วารสารการแพทย์แผนจีนในประเทศไทย
Thailand Journal of Traditional Chinese Medicine
泰国中医药杂志

Original Article

Rhizoma Zingiberis alleviate the neurotoxicity of *Pinellia ternata*

Tan Lu[1,2], Kuang Qixuan[1], Guo Wenxiu[1], Li Xinai[1], Lei Lirong[1], Gong Leiqiang[1], Huang Lijun[3], Guo Dale[1], Wang Dong[3], Deng Yun[1]

[1] *State Key Laboratory of Characteristic Chinese Medicine Resource of Southwest China, School of Pharmacy, Chengdu University of Traditional Chinese Medicine, China*

[2] *Chongqing Dadukou District Traditional Chinese Medicine Hospital, China*

[3] *School of Basic Medical Sciences, Chengdu University of Traditional Chinese Medicine, China*

Abstract: Objective of this study was to evaluate the toxicity mitigation effect of Rhizoma Zingiberis on the neurotoxicity of Banxia (the tuber of *Pinellia ternata*). Methods: Using the zebrafish embryo stained with acridine orange and zebrafish behavioral evaluation system, the neurotoxicity of blank control group, Raw Banxia group (RB), Rhizoma Zingiberis processed Banxia group (RPB), and 6-gingerol processed Banxia (6-GPB) group were compared on the basis of the moving distance and speed as well as the degree and distribution of apoptotic cells. Results: The moving distance and average speed of zebrafish in the RB group were significantly higher than those in the blank control group ($p<0.001$), while the RPB groups and 6-GPB were lower than those of the RB group. Conclusion: Rhizoma Zingiberis can alleviate the neruotoxicity of Banxia and 6-gingerol can partly account for the alleviative effect.

Keywords: *Pinellia ternata*; zebrafish; behavioral analysis; neurotoxicity; 6-gingerol

Corresponding author: Wang Dong: wangdong@cdutcm.edu.cn;
Deng Yun: dengyun@cdutcm.edu.cn

Received 19 October 2021 Revised 20 November 2021 Accepted 27 November 2021

Introduction

The dry tuber of *Pinellia ternata* (Banxia) is a famous traditional Chinese medicine (TCM) which had been used to treat many diseases including excessive phlegm, headache, nausea and vomiting.[1] However, Banxia was listed as a toxic TCM by the Shennong Classic of Materia Medica (Shen Nong Ben Cao Jing).[2] For instance, Banxia was depicted can harm the throat due to its pungency. Nowadays, there are lots of reports about the neural, renal, and enceinte toxicities of Banxia.[2] Processing with Rhizoma Zingiberis is a useful way to alleviate the toxicity of Banxia, which had been depicted in Handbook of Prescriptions for Emergencies (Zhou Hou Bei Ji Fang).

As the behavioral profiling of larval zebrafish can reveal the conserved functions of

psychotropic molecules, larval zebrafish locomotor behavior assay can be used as an effective tool to estimate the toxicity alleviating effect of Rhizoma Zingiberis and its related compounds on Banxia.[3-7]

Recently, we have studied the toxic alleviation of Rhizoma Zingiberis. Larval zebrafish was applied to estimate the toxicity of raw Banxia, Rhizoma Zingiberis processed Banxia and 6-gingerol (a typical compound of Rhizoma Zingiberis) processed Banxia. The result revealed that 6-gingerol and Rhizoma Zingiberis processed Banxia showed a weaker toxicity to the behavior of larval zebrafish. In this paper, the details of alleviate effect of Rhizoma Zingiberis against Banxian-induced neurotoxicity were described.

Materials and Method

1. General experimental procedures

Zebrafish behavior was detected under a Noldus DanioScope (Noldus Information Technolog); the distribution of apoptotic neural cell of larval zebrafish was detected with a Leica M165 FC fully apochromatic corrected stereo microscope (Leica Microsystems); Banxia and Rhizoma Zingiberis processed Banxia was purchased from TongRenTang, 6-gingerol was purchased from Chengdu Must Biotechnology Co.,Ltd.; sodium carboxymethylcellulose (CMC-Na) was purchased from Dalian Meilunbio Co., Ltd.; dimethyl sulfoxide and acridine orange were purchased from Sigma.

