Candidalysin impedes the development of embryonic zebrafish (*Danio rerio*)

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Abstract

Candidalysin is a cytolytic peptide secreted by *Candida albicans*, an opportunistic human fungal pathogen that was widely spread in clinic and in the environment. However, its impacts on the development of aquatic organisms remain limited. Herein, we explored the developmental effects of candidalysin on embryonic zebrafish at concentrations from 0.008 to 160 mg/L. In acute exposure, decreased survival rate with EC_{50} at 49.5 mg/L were observed. Meanwhile, in high-dose exposure groups (5-160 mg/L), an increased swimming frequency were observed at candidalysin concentrations of 10 mg/L and above. In chronic exposure, decreased spontaneous contraction, hatching rate and heart rate were identified in low-dose exposure groups (0.008-5 mg/L). Especially for the heart rate, significant changes were observed at candidalysin concentrations as low as 0.008 mg/L (environmentally relevant). The present study first time pointed out the developmental toxicities of candidalysin on fish that was rarely investigated, and thus contributed to its environmental risk assessment.

1. Introduction

Secondary metabolites produced by fungi, especially mycotoxin such as aflatoxin, are mostly teratogenic, mutagenic, carcinogenic, and endocrine disruptive factors and can contaminate agricultural products resulting in detrimental effects on public health and economic losses (Chanda et al. 2016). The trait of fast excretion and low concentration challenge its detection in various samples. *Candida albicans* (*C. albicans*), one of the leading causes of hospital-acquired fungal infections, can survive for extended period of time in aquatic environment including marine and fresh water, and often cause crude mortality rate and rising antifungal resistance. It is a ubiquitous commensal of the native microbiota and asymptomatically colonizes many niches (Lohse et al. 2018), and can cause superficial and life-threatening systemic infections through its morphological switch between yeast and hyphae.

Fungal infections occur in all age groups. Oral candidiasis is most encountered in the newborn period, with an incidence of 20%- 40% within the first year of life (Alter et al. 2018). C. albicans is greatly contributing to caries pathogenesis, particularly in adolescents, with high prevalence of up to 47% in the oral cavity (Bliss et al. 2012). Positive association was identified between oral *Candida* carriage and caries experience, with detection rates up to 89% in caries adolescents versus 2–22% in caries-free adolescents (Xiao et al. 2018). Furthermore, childhood vulvovaginitis is one of the most common gynecological conditions in adolescent females (Barousse et al. 2004). Adolescent females with vulvovaginal candidiasis, is frequently caused by *C. albicans* with an incidence of 27.5%- 29.37 %. It mainly related with hormonal changes, poor hygiene, decreased immunity, and deficiency of parents' care and knowledge of reproductive health (Xu et al. 2020). Hence, the increasing prevalence of *C. albicans* infection in adolescents should attract sufficient attention.

It is recently discovered that the hyphal form of *C. albicans* secretes a cytolytic peptide toxin namely candidalysin, the first cytolytic toxin identified in human fungal pathogens (Moyes et al. 2016). It suited the description of classical virulence factor in that it caused cellular damage and membrane

permeabilization, induced calcium influx, and subsequent translocation of *C. albicans* across the intestinal epithelia, providing escape route from the hostile phagosome environment (Moyes et al. 2016; Ho et al. 2021). Furthermore, it acted as an immunomodulatory molecule by activating host immunity (Ho et al. 2019), and is associated with disease severity and mortality in patients with alcoholic hepatitis (Chu et al. 2020). While several studies had demonstrated that candidalysin can activate MAPK signaling and cytokine responses, and was critical for neutropil recruiting and epithelial damage in the zebrafish swimbladder model of mucosal infection (Moyes et al. 2016; Ho et al. 2019). However, as a secreted toxin capable of causing cell damage, the potential toxic effects of candidalysin on development of aquatic organisms are poorly understood.

Herein, we focus on the toxicity effects of candidalysin in embryonic zebrafish. Multiple developmental parameters including spontaneous contraction, survival rate, hatching rate, heartbeat rate, and swimming behavior were systematically assessed. Our findings point out the novel insights into candidalysin-medicated *C. albicans* infection to wild aquatic organism and its potential risks in adolescents *Candida* infection.

