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EXPERIMENTAL STUDY

Zebrafish model for assessing induced organ toxicity by Strychnos nux-vomica

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Abstract

OBJECTIVE: To assess the acute organ toxicity of Strychnos nux-vomica with zebrafish model visually.

METHODS: To assess acute toxicity, we initially determined the lethal concentration after Strychnos nux-vomica treatment for 24 h. Zebrafish was treated with five concentrations ≦ LC10 for 24 h, and the effects of Strychnos nux-vomica on morphology, function of heart, central nervous system, liver, kidney and organ-specific cell death were assessed. Next, we assessed the reversibility of toxic effect.

RESULTS: Strychnos nux-vomica has an effect on the different organs of zebrafish, including heart, central nervous system, liver, and kidney, and cadiotoxicity induced by Strychnos nux-vomica was reversible to some extent.

CONCLUSION: Zebrafish model is suitable for confirming the toxic target organs for Chinese traditional medicine.

Key words: Zebrafish; Strychnos nux-vomica; Toxicity

INTRODUCTION

Strychnos nux-vomica, in the family Loganiaceae, is dry mature seeds of Strychnos nux-vomica Linn. It has a long history as medicine and is widely used to treat various diseases, including rheumatoid arthritis and arthralgia. The Chinese Pharmacopoeia 2010 Edition records that it is warm, bitter and poisonous,[1] distributed to liver and spleen channels. It has the effect of treating swelling, inflammation, exciting central nervous system, and alleviating pain. However, its therapeutic use is often hindered by problems such as its strong toxicity.[2] Therefore, it is important to strengthen the research on the compatibility of Strychnos nux-vomica, explore its effective compatibility, decrease its toxicity and increase its efficacy.

Recently, it has widely proved that zebrafish is an inexpensive and alterative model for the rapid evaluation of the potential toxicity for drug candidates since it shares the physiological, morphological, and histological similarities with mammals and human.[3,4] The gene homology between zebrafish and humans is as high as 85%. In signal transduction pathways and at the protein level,

the functional domains are highly conserved or structurally identical.[4,5] Compared with the traditional *in vivo* model like mouse and *in vitro* cell culture model, transparent zebrafish embryos offer unique advantages for assessing the drug effects on various developmental events because multiple organs can be observed under the microscope.[6] As a consequence, pharmacodynamic, pharmacokinetic, and metabolite activities can be evaluated easily.[7] In addition, low cost, short cycle, fewer test compounds, and high throughput also make it a promise and successfully used model in studying developmental toxicity,[8] teratogenicity, cardiovascular toxicity,[9] liver toxicity,[10] behavioral toxicity,[11] kidney toxicity,[12] and a series of evaluation assays.[13,14]

In this study, the acute toxicities and organ toxicity induced by Strychnos nux-vomica was assessed in zebrafish. The zebrafish model was found to be an alternative or even better predictive toxicity model compared with conventional system in Strychnos nux-vomica safety assessment.

METHODS AND MATERIALS

Zebrafish breeding and handling

Embryos were generated by natural pairwise mating as described in The Zebrafish Book[15] (Westerfield, 1993). Embryos were maintained at 28.5 ℃ in fish water (0.137 mol/L NaCl, 5.4 mmol/LKCl, 0.25 mmol/L Na_2HPO_4, 0.44 mmol/L K_2HPO_4, 1.3 mmol/L $CaCl_2$, 1.0 mmol/L $MgSO_4$, and 4.2 mmol/L $NaHCO_3$ (pH 7.0-7.2, conductivity 500-750 μs/cm)). Embryos were cleaned (dead and unfertilized embryos removed) and staged at 4 h post fertilization (hpf). Because embryos received nourishment from an attached yolk sac, no feeding was required for 7 days post fertilization (dpf).

Plant material and extraction

Strychnos nux-vomica was supplied blinded by the Anguo Shengshan Pharmaceutical Co., Ltd., (Anguo, China). Smashed into powders of 25.4 mm, the Strychnos nux-vomica (50 g) was extracted with distilled water ten-times volume of weight by refluxing for two h. After the crude extract was filtered, the supernatant was collected for further procedure, and the residue was successively extracted with distilled water ten-times volume of weight under reflux twice, once an hour. The water extract was mixed, centrifuged (3000 × *g*) for 30 min (RJ-LDL-50G, Wuxi Ruijiang Analysis Instrument Co., Ltd., Wuxi, China) and the supernatant was concentrated under reduced pressure using a vacuum rotary evaporator. After that, the water separation was collected and dried under vacuum at room temperature. The dried extract was kept in a desiccator until it was used. Prior to the following biological assays, the whole extract was re-dissolved into the stock solution (400 μg/mL) with fisher water, and then diluted to the working concentrations.

Drug treatment

Different organs of zebrafish form at different stages. To ensure comparability for assessing drug-induced lethality and acute toxicity, central nervous system (CNS) and heart were assessed after treating 2 dpf zebrafish for 24 h, and liver and kidney were assessed after treating 4 dpf zebrafish for 24 h. For each concentration, 20 zebrafish were distributed into 24-well plates containing 2 mL sterile fish water, each well two larva fish. During early embryogenesis, a protective chorion membrane, which might interfere with compound uptake, was present. Therefore, to facilitate drug delivery, 2-dpf zebrafish were de-chlorinated using a 0.5 mg/mL of protease solution (P6911, Sigma-Aldrich Co., St. Louis, MO, USA) for 15 min at room temperature. 2-dpf or 4-dpf zebrafish were then incubated with compounds for 24 h. For each experiment, untreated zebrafish were used as assay control. If > 10% of embryos in the untreated control group were dead or malformed, the experiment was considered invalid and aborted.

Lethality assessment

To assess lethality, 10 concentrations were initially tested: 0.01, 0.5, 0.1, 0.5, 1, 5, 10, 50, 100, and 400 μg/mL. Based on the preliminary experiment, we set up six concentrations at last. To establish lethality curves, after treatment for 24 h, surviving zebrafish exposed to different concentrations were counted, and dead embryos were removed since dead zebrafish can disintegrate, impeding counting. Experiments were performed three times. LC_{50} and LC_{10} were calculated using logistic regression analysis (SPSS Statistics for Windows, Version 17.0., SPSS Inc., Chicago, IL, USA).

Assessment of organ morphology / function toxicity

Six concentrations ≦ LC10, determined in lethality studies, were used to assess organ-specific toxicity. Drug treatment was performed as described above. After heart rate and circulation were assessed, zebrafish were anesthetized with 0.5 mm M3-aminobenzoic acid ethyl ester (MESAB-22, Sigma-Aldrich Co., St. Louis, MO, USA) and visually assessed using a TE-2000-S-stereomicroscope (Nikon Chansn Instrument Co., Ltd., Beijing, China) equipped with a SPOT Insight digital camera. Three percent of methylcellulose (Sigma-Aldrich Co., St. Louis, MO, USA) was used to immobilize zebrafish to facilitate imaging. For different organs, the end points for organ morphology or function toxicity are different. Abnormal heart rate, tachycardia, arrhythmia, abnormal circulation, pericardial edema and abnormal heart chamber morphology were used for evaluation standard of cardiac toxicity, misshapen brain was for CNS toxicity, liver size and color was for hepatotoxicity, and fluid accumulation around the kidney, cyst formation, or trunk edema was for renal toxicity.