

Quantification of heart rate in adults *Danio rerio* (Hamilton, 1822) exposed to an extract of the Indo-Pacific *Archaster typicus*, Müller & Troschel, 1840

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Abstract. This study used a repeated-measures design to evaluate the effects of different concentrations (25, 50, 100, and 200 ppm, including a control) and exposure durations (0, 24, 48, and 72 h) of an alcoholic extract derived from the echinoderm *Archaster typicus* (Müller & Troschel, 1840) on the heart rate of adult zebrafish, *Danio rerio* (Hamilton, 1822). Each treatment was conducted in triplicate tanks containing seven fish each, and data were analyzed using a linear mixed-effects model. Exposure to the extract significantly affected heart rate, with variation observed across both concentration and time. The baseline heart rate of untreated fish was 110 bpm, increasing to 127 bpm at 24 h and remaining elevated through 72 h. Heart rate differed significantly among concentrations, with the highest mean observed at 50 ppm (150±4.97 bpm) and the lowest at 100 ppm (129±10.4 bpm). At 72 h, all treatment groups exhibited increased heart rates relative to the control. Temporal patterns indicated mild increases at 24 h, followed by more pronounced elevations at 48 h and 72 h across treatments. A significant interaction between concentration and exposure duration indicated that the effects of the extract developed progressively over time. Additionally, heart rate in the control group increased moderately during the experiment, likely reflecting handling or experimental conditions. These findings demonstrate that the cardiac response of *D. rerio* to *A. typicus* extract is influenced by both concentration and exposure duration, with delayed effects emerging under prolonged exposure.

Key Words: heart rate, *Archaster typicus*, zebra fish, extract, bioactive compounds.

Introduction. Zebrafish, *Danio rerio* (Hamilton, 1822), is a dynamic model organism because of its intrinsic characteristics like sharing of genetic similarity to humans, size, short life cycle, easy handling, ease of breeding, high fecundity, and cost-effectiveness for screening drugs (Vliegenthart et al 2014; Rennekamp et al 2015; Echeazarra et al 2021). Various studies on toxicity substantiate that the mammalian model and the *D. rerio* model are outstandingly comparable. In the early stages of drug development, *D. rerio* can be used to purge potentially unstable compounds rapidly to evaluate and prioritize compounds for future preclinical and clinical studies (Ali et al 2011; Kais et al 2013; He et al 2014). Marine ecosystems provide a rich reservoir of novel bioactive chemical entities with significant medicinal potential (Khalifa et al 2019). Marine species can create bioactive chemicals to protect themselves against environmental stressors (Catanesi et al 2021). Since these pharmacologically effective secondary metabolites are secreted as a metabolic product during as part of survival mechanisms, a preliminary assessment of these compounds is necessary to evaluate human risk (Sumitha et al 2019).

Currently, more than half of commercially available drugs are either derived from natural sources or are synthesized by using natural products as templates or starting material (Geigert 2023). A few new compounds were reported as metabolites of

asterosaponins from the sea star *Archaster typicus* (Müller & Troschel 1840) showing cytotoxic activity against the human cervical cell line and the mouse epidermal cell line (Kicha et al 2010). Three highly oxygenated sterols were isolated from the methanolic extract of the starfish *A. typicus*. This extract contains flavonoids, tannins, and saponins (Mailoa et al 2025).

The heart rate (HR) and cortisol levels of *D. rerio* are commonly measured to elucidate the pharmacological effects of chemical substances (Harada et al 2022). The therapeutic and toxic responses for both cardiac and non-cardiac drugs testing can be performed by simply immersing the fish in the intended drug (Langheinrich 2003; Milan et al 2006). However, accurate measurements of cardiovascular variables can only be achieved by immobilizing the fish during recordings (Muntean et al 2010). Anesthetics can suppress or compromise autonomously regulated homeostatic functions (such as breathing, heartbeat, and blood pressure) to varying degrees (Mashour et al 2011). The average HR of the *D. rerio* is around 148 beats minute⁻¹ (bpm) (Haverkamp et al 2000). HR at 28°C increased from about 125 bpm in embryos to a peak of ~175 bpm at days 10-30 and then fell to ~130 bpm by day 100 (Barrionuevo et al 1999).

