata*). (A) atrium, (V) ventricle, (BA) bulbous arteriosus, (SV) sinus venosus.

The heart and blood vessels of fish embryos constitute two systems that are commonly studied in toxicity tests. They often manifest as changes in heart rate rhythm and reductions in blood flow, as observed in studies such as Zoupa & Machera (2017). Furthermore, studying these systems offers the potential to identify additional toxicological effects on the morphology and function of the heart. The present study presents the morphological defects of the heart in Table 2.

Table 2. Morphological abnormalities of wader pari fish embryo's heart which were exposed to mangosteen peel decoction.

Heart Defects	Concentration (μg/mL)				
	0	0.5	1	5	25
Heart edema	+	+	+	-	-
Bending of the heart, abnormal heart	+	+	+	-	-
Heart rate disorders	+	+	-	+	+
Heart tube	+	+	+	+	+

+ = Normal
- = Abnormal

One of the toxicity parameters involves the treatment's effect on the heart of fish embryos, categorised based on the measurement of the distance between the venous sinus and the arterial bulb (SV-BA interval) as described by Li et al. (2018). The Venous Sinus-Arterial Bulb (SV-BA) distance varied from 61.6 to 64.3 μm (Figure 6).

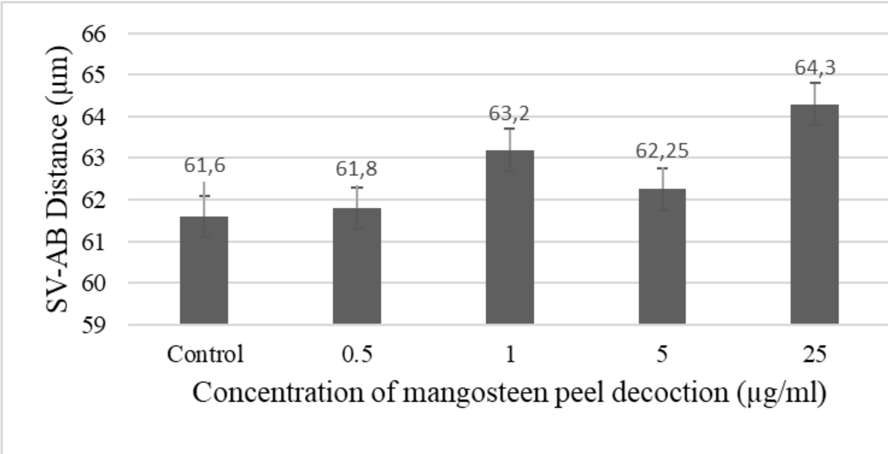

Figure 6. The Venous Sinus-Arterial Bulb (SV-BA) distance graph of 72 hpf larvae in the control group and treatment exposure at concentrations of 0.5, 1, 5, and 25 μg/mL showed no significant differences between treatments ($p < 0.05$).

Cardiac development is a parameter used to assess the toxic effects of certain substances on fish embryonic development. The heart is the first organ to form during embryonic development. Consequently, exposure to toxic chemicals at this early stage can result in malformations in the heart's morphology (Antkiewicz et al. 2005). The results of cardiac morphology observations indicated that the embryonic heart developed normally in both the control group and the lower concentrations of the treatment. However, higher concentrations of the treatment led to heart malformations or abnormalities, such as cardiac edema.

Notably, marked cardiac or pericardial edema was observed at the distance between the attachment points of the heart to the inlet and outlet channels (SV-AB). The formation of pericardial edema can separate the points of attachment for inflow and outflow, causing an elongated cardiac morphology (Antkiewicz et al. 2005).

The assessment of the venous sinus-arterial bulb (SV-BA) distance was conducted to determine the treatment's effect on the hearts of wader fish larvae. The SV-BA distance essentially serves as an index of cardiac loops that can be utilised to investigate cardiac malformations (Antkiewicz et al. 2005). The results indicated that the SV-BA distance (Figure 6) did not show a significant difference between the control group and all treatment groups. This suggests that exposure to mangosteen peel decoction did not impact the development of cardiac looping in the fish embryo.

CONCLUSION

Exposure to mangosteen peel decoction at a concentration of 25 µg/mL and higher proved to be toxic to wader fish embryos, leading to reduced hatchability, survival rates, and influencing heart rate as well as heart and body morphology.

AUTHOR CONTRIBUTIONS

P.P. and L.U.K. designed and conducted experiments, conducted statistical analysis, visualised data, and compiled papers. B.R. assisted in designing experiments, visualising data, and compiling the papers. All the authors have read and approved the last manuscript.

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CONFLICT OF INTEREST

The authors declare that there are no competing interests.

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