2. Materials And Methods

2.1 Candidalysin synthesis.

The candidalysin peptide was synthesized from Shanghai Top-Peptide Biotechnology Co.Ltd. (China) and stored in -20 ℃ until use. The amino acid sequence of candidalysin is as follows: SIIGIIMGILGNIPQVIQIIMSIVKAFKGNK.

2.2 Maintenance of Zebrafish.

Blastula-stage fish embryos (Danio rerio, AB strain), at 2 – 3 hours post fertilization (hpf), were randomly placed into 100 mL covered glass beakers with 50 mL standard E3 medium (containing 5 mM NaCl, 0.17 mM KCl, 0.33 mM MgSO4 and 0.33 mM CaCl2) with a conductivity between 500–550 μS/cm and the pH value between 6.8–7.5. The temperature was set at 28 ± 1 ℃. The photoperiod was set at 14:10 h light/dark.

2.3 Exposure to candidalysin.

The exposures were performed as described previously with some modifications (Zhao et al. 2016; Zhao et al. 2015). For acute exposure, zebrafish embryos at 48 hours post fertilization (hpf) were employed. The experimental setup of high-dose acute exposure was consisted of one solvent control and six exposure groups of candidalysin at concentrations of 5, 10, 20, 40, 80, and 160 mg/L for the evaluation of embryo survival rates and swimming behavior (swim bouts per minutes). Each treatment consisted of eight replicates. Survival rate and swimming behavior of embryos at 72 hpf were examined subsequently under a stereomicroscope (Nikon, SMZ745T).

For low-dose chronic exposure, blastula-stage embryos, at 2–4 hpf, were randomly placed in 100 mL covered glass beakers containing 50 mL of reconstituted water at 27 ± 1°C with the appropriate concentration of candidalysin. The experiment was consisted of one solvent control (0.1% DMSO) and five concentrations of candidalysin at concentrations of 0.008, 0.04, 0.2, 1, and 5 mg/L for evaluation of spontaneous contraction, hatching rate and cardiovascular functions of embryos. Every 24 hours, embryonic development status was checked and dead embryos were removed. The standard E3 medium was completely changed every day with freshly prepared E3 medium with appropriate candidalysin concentrations. Spontaneous contraction of embryos at 24 hpf, hatching rate at 48 hpf, heartbeat rate at 56 hpf, and survival rate of embryos at 72 hpf were examined as previously described (Zhang et al. 2020). Spontaneous contraction was checked under a stereomicroscope (Nikon, SMZ745T), while the cardiac morphology (heartbeat rate) was measured via a high-speed digital camera (Zyla 5.5, Andor, Belfast, UK) mounted on an upright microscope (Eclipse Ti2-E, Nikon, Japan) as described in the previous study (Zhao et al. 2016; Zhao et al. 2015).

2.4 Measurement of Cardiac Physiology.

The cardiac function evaluation was performed as described previously (Zhang et al. 2020). Individual embryo was positioned on a glass depression slide in a lateral position and acclimated to the microscope illumination for 10 seconds. Digital video recordings of embryonic heart section (20 seconds) were captured with a high-speed digital camera (Zyla 5.5, Andor, Belfast, UK) at 30 frames per second (fps). The digital video analysis for heartbeat rate was done with Image J (NIH, Bethesda, USA; https://imagej.nih.gov/ij/).

2.5 Data Analysis and Statistics.

The significance of differences between the control and candidalysin-exposed embryonic developmental parameters were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's corrected post-hoc comparisons via SPSS software (Version 20., Chicago, IL, USA). Before running the ANOVA, data were examined for normality and homogeneity of variances via Shapiro-Wilk and Levene's tests. Results were given as mean ± standard deviation (SD), and differences were considered significant when $p < 0.05$.

3. Results

Previous studies showed that the cytolytic peptide, candidalysin (Fig. 1), can induce tissue damage in an adult zebrafish swimbladder model of mucosal infection (Moyes et al. 2016; Ho et al. 2019). However, the effect of candidalysin on the embryonic development of zebrafish remains unknown. Therefore, in this study, we performed an independent embryonic exposure to evaluate the impact of candidalysin.