Given the adverse side effects associated with many synthetic drugs used in the treatment of cardiovascular diseases, there is increasing interest in identifying natural bioactive compounds with potential therapeutic applications. In this context, the present study aimed to evaluate the effects of the alcoholic extract of *A. typicus* on the HR of adult *D. rerio*.

Material and Method

Description of the study area. *A. typicus* was collected from the intertidal zone of Barangay Poblacion, Bacolod, Lanao del Norte, Philippines, during peak low tide. The sampling site was located at 8°11'52.26"N and 124°01'08.31"E (Figure 1)

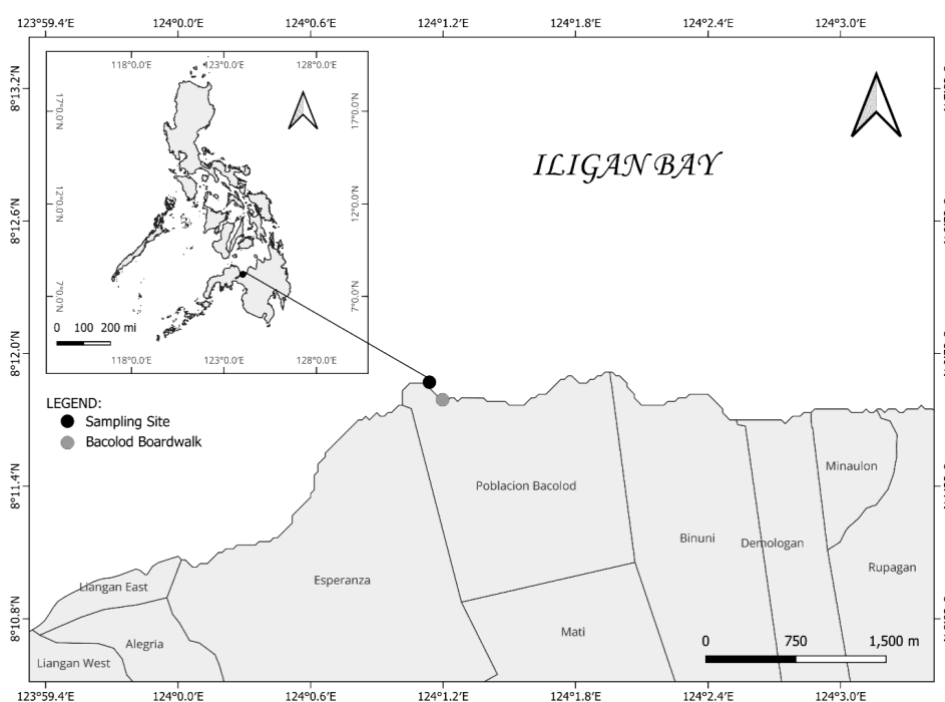


Figure 1. Location map (Source: QGIS v. 3.34.0).

Permits and echinoderm collection. All experimental procedures involving *Danio rerio* were approved by the Institutional Animal Care and Use Committee (IACUC) of MSU-Iligan Institute of Technology and conducted in accordance with institutional and international guidelines. Collection of *A. typicus* was authorized by local government officials of Bacolod, Lanao del Norte, Philippines.