2. Zebrafish Rearing and Embryo Collection

Zebrafish experimental operations were performed according to the National Institutes of Health Guide for the use and care of experimental animals and were approved by the animal experimentation ethics committee of Chengdu university of TCM. Wild AB zebrafish were supported independently by the zebrafish experimental platform maintained at 28.5˚C water (PH 7.2-7.5; conductivity 500-550μs/cm) under a 14 h light/ 10h dark cycle. 4-8 months old zealth zebrafish were paired to obtain zebrafish embryos and larvae.[5]

3. Preparation of samples

Raw Banxia (5.00 g) and Rhizoma Zingiberis processed Banxia (5.00 g) was powdered and refluxed with 125 mL methanol under 70˚C (three times, 2 h each time). The methanol solution was filtered was dried under vacuum to yield the extracts of raw banxia (RB) and Rhizoma Zingiberis processed banxia (RPB). 2.00 g raw Banxia was boiling with 6.75 mg 6-gingerol (the contents of gingerols in Rhizoma Zingiberis is about 0.96-2.96 %)[8] under 50 mL water twice (2 h each time), the water was filtered was dried under vacuum to yield the extracts of 6-gingerol processed Banxia (6-GPB). The extracts of RB, RPB and 6-GPB were dissolved with DMSO at the concentration of 20 mg/mL to yield samples for subsequent behavior and neurotoxic assays.

4. Neurotoxicity of larvae zebrafish

Forty-five healthy 1 dpf zebrafish larvae were divided into four group (fifteen zebrafish larvae each group) in a 24-well plate, and treated with 5 mL culture solution which containing 40 μg/mL each sample [blank group (40 μg/mL DMSO), RB group, RPB group, and 6-GPB group] in 5 min. After 24 h treatment, zebrafish larvae was dyed with 2 μg/mL acridine orange about 20 min and then washed three times with culture solution. Finally each zebrafish larvae was anaesthetized with tricaine and immobilized on a slide with 1% CMC-Na. The fluorescence intensity of the brain and spinal cord of zebrafish larvae was observed and photographed under a fluorescence microscope. ImageJ software was used to obtain the IOD of fluorescence intensity.

5. Behavior analysis of larvae zebrafish

Forty-five healthy 6 days post fertilization (dpf) zebrafish larvae were divided into four

group (fifteen zebrafish larvae each group) in a 6-well plate, and treated with 5 mL culture solution which containing 40 μg/mL each sample [blank group (40 μg/mL DMSO), RB group, RPB group, and 6-GPB group] in 5 min. Afterwards, each zebrafish larvae was transfer to a 96-well plates, and each well was added with 100 uL culture solution containing sample at a concentration of 40 μg/mL. Finally, the 96-well plate was monitored with a Noldus DanioScope. The behavior of larvae zebrafish was recorded 20 min after first two adaptive minutes. Moving distance and speed of larvae zebrafish was analysis with an Ethovision XT software (Noldus Information Technology).

6. Statistical analysis

Data were presented as the mean±standard error of the mean (SEM) and analyzed using GraphPad Prism 7.0 software (San Diego, CA, USA). The differences between different groups were evaluated through an unpaired two-tailed Student's t-test; and p-value <0.05 was considered statistically significant.

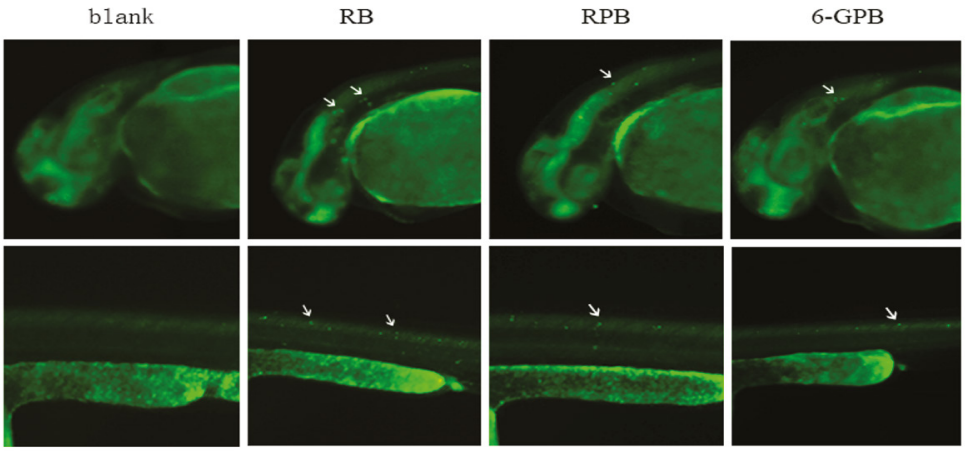

Figure 1. Distribution of apoptotic cells in 2 dpf embryos after administration of raw Banxia (RB), 6-gingerol processed Banxia (6-GPB) and Rhizoma Zingiberis processed Banxia (RPB)