3.1 High-concentration of candidalysin causes mortality and changes of the swimming behavior of embryonic zebrafish.

High exposure concentrations of candidalysin (80 and 160 mg/L) resulted in embryo death (Fig. 2a). The EC50 was at 49.5 mg/L. The Swimming behavior of zebrafish was significantly changed after candidalysin exposure at 72 hpf. As the concentration of candidalysin increased, the swim bout frequency of embryos increased. For instance, candidalysin dramatically increased the swim bout frequency of embryos by 4.57, 3.86 and 9.14 times compared to controls at concentrations of 10, 20, and 40 mg/L, respectively, suggesting neurotoxicity occurred (Willi et al. 2018) (Fig. 2b). These results suggest that candidalysin exposure induces significant behavioral effects caused by damaging the fish embryo neural development.

3.2 Low-concentration of candidalysin affects embryonic development.

During the 56 h low-dose chronic exposure period, the spontaneous contraction, hatching success and heartbeat rate were evaluated sequentially. There were no remarkable alterations of survival rate observed in groups exposed to increasing concentrations of candidalysin at 24 hpf (Fig. 3a). However, developmental changes were identified for candidalysin. Candidalysin led to a dose-dependent (except 0.2 mg/L) decrease in spontaneous contractions of embryonic zebrafish at 24 hpf. Among them, high concentration (1 mg/L and 5 mg/L) of candidalysin induced significantly decreased spontaneous contractions by 1.85 and 2.53 times, respectively (Fig. 3b). Surprisingly, the hatching rate at 48 hpf was evidently decelerated after exposure of relatively low concentration of candidalysin (0.008 mg/L). At this time, the hatching rate was 73.9%, while 92.2% hatched in the control group ($p = 0.0148$). (Fig. 4a). Similarly, at 56 hpf, zebrafish embryo heartbeat rates were significantly decreased even at 0.008 mg/L candidalysin, with ranged down about 5.5% ($p < 0.0001$) (Fig. 4b).

4. Discussion

C. albicans is an opportunistic pathogen whose association with human disease is well documented (Ho et al. 2021). Colonization with *Candida* is common in human, even in premature infants (Bliss et al. 2012). The dietary habits and other environmental exposures can potentially shape microbial composition, and might affect the susceptibility of *C. albicans* infection in adolescents. *C. albicans* is widely isolated from different aquatic environments, and has already been advised as a fecal microorganism indicator for aquatic environments (Buck 1977). Candidalysin is a recently described cytolytic peptide toxin exclusively secreted by the pathogenic hyphal forms of *C. albicans*, contributing to mucosal tissue damage and the establishment of systemic infection (Hanaoka and Domae 2021). Therefore, candidalysin is not yet well investigated and should be further examined for its environmental occurrence, fate in the environment, and its effects to aquatic microbiota.

Environmental chemical exposure is strongly linked to neurodevelopmental impact. Zebrafish (*Danio rerio*) is one of the most popular vertebrates used in environmental toxicants identification due to its rapid life cycle, small size, rapid development, features like external fertilization and embryonic transparency. The analysis based on the behavior profile of zebrafish at embryonic stage can evaluate the impacts of

environmental exposure (Zhang et al. 2021). Here, we use zebrafish larvae exposure model, which might reflect candidalysin's effect in Candida infection in adolescents.

The exposure of low and high concentration of candidalysin reflects chronic and acute exposures respectively, which are representative factors of the risk of candidalysin. In this study, effects of low and high concentration of candidalysin were both investigated, as low concentrations of candidalysin represented environmentally realistic doses' effect on embryonic zebrafish (Christen et al. 2010), while high concentrations were chosen as pharmacologically relevant. We demonstrated that exposure of zebrafish embryos to low-concentrations of candidalysin affected physiological end points, including decreased spontaneous contraction, heartbeat rate and hatching rate. Spontaneous contraction of zebrafish is initiated and regulated by the embryonic neural circuity within the spinal cord and normally serves as an important indicator for neural developmental effects. The decreased spontaneous contractions pointed out the potential neurotoxic effects of candidalysin which needs further exploration. The hatching rate decreased significantly while stimulated by relatively low-dose of candidalysin (0.008 mg/L), suggesting that candidalysin is a strong candidate imposing stress on embryonic development at low concentrations. Furthermore, the heartbeat and hatching success showed similar decreases at concentration as low as 0.008 mg/L, indicating that candidalysin could impose stress on embryonic development at relatively low concentrations.