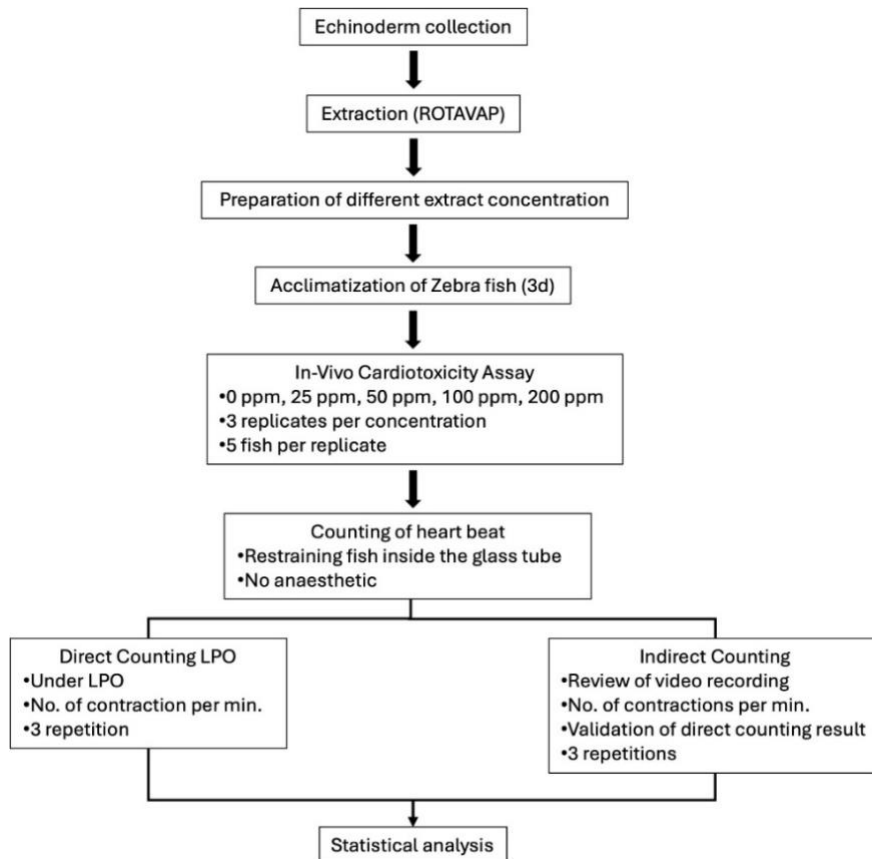


Figure 2. Flow diagram of the method.

The permit to collect the echinoderm was issued by the local officials of Bacolod municipality, Lanao del Norte, Philippines. The echinoderms were hand-picked on the intertidal area of Barangay Poblacion, Bacolod municipality. The specimens were distributed in the exposed sandy-rocky portion of the intertidal area with patches of seagrass, as well as in the shallow portion of the lower intertidal zone during peak low tide. They were thoroughly rinsed with seawater, debris removed, and stored in a cold storage thermal-resistant box with seawater and immediately transported to MSU-Iligan Institute of Technology, Marine Science Laboratory for processing.

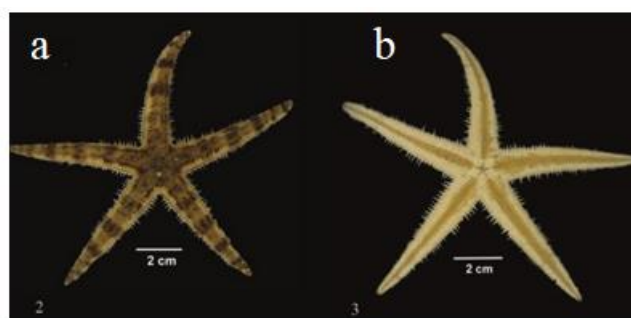


Figure 3. *Archaster typicus* (Müller & Troschel, 1840). a. Dorsal view; b. Ventral view.

Processing and extraction. Whole bodies of adult *A. typicus* were chopped into small, thin slices using a sharp knife, and the finely-chopped materials were placed in a clear glass bottle, soaked in hexane, tightly sealed and stored at room temperature. After two weeks, the liquid was decanted and filtered using Whatman II filter paper.

Extraction was carried out following the method described by Shushizadeh et al (2019) with minor modifications. During extraction, ice cubes were placed in the water circulator. The round-bottomed flask was filled with the filtrate not more than half-full and

connected to the bump trap with a plastic clip. The flask was turned slowly, lowering it into the water partially submerged, keeping the plastic clip above the water. The vacuum source was turned on, and the flask was rotated at a moderate rate at 110 rpm, one-third of its maximum value. The stopcock on the evaporator was closed by turning it perpendicular to the bleed valve. The liquid was allowed to evaporate completely at reduced pressure and a temperature of 45 °C until solid material formed. The concentrated crude residue was transferred to a lightly opaque container and stored at 4 °C for subsequent preparation of the different concentrations of the extract.