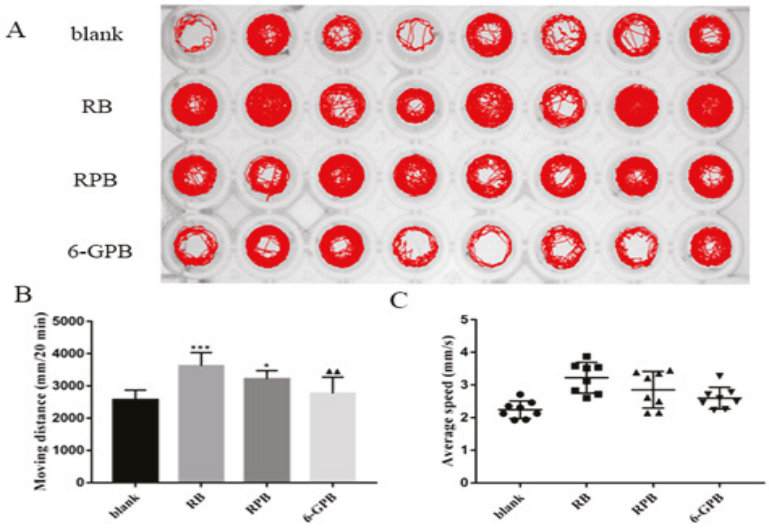

Figure 2. Effect of raw Banxia (RB), Rhizoma Zingiberis processed Banxia (RPB) or 6-gingerol processed Banxia (6-GPB) on the motility of zebrafish juveniles

Results and Discussion

Due to the fact that acridine orange can get through the death cell membrane and bind with the DNA of and then generate green fluorescence under 450 nm, zebrafish larvae with apoptotic cells always can generate a brighter fluorescence.[6] As shown in Figure 2, the intensity of fluorescence of zebrafish larvae in RPB group and RB group are stronger than the blank group which indicated the neurotoxicity of Banxia. The fluorescence of zebrafish larvae in 6-GPB and RPB group are weak than those of RB group also revealed the toxic-alleviation of Rhizoma Zingiberis.

Compared with healthy one, zebrafish larvae with nerve injury always have an abnormal behavior.[7] For example, those who have apoptotic cells always move more strenuous. As we can see from Figure 1, compared with the zebrafish larvae in blank group, the moving distance Figure 2b) and speed (Figure 2c) of zebrafish larvae in RB group, RPB group, and 6-GPB group was improved, revealing the neurotoxicity of Banxia. Compared with the PB group, the moving distance (Figure 2b) and speed (Figure 2c) of zebrafish larvae in RPB group, and 6-GPB group was declined.

This work indicated Rhizoma Zingiberis showed obvious neuroprotective effect against the raw Banxia induced apoptosis, and 6-gingerol might be the material basis and could partly account for the alleviative effect of Rhizoma Zingiberis.

Acknowledgments

This study was funded by the National Natural Science Foundation of China (81973460, U19A2011) and Project of First-Class Disciplines Development supported by Chengdu University of Traditional Chinese Medicine (CZYJC1905).

References

1. Chinese Pharmacopoeia Commission. Chinese pharmacopoeia. Beijing: The Medicine Science and Technology Press of China; 2020. p.123.
2. Zhong LY. Studies on irritant component of Pinelliae tuber and the attenuation mechanism and technology of processing. Nanjing: Nanjing University of Chinese Medicine; 2007. (In Chinese)
3. Rihel J, Prober DA, Arvanites A, et al. Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. Science. 2010;327(5963):348-51.
4 Wang YN, Hou YY, Sun MZ, et al. Behavioural screening of zebrafish using neuroactive traditional Chinese medicine prescriptions and biological targets. Sci Rep. 2014; 4(24):5311.
5 Westerfiled M. The zebrafish book. 4th ed. Eugene: University of Oregon Press, 2000.
6 Ninkovic J, Bally-Cuif L. The zebrafish as a model system for assessing the reinforcing properties of drugs of abuse. Methods. 2006; (39):262-74.
7 Carvan MJ, Loucks E, Weber DN, et al. Ethanol effects on the developing zebrafish: neurobehavior and skeletal morphogenesis. Neurotoxicol. Teratol. 2004;(26):757-68.
8 Jin YP. Study on the mechanism of gingerols antagonize the inflammatory effect of Pinella pedatisecta and *Pinellia ternata*. Nanjing: Nanjing University of Chinese Medicine; 2016

วารสารการแพทย์แผนจีนในประเทศไทย
Thailand Journal of Traditional Chinese Medicine
泰国中医药杂志