Embryonic zebrafish showed increasing swimming frequency when stimulated by high-concentration of candidalysin at 72 hpf. The swimming behavior (bout per min) of 72 hpf embryos showed a significant increase at 10 mg/L, and displayed dose-dependent pattern (except 20 mg/L). This was possibly because of the imposed stress on embryonic zebrafish by high doses of candidalysin, which affected the fitness of the embryos and consequently behavior and survival of embryos. The embryos were dead when stimulated by 80 mg/L and 160 mg/L of candidalysin, which prompted its ecotoxicological risk. However, this current research provides one perspective on the effectiveness of candidalysin on embryonic zebrafish. There is an urgent need to further analyze the consequences of candidalysin in additional aquatic organisms, and forthcoming studies is needed within this field to elucidate the molecular mechanisms underlying the developmental disruptions.

In the present study, significant effects of candidalysin including decreased spontaneous contraction, hatching rate, heartbeat rate, and increased swimming frequency were observed in embryonic zebrafish. In addition, candidalysin treatment leads to embryo death at relatively high concentrations. Overall, the present study demonstrates that candidalyin can impair the development of embryonic zebrafish and provides insights into unexpected and potentially environmental hazard relevant health impacts of candidalysin on aquatic organisms, but also be important for humans (especially adolescents), as *C. albicans* is an important human fungal pathogen.

Declarations

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Conflicts of interest

The authors declare that there is no conflict of interest.

Availability of data and material

Not applicable.

Code availability

Not applicable.

Authors' contributions

Not applicable.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

References

1. Chanda A, Gummadidala PM, Gomaa OM (2016) Mycoremediation with mycotoxin producers: a critical perspective. Appl Microbiol Biotechnol 100:17–29. https://doi.org/10.1007/s00253-015-7032-0

2. Lohse MB, Gulati M, Johnson AD, Nobile CJ (2018) Development and regulation of single- and multi-species Candida albicans biofilms. Nat Rev Microbiol 16:19–31. https://doi.org/10.1038/nrmicro.2017.107
3. Alter SJ, McDonald MB, Schloemer J et al (2018) Common Child and Adolescent Cutaneous Infestations and Fungal Infections. Curr Probl Pediatr Adolesc Health Care 48:3–25. https://doi.org/https://doi.org/10.1016/j.cppeds.2017.11.001
4. Bliss JM, Wong AY, Bhak G et al (2012) Candida Virulence Properties and Adverse Clinical Outcomes in Neonatal Candidiasis. J Pediatr 161:441–447. .e2 . https://doi.org/https://doi.org/10.1016/j.jpeds.2012.02.051
5. Xiao J, Huang X, Alkhers N et al (2018) Candida albicans and Early Childhood Caries: A Systematic Review and Meta-Analysis. Caries Res 52:102–112. https://doi.org/10.1159/000481833
6. Barousse MM, Van Der Pol BJ, Fortenberry D et al (2004) Vaginal yeast colonisation, prevalence of vaginitis, and associated local immunity in adolescents. Sex Transm Infect 80:48 LP – 53. https://doi.org/10.1136/sti.2002.003855
7. Xu L, Hu Z, Yu F, Tang Y (2020) Analysis of characteristics of vulvo-vaginal infections in 14- to 18-year-old girls in late puberty. J Int Med Res 48:0300060520946506. https://doi.org/10.1177/0300060520946506
8. Moyes DL, Wilson D, Richardson JP et al (2016) Candidalysin is a fungal peptide toxin critical for mucosal infection. Nature 532:64–68. https://doi.org/10.1038/nature17625
9. Ho J, Camilli G, Griffiths JS et al (2021) Candida albicans and candidalysin in inflammatory disorders and cancer. Immunology 162:11–16. https://doi.org/https://doi.org/10.1111/imm.13255
10. Ho J, Yang X, Nikou S-A et al (2019) Candidalysin activates innate epithelial immune responses via epidermal growth factor receptor. Nat Commun 10:2297. https://doi.org/10.1038/s41467-019-09915-2
11. Chu H, Duan Y, Lang S et al (2020) The Candida albicans exotoxin candidalysin promotes alcohol-associated liver disease. J Hepatol 72:391–400. https://doi.org/https://doi.org/10.1016/j.jhep.2019.09.029
12. Zhao Y, Zhang K, Fent K (2016) Corticosteroid Fludrocortisone Acetate Targets Multiple End Points in Zebrafish (Danio rerio) at Low Concentrations. Environ Sci Technol 50:10245–10254. https://doi.org/10.1021/acs.est.6b03436
13. Zhao Y, Castiglioni S, Fent K (2015) Synthetic Progestins Medroxyprogesterone Acetate and Dydrogesterone and Their Binary Mixtures Adversely Affect Reproduction and Lead to Histological and Transcriptional Alterations in Zebrafish (Danio rerio). Environ Sci Technol 49:4636–4645. https://doi.org/10.1021/es505575v
14. Zhang K, Yuan G, Werdich AA, Zhao Y (2020) Ibuprofen and diclofenac impair the cardiovascular development of zebrafish (Danio rerio) at low concentrations. Environ Pollut 258:113613. https://doi.org/https://doi.org/10.1016/j.envpol.2019.113613