Preparation of the different concentrations of the alcoholic extract. Test solutions (25, 50, 100, and 200 ppm) were prepared by diluting a 300 ppm stock solution with distilled water. Concentrations were selected based on preliminary LC50 determination (> 400 ppm), with 100% survival observed at the highest concentration tested.

Procurement and acclimatization of *Danio rerio*. A healthy, pink, 6-month-old, wild-type (WT) strain of *D. rerio* was purchased at licensed local pet fish sellers in Iligan City, Lanao del Norte, Philippines. Fish was maintained and acclimatized for 2 weeks in a 45 L tank at 28 °C with a 14 h/10 h light/dark cycle, fed regularly, and provided with aeration. The water was monitored daily for dissolved oxygen content, salinity, temperature, and pH using calibrated portable, handheld meters. Water in the tank was changed every 3 days.

In-vivo cardiotoxicity assay. After acclimatization, a group of seven fish, regardless of sex, were randomly picked from the acclimatization tank and were assigned to an experimental tank with a code label corresponding to an extract concentration and replicate number. Immediately, their baseline heart rates (BHR) were counted (0h) and the completion time was labelled on the tank. Recording the heart rate of all individuals in a group was first completed before transferring another group. Extract concentrations include 25, 50, 100 and 200 ppm, each with three replicates. A control setup was provided with water only. The tanks were provided with moderate aeration and monitored until the experiment was terminated at 72 h.

To obtain accurate count of the heartbeat, restraining the fish was necessary. Anesthetic was not used to resolve issues regarding the adverse effects of the drug on breathing, heartbeat, and blood pressure (Mashour et al 2011). The fish was carefully guided to enter one opening of a transparent glass tube, completely immersed in water with a diameter larger than the body of the fish, but just enough to minimize its movement inside. Counting the heartbeat of the adult *D. rerio* under a microscope was adapted from Sumitha et al (2019) method for embryos with modifications. The Petri dish was mounted on a Trinocular Compound Microscope provided with a 10 MP digital camera (UB1031 Model) with a cable attached to a computer. The pulsating heart was focused under the LPO and the number of ventricular contractions of the heart in three full one minute was counted. Each minute was considered one replicate. The results from direct counting were validated by reviewing the video which was saved in the computer. The review of the video recordings was only a confirmatory procedure and did not generate a separate data set. The whole procedure was repeated in the 24, 48, and 72 hour monitoring periods.

Statistical analysis. The aim of the study was to evaluate the effects of *A. typicus* extract on adult *D. rerio* across a 72-hour exposure period. Specifically, the study investigated whether different extract concentrations (0, 25, 50, 100, and 200 ppm) influence heart rate over time and whether the magnitude of this effect changes across monitoring intervals (0 h, 24 h, 48 h, and 72 h).

Each individual fish was repeatedly measured at the four specified time points. Since multiple fish were housed within replicate tanks and treatments were administered at the tank level (3 replicate tanks per concentration; total = 15 tanks), the data exhibited a hierarchical and longitudinal structure. Consequently, observations were not independent, as fish within the same tank shared environmental conditions and repeated measurements within the same fish were correlated.

To account for this experimental structure, a linear mixed-effects model (LMM) was fitted. In this framework, concentration, time, and their interaction were treated as fixed effects representing systematic treatment influences. Random intercepts were included for replicate tanks and for individual fish nested within tanks to account for tank-level variability and fish-level biological variation.

Categorical predictors were coded using treatment (dummy) coding, with 0 ppm (control) for concentration and 0 h for time specified as the reference (baseline) levels. Accordingly, the model intercept represents the expected mean heart rate under baseline conditions (0 ppm at 0 h), and all fixed-effect estimates, including interaction terms, were interpreted relative to this reference group.

Fixed effects were evaluated using Type III analysis of variance with Satterthwaite's approximation for denominator degrees of freedom.