นิพนธ์ต้นฉบับ

ผลของขิงในการลดความเป็นพิษต่อระบบประสาทของปั้นเซี่ย

ถาน ลู่[1,2], ขว้าง ฉีเสวียน[1], กัว เหวินซิ่ว[1], หลี่ ซินอ้าย[1], เหลย หลีหรง[1], กง เหล่ยเฉียง[1], หวง ลี่จวิน[3], กัว ต้าเล่อ[1], หวาง ต้ง[3], เติ้ง ยวิน[1]

[1]ห้องปฏิบัติการหลักการแพทย์แผนจีนพิเศษภาคตะวันตกเฉียงใต้ คณะเภสัชศาสตร์ มหาวิทยาลัยการแพทย์แผนจีนเฉิงตู

[2]โรงพยาบาลการแพทย์แผนจีนฉงชิ่ง เขตต้าตู้โข่ว

[3]สถาบันการแพทย์แผนจีนพื้นฐานแห่งมหาวิทยาลัยการแพทย์แผนจีนเฉิงตู

บทคัดย่อ: บทความนี้มีวัตถุประสงค์เพื่อศึกษาผลของขิง (Rhizoma Zingiberis) ในการลดความพิษต่อประสาท ของตัวยาปั้นเซี่ย (ลำต้นใต้ดินของ *Pinellia ternata*) โดยประเมินจากตัวอ่อนของปลาม้าลาย (zebrafish) ที่ย้อมด้วย acridine orange พฤติกรรมของปลาม้าลาย ระยะทางการเคลื่อนที่ ความเร็ว ระดับและการกระจายตัวของการเกิดการตายของเซลล์แบบ apoptosis โดยเปรียบเทียบกลุ่มที่ได้ปั้นเซี่ย (RB) กลุ่มที่ได้ปั้นเซี่ยเผ้าจื้อกับขิง (RPB) และกลุ่มที่ได้ปั้นเซี่ย (RB) เผ้าจื้อกับสาร 6-gingerol (สารสำคัญในขิง) (6-GPB) ผลการศึกษาพบว่า กลุ่ม RB ปลาม้าลายจะมีการเคลื่อนที่และค่าเฉลี่ยของความเร็วสูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ($p<0.001$) ในขณะที่กลุ่ม RPB และกลุ่ม 6-GPB มีค่าน้อยกว่ากลุ่ม RB ผลการศึกษานี้สรุปได้ว่า ขิงมีส่วนช่วยลดความเป็นพิษต่อระบบประสาทของปั้นเซี่ยได้ และสารสำคัญคือ 6-gingerol

คำสำคัญ: ปั้นเซี่ย; ปลาม้าลาย; การประเมินพฤติกรรม; ความเป็นพิษต่อระบบประสาท; สาร 6-gingerol

ผู้รับผิดชอบบทความ: หวาง ต้ง: wangdong@cdutcm.edu.cn
เติ้ง ยวิน: dengyun@cdutcm.edu.cn

วารสารการแพทย์แผนจีนในประเทศไทย
Thailand Journal of Traditional Chinese Medicine
泰国中医药杂志

原创论文

姜制对半夏神经毒性的缓解作用

谭璐[1,2]，旷歧轩[1]，郭文秀[1]，李新爱[1]，雷力荣[1]， 龚雷强[1]，黄莉钧[3]，郭大乐[1]，王栋[3]，邓赟[1]

[1]成都中医药大学药学院西南特色中药国家重点实验室

[2]重庆大渡口区中医院

[3]成都中医药大学基础医学院

摘要：本研究为评估姜制对半夏神经系统方面的毒性缓解作用。方法：设空白对照组、半夏组、姜半夏组及6-姜酚处理组评估其对斑马鱼行为学影响的比较实验。同时采用吖啶橙对斑马鱼胚胎整体染色，观察各组处理后斑马鱼胚胎内细胞凋亡程度和分布。结果：半夏组的斑马鱼移动距离和游动平均速度均显著高于空白对照组，而姜半夏组及6-姜酚处理组移动距离和游动平均速度均低于半夏组；空白对照组胚胎内没有出现凋亡细胞，半夏组和姜半夏组胚胎内出现凋亡细胞，姜半夏组及6-姜酚处理组相对于半夏组胚胎神经细胞凋亡的程度有所缓解。结论：半夏对神经系统有一定的毒性，姜制能缓解半夏在神经系统方面的毒性，姜酚类成分可能为生姜解半夏毒的物质基础。

关键词：半夏；斑马鱼；行为分；神经损伤；6-姜酚

通讯作者：王栋：wangdong@cdutcm.edu.cn
邓赟：dengyun@cdutcm.edu.cn