15. Willi RA, Faltermann S, Hettich T, Fent K (2018) Active Glucocorticoids Have a Range of Important Adverse Developmental and Physiological Effects on Developing Zebrafish Embryos. Environ Sci Technol 52:877–885. https://doi.org/10.1021/acs.est.7b06057
16. Buck JD (1977) Comparison ofin Situ andin Vitro survival ofCandida albicans in seawater. Microb Ecol 4:291–302. https://doi.org/10.1007/bf02013273
17. Hanaoka M, Domae E (2021) IL-1α released from oral epithelial cells upon candidalysin exposure initiates an early innate epithelial response. Int Immunol 33:161–170. https://doi.org/10.1093/intimm/dxaa070
18. Zhang K, Liang J, Brun NR et al (2021) Rapid Zebrafish Behavioral Profiling Assay Accelerates the Identification of Environmental Neurodevelopmental Toxicants. Environ Sci Technol 55:1919–1929. https://doi.org/10.1021/acs.est.0c06949
19. Christen V, Hickmann S, Rechenberg B, Fent K (2010) Highly active human pharmaceuticals in aquatic systems: A concept for their identification based on their mode of action. Aquat Toxicol 96:167–181. https://doi.org/https://doi.org/10.1016/j.aquatox.2009.11.021

Figures

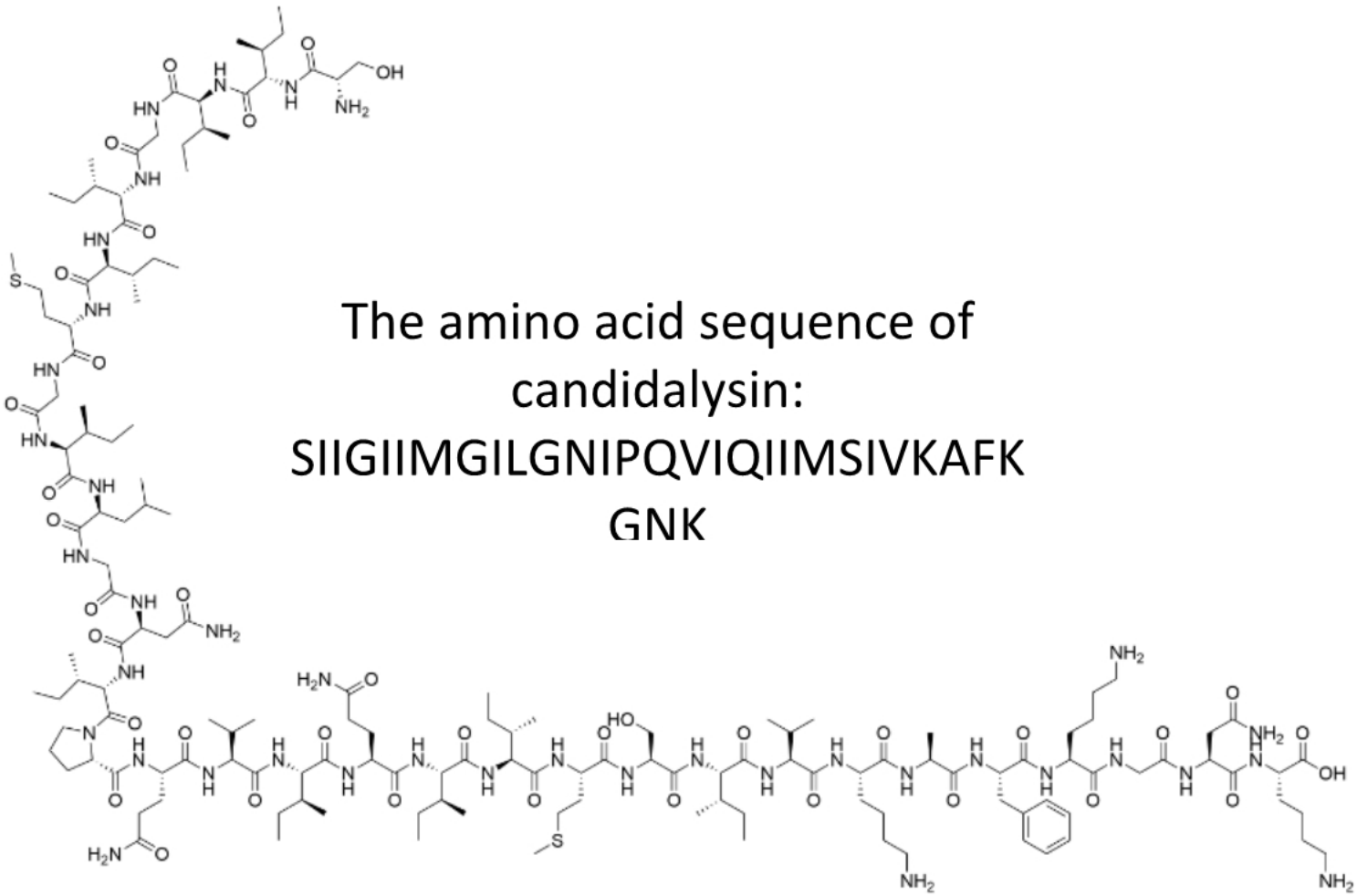

Figure 1

Molecular formula of candidalysin.