Results

Baseline heart rate (BHR). The BHR at 0 h was 110 ± 0.37 bpm in the control group (Figure 4, Table 1). In treatment groups, BHR was 9%, 26.4%, and 1.8% higher at 25, 50, and 200 ppm, respectively, while it was 0.9% lower at 100 ppm compared to control.

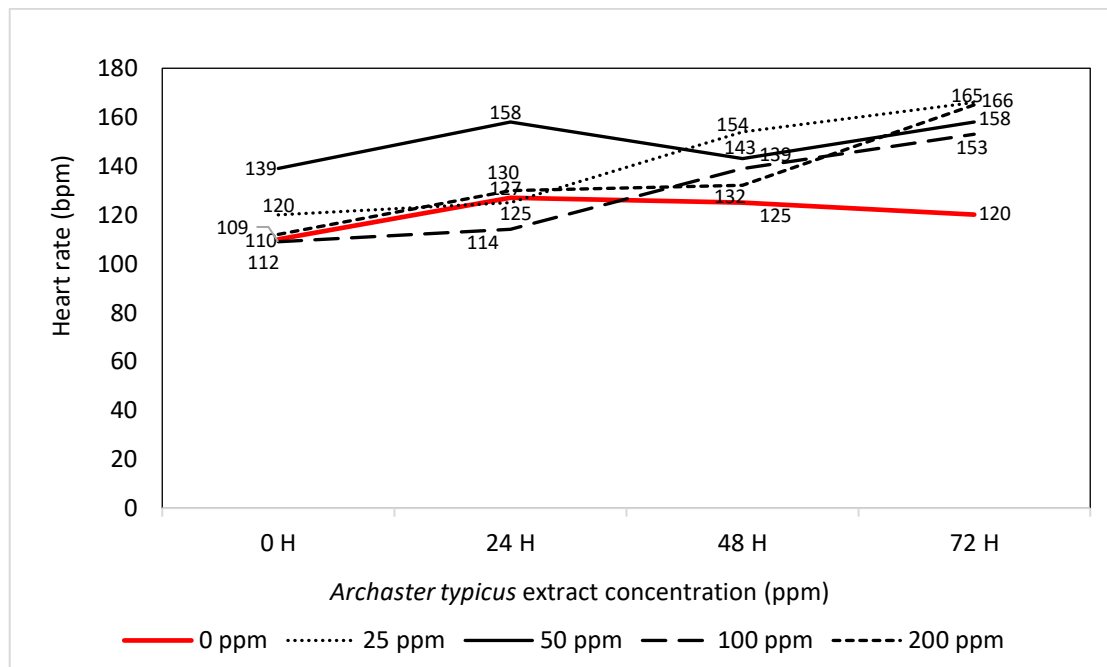


Figure 4. Heart rate profiles of *Danio rerio* within 72 h.

Temporal variation with treatments. The control HR increased from 110 ± 0.37 to 127 ± 0.29 bpm in 24 h, then slightly decreased and stabilized between 120 ± 0.4 to 125 ± 0.32 bpm from 48 to 72 h (Figure 4, red line). In the treatment groups, heart rate at 24 h decreased by 1.5% in 25 ppm and -19.2% in 100 ppm, increased by 24% in 50 ppm and 2.4% in 200 ppm from the control (0 ppm). All treatment groups showed increased HR of 6%-23% in 48 h and a larger increase of 28-38% in 72 h from the control (0 h) (Table 1). The increase in HR at 72 h with respect to the baseline HR of each group (0 h) was highest in 200 ppm (47%), followed by 25 ppm (38%) and lowest in 50 ppm (14%). (Figure 5).

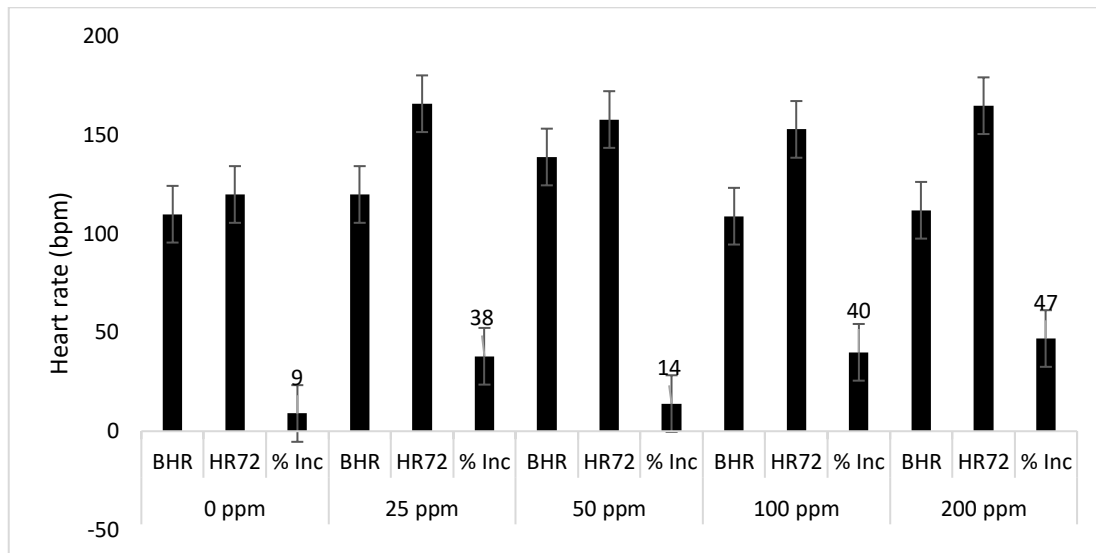


Figure 5. Baseline HR, heart rate at 72 h (HR72), and percentage increase (%Inc) in HR of *Danio rerio* in control and treatment groups after 72 h exposure to *Archaster typicus* extract.

Table 1
Mean heart rates (bpm) per treatment group in the four monitoring periods.

Time (h)	Extract concentration (ppm)				
	0	25	50	100	200
0	110±0.37	120±10.09	139±2.39	109±2.2	112±4.11
24	127±0.29	125±10.72	158±3.28	114±2.27	130±5.7
48	125±0.32	154±2.49	143±1.33	139±4.36	132±4.58
72	120±0.4	166±4.47	158±7.59	153±5.11	165±9.16
Mean	121±3.8	141±11.1	150±4.97	129±10.4	135±11.0

Comparison between concentrations. The concentration 50 ppm showed the highest BHR of 139±2.39 bpm, while 100 ppm and 200 ppm showed the lowest BHR of 109±2.2 and 112±4.11 bpm, respectively. HRs were consistently high in 50 ppm from 0 to 72 h (Figure 4, Table 1). The biggest increase in HR relative to the control was 23% in 25 ppm at 48 h and 38% in 25 ppm and 200 ppm at 72h. Mean HR was highest in the 50 ppm treatment group, and values were higher compared to the mean HR of the control.

Statistical outcomes. A linear mixed-effects model revealed that exposure time significantly affected heart rate ($F(3,210) = 74.94, p < 0.001$), and a significant interaction between concentration and time was observed ($F(12,210) = 9.72, p < 0.001$). In contrast, the main effect of concentration was not statistically significant ($F(4,10) = 2.26, p = 0.135$).

Table 2
Type III tests of fixed effects (Linear Mixed-Effects Model)

Effect	df1	Df 2	F	p-value
Concentration	4	10	2.26	0.135
Time	3	210	74.94	2.2×10^{-16} ***
Concentration × Time	12	210	9.72	5.52×10^{-15} ***

*** $p < 0.001$, significant interaction.

Table 3 shows notable variability among replicate tanks and among fish nested within tanks, supporting the inclusion of random intercepts to account for clustering and repeated observations. Variability among tanks was greater than variability among individual fish, indicating that tank level conditions contributed substantially to HR variation.