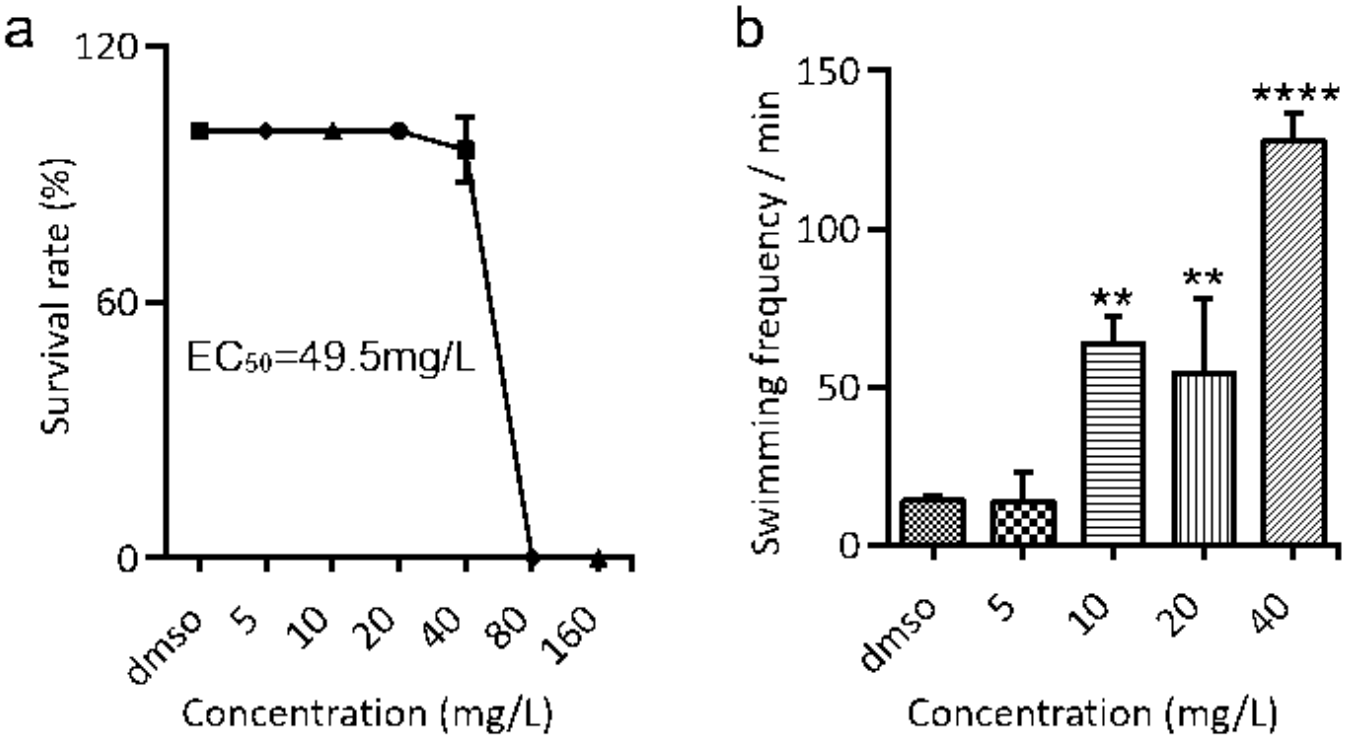

Figure 2

Survival rate and swimming behavior of zebrafish embryos exposed to high concentration of candidalysin. a) Survival rates of 72 hpf zebrafish embryos upon different concentrations of candidalysin exposures. b) Swimming behavior (swim bouts per minute) of 72 hpf zebrafish embryos exposed to different concentrations of candidalysin. Each bar represents mean value ± SD of three replicates. Each data represents the mean value ± SD of three independent replicates. In all panels: **, $p < 0.01$; ****, $p < 0.0001$.

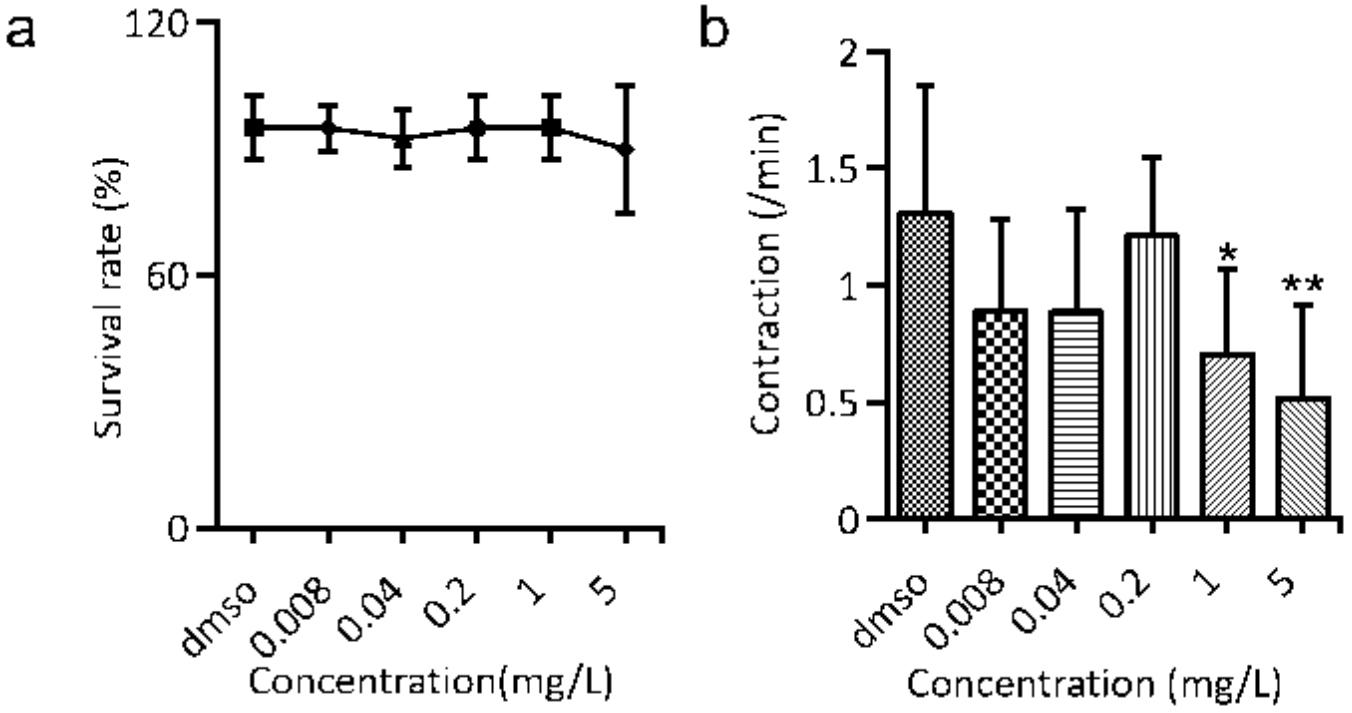

Figure 3

Survival rate and contractions of zebrafish embryos exposed to low concentration of candidalysin. a) Survival rate of 24 hpf zebrafish embryos exposed to increasing concentration of candidalysin. b) Spontaneous contractions of 24 hpf zebrafish embryos exposed to candidalysin at different concentration.