Table 3

Random effects variance components

<i>Random effect</i>	<i>Variance</i>	<i>Std. Dev.</i>
Tank (Intercept)	134.40	11.59
Fish within tank (Intercept)	94.93	9.74
Residual	205.84	14.35

Table 4

Fixed effects parameter estimates (reference: 0 ppm at 0 h)

<i>Parameter</i>	<i>Estimate</i>	<i>SE</i>	<i>df</i>	<i>p-value</i>
Intercept (0 ppm, 0h)	110.00	8.05	14.11	< 0.001
Concentration 25 ppm	10.00	11.39	14.11	0.395
Concentration 50 ppm	29.20	11.39	14.11	0.022
Concentration 100 ppm	-1.00	11.39	14.11	0.931
Concentration 200 ppm	2.00	11.39	14.11	0.863
Time 24 h	17.13	5.24	210.00	0.001
Time 48 h	15.00	5.24	210.00	0.005
Time 72 h	10.00	5.24	210.00	0.058

Note: The reference level for mean heart rate was 0 ppm at 0 h; p-values are presented for each parameter; SE: standard error; df: degrees of freedom; statistical significance was evaluated at $\alpha=0.05$ using Type III ANOVA with Satterthwaite's approximation for denominator degrees of freedom.

A significant increase in heart rate was observed at 50 ppm ($p = 0.022$), while other concentrations showed no significant differences. Time significantly affected heart rate at 24 h ($p = 0.001$) and 48 h ($p = 0.005$), but not at 72 h ($p = 0.058$).

Table 5

Concentration \times Time interaction effects

<i>Interaction</i>	<i>Estimate</i>	<i>SE</i>	<i>df</i>	<i>p-value</i>
25 ppm \times 24 h	-12.33	7.41	210	0.097
50 ppm \times 24 h	1.27	7.41	210	0.864
100 ppm \times 24 h	-12.13	7.41	210	0.103
200 ppm \times 24 h	1.27	7.41	210	0.864
25 ppm \times 48 h	18.87	7.41	210	0.012 ***
50 ppm \times 48 h	-11.47	7.41	210	0.123
100 ppm \times 48 h	15.00	7.41	210	0.044 ***
200 ppm \times 48 h	5.13	7.41	210	0.489
25 ppm \times 72 h	35.60	7.41	210	< 0.001 ***
50 ppm \times 72 h	8.87	7.41	210	0.233
100 ppm \times 72 h	34.07	7.41	210	< 0.001 ***
200 ppm \times 72 h	42.73	7.41	210	< 0.001 ***

*** $p < 0.001$, significant interaction.

No significant interaction effects were observed at 24 h. At 48 h, significant interactions were detected for 25 ppm ($p = 0.012$) and 100 ppm ($p = 0.044$). At 72 h, significant interaction effects were observed at 25 ppm, 100 ppm, and 200 ppm (all $p < 0.001$)

Discussion. Based on statistical analysis results using the LMM, HR differed significantly among extract concentrations. The mean HR was highest in 50 ppm with 150 ± 4.97 bpm, and lowest in 100 ppm with 129 ± 10.4 bpm. Compared to control (0 ppm), HR at 72h was higher by 38.3% in 25 ppm, 31.6% in 50 ppm, 27.5% in 100 ppm, and 37.5% in 200 ppm (Table 1). These results indicated that the changes in HR of the fish were concentration-related but a non-linear response.

HR also varied significantly across monitoring periods and a significant concentration x time interaction. The significant interaction between concentration and time indicated that the effect of the extract concentration on HR depends on exposure duration. The overall main effect of the concentration (averaged across time) was not statistically significant after accounting for tank-level replication (Table 2). With respect to the BHR, mild increases were observed in 24 h, however, strongly elevated HRs were observed in all treatment groups at 48 and 72 h monitoring periods (Table 1). Fifty ppm maintained elevated HR from 0 h until 72 h (Figure 4). This suggested that the effect of the extract concentrations was an increase in HR or tachycardia after prolonged exposure to the extract (48-72h). The increased HR or tachycardia may be associated with mechanisms such as altered ion channel activity; however, electrophysiological studies are required to confirm this hypothesis. Results of random effects variance components (Table 3) suggested that individual-level physiological variation contributed more to HR differences than tank-to-tank environmental variability. This supports the appropriateness of including random intercepts for both tank and fish within tank to account for hierarchical clustering.