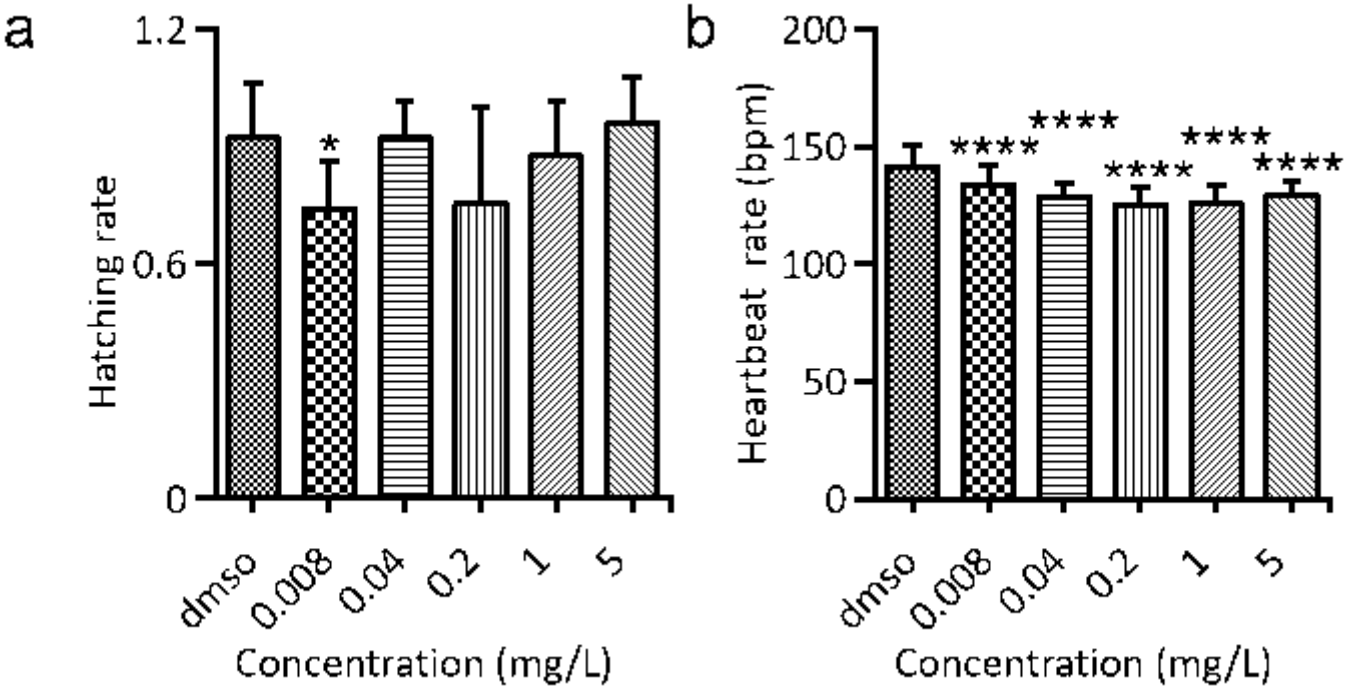

Figure 4

Hatching rate and heartbeat rate of zebrafish embryos exposed to low concentration of candidalysin. a) Hatching rate of 48 hpf zebrafish embryos upon different concentrations of candidalysin exposures. b) Heartbeat rate (bpm) of 56 hpf zebrafish embryos upon different concentrations of candidalysin exposures. Each data represents the mean value ± SD of eight replicates. In each group: control (n= 41), 0.008 mg/L (n= 51), 0.04 mg/L (n= 58), 0.2 mg/L (n= 52).1 mg/L (n=46), 5 mg/L (n=53). Data represents as mean value ± SD. In all panels: *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.0001$.