At the reference condition (0 ppm at 0 h), the estimated mean HR was 110 ± 0.37 bpm (Figure 4 and Table 1). This represented the BHR of untreated adult *D. rerio*. Current findings on control HR were comparable to the findings of Leong et al (2010) that the adult *D. rerio* heartbeat is 110 and 130 bpm. The current finding was also comparable to the adult zebra fish HR of 122.58 ± 2.15 bpm in males and 121.37 ± 2.63 bpm in females (Mousavi et al 2020); 118 ± 14 bpm (Liu et al 2016); 149 ± 8 bpm (Nemtsas et al 2010); and 120 to 180 bpm (De Luca et al 2014). At 0 ppm (control), HR increased significantly over time. The increase of approximately 15-17 bpm at 24-48 h indicated that even in the absence of extract (0 ppm), prolonged handling and experimental conditions may elevate HR. These results showed the importance of including a time factor in the analysis since HR is not static and naturally fluctuates during sustained laboratory monitoring.

At immediate exposure (0h), 50 ppm caused a strong effect on *D. rerio* HR, registering the highest baseline HR of 139 ± 2.39 bpm. The elevated HR was sustained until 72 h with 158 bpm, equivalent to 14% increase. The concentrations of 100 and 200 ppm did not immediately alter HR (Figure 5). Higher concentration effects included delayed responses with individual variability, suggesting prolonged exposure amplified impacts beyond initial periods. The lack of immediate effect at 100 and 200 ppm may indicate delayed physiological responses at higher doses (Table 4, Figure 5). No interaction terms were statistically significant at 24h. At 48h, 25 ppm and 100 ppm produced additional HR increases beyond the sum of their individual main effects, suggesting delayed effects emerging after prolonged exposure. There was a strong, significant interaction between concentrations 25 ppm, 100 ppm, and 200 ppm at 72 h with $p = 2.94 \times 10^{-6}$, 7.36×10^{-6} , 2.85×10^{-8} , respectively (Table 5).

These results indicated pronounced delayed cardiac stimulation at higher concentrations after extended exposure of 48 to 72 h. Results suggested that the delayed emergence of strong effects supported the hypothesis that cardiotoxicity is not immediate but develops with sustained exposure. This pattern was biologically meaningful because chronic exposure effects are often more indicative of toxic stress than transient acute responses. The significant interaction between concentration and time indicated that the response of *D. rerio* HR to *A. typicus* extract was not immediate but developed progressively over exposure duration. HR increased over time even in control conditions. The hierarchical mixed-effects framework validated that these treatment effects persisted even after accounting for individual biological variability and tank-level clustering.

The decrease in HR in 100 ppm at 24 h (Table 1) can be viewed as a transient bradycardia. This observation aligned with the earlier reports of Patole (2022), Abdul-Ghani et al (1997), and Salahdeen et al (2004) that the reduced HR may be associated with the effect of the bioactive components of the extract.

Current results differed from the effect of the extract of another genus of star fish, *Stellaster equestris*, on the HR of adult *D. rerio* (Sumitha et al 2019). The results of the cardiotoxicity assay suggested that the number of pulses per minute compared with the negative control was stable and did not show any change in HR. In contrast, the HR s in

the current study varied with concentration and with the duration of exposure to *A. typicus* extract. Further, in the current study, HR was lower by 4% in 50 ppm, 6.47% in 100 ppm, and 18.6% in 200 ppm at the same incubation period of 48h. Survival rate of the fish was 100% in both the control and the treatment groups. All fish behaved normally after the termination of the experiment at 72h.

Conclusions. The alcoholic extract from *A. typicus* modulated HR in adult *D. rerio* in a manner dependent on both concentration and duration of exposure. Lower concentrations showed mild HR increases at 24h, while more pronounced and significant elevations occurred at 48 and 72 h in the treatment groups. No mortality was observed at the tested concentrations during the 72 h exposure. It is recommended to purify and isolate the bioactive ingredients of *A. typicus* extract as this warrants further investigation of its bioactive constituents and cardiac effects. It is also recommended to evaluate the effect of the extract on the reproductive potential and embryogenesis of *D. rerio*.

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Conflict of interest. The authors declare that there is no conflict of interest.

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