Rongchang and Xifeng capsules suppress pentylenetetrazole-induced seizures and change rest/wake behavior in zebrafish larvae

Zhang Shuhui, Xu Jia, Wang Chao, Feng Daofu, Dai Liangti, Kuang Xiangyu, Zhu Shuyi, Fang Yongchun, Chen Dongyan, He Xianghui, Feng Xizeng

OBJECTIVE: To evaluate the effects of Rongchang capsule and Xifeng capsule on pentylenetetrazole-induced epilepsy in zebrafish larvae and to explore the possible mechanisms behind their actions.

METHODS: We utilized a trajectory tracking system to monitor seizures in zebrafish larva to confirm that certain concentrations of Rongchang capsule and Xifeng capsule produce antiepileptic effects. c-fos expression was assessed by quantitative reverse transcription-polymerase chain reaction to validate the efficacy of the capsules. Rest/wake behavior and correlation analysis predicted the targets of Rongchang capsule and Xifeng capsule.

RESULTS: Larval movement times and total distances traveled by zebrafish larvae experiencing pentylenetetrazole (PTZ)-induced seizures were decreased by valproate treatment. Rongchang (500 μg/mL) and Xifeng (200 μg/mL) rescued the epileptic behaviors and down-regulated c-fos expression in the brains of larvae, which indicated antiepileptic effects. The rest/wake behavioral profiles showed that Rongchang and Xifeng differentially decreased rest time at night and increased larval locomotor activities during the day. Based on correlation between the actions of the two capsules and known compounds, we predicted that they might change rest/wake behaviors by affecting serotonin, GABAergic and histamine signaling pathways.

Abstract
CONCLUSION: The efficacy of Rongchang capsule and Xifeng capsule in alleviating epilepsy-like behaviors and molecular responses was confirmed. Our study provides insight into the capsules' effect on epilepsy.

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Keywords: Zebrafish; Larva; Anticonvulsants; Rongchang capsule; Xifeng capsule; Rest/wake behavior

INTRODUCTION

Epilepsy is one of the most widespread neurological diseases that affects 1%-2% of the world’s population.1,2 Epilepsy is usually characterized by recurrent seizures attributed to excessive and hypersynchronous electrical discharges of brain nerve cells, which cause damage to the brain. In addition, excessive glutamatergic and/or γ-aminobutyric acid (GABAergic) function results in a disrupted ratio between excitatory and inhibitory neurotransmission, which can lead to the initiation of seizures.3 However, the mechanism of epilepsy is complicated and not completely understood. As a result, epileptic seizures can usually be controlled but not cured with available antiepileptic drugs (AEDs). Epilepsy can occur at any age. Pediatric epilepsy is one of the most common chronic nervous system problems in childhood. The possibility of developing intractable seizures and the side effects of some AEDs affect the quality of life of epileptic patients. For example, valproic acid (2-n-propylpentanoic acid, VPA) is a classical antiepileptic drug used to treat all forms of seizures. It inhibits the activities of histone deacetylases and GABA transaminase, and increases mitochondrial adenosine-triphosphate (ATP) production, which reduces the production of phosphoinositides.4 The side effects of VPA include pancreatitis, teratogenesis and acute hepatic failure. Treatment of children and pregnant women with VPA is contraindicated.5 Therefore, many studies have focused on natural medicines, including Traditional Chinese Medicine (TCM) and other medicinal plants for the treatment of epilepsy. Compounds, such as ginkgotoxin, tanshinone A, steroid glycosides and Xifeng capsules have shown antiepileptic action.6-9 Animal models of epilepsy, especially rodent models, have been generated to investigate the pathogenetic mechanism underlying epileptic seizures and for the development of new antiepileptics.10-15 However, animal models are relatively expensive and are unsuitable for high-throughput screening of AEDs. Zebrafish seizure models are similar to the human condition with respect to neurotransmitter systems and stress axis organization. They are also easy to handle and relatively efficient for high-throughput in vivo screening.16 Quantitative reverse transcription-polymerase chain reaction (RT-PCR), whole-mount in situ hybridization and antibody staining allow the detection of specific DNAs, RNAs or proteins in the whole developing animal.17 Seizures can be induced in zebrafish embryos (2 d post fertilization) and larvae (4 or 7 d post fertilization) by pentylenetetrazole (PTZ, a commonly used experimental convulsant) by immersion in fish water containing PTZ, and can be ameliorated by administration of antiepileptic drugs, such as VPA.18,19,20 Larvae normally exhibit gently spontaneous movements and infrequent darting swimming, whereas those exposed to PTZ present typical locomotor patterns: dramatically increased activity and swimming speed, rapid swimming in a motion circular, switching, loss of posture and incongruous jerky movements. In addition, c-fos gene expression is upregulated.21-23 Zebrafish as young as 4 d post fertilization (dpf) have active and sleep-like states. Continuous tracking of larvae movement behaviors during light and dark cycles can be recorded by a camera and computer. High throughput screening for behavioral changes has identified new uses for poorly characterized compounds.24-26 Based on behavioral phenotypes and drugs known to act on specific targets, the mechanisms of action of unknown compounds and TCMs can be predicted.27

Rongchang capsule is a Chinese patent drug developed by Professor MA Rong. It was developed to alleviate the cognitive impairment caused by epilepsy and also to control the onset of epilepsy. Xifeng capsule has an antiepileptic effect, while Rongchang capsule is also beneficial for the development of cognitive function intelligence based on an antiepileptic action. The composition of Chinese patent drugs is complex and their means of production and the ratio of traditional Chinese medicine herbs are confidential. We initially investigated the anticonvulsant activities of Chinese patent drugs using a zebrafish model system. Rongchang capsule and Xifeng capsule are effective for treating pediatric epilepsy. In this article, we describe effects of the two capsules in an animal model of epilepsy, which support the curative effects of the two capsules. We based our anti-epileptic assay on a trajectory tracking system of zebrafish larval seizures. This system is highly accurate and easy to implement.28 According to Baxendale et al.,4 we employed a zebrafish larva seizure model using 20 mmol/L PTZ. We then confirmed that certain concentrations of Rongchang capsule and Xifeng capsule exhibited anti-epileptic effects comparable to VPA, including down-regulation of c-fos expression. The formulae for proprietary Chinese medicines are complicated. Therefore, delineating the mechanisms of action of the two capsules is a significant study. We therefore predicted the biological targets of the two capsules by combining the rest/wake behavioral phenotypes with profiles of previously described drugs.
MATERIALS AND METHODS

Zebrafish
Zebrafish (AB strain) were maintained in a fish-farming system. There was a constant temperature of 28.5 °C, a constant light cycle (14 h light/10 h dark) and circulating water (KCl 0.05 g/L, NaHCO₃ 0.025 g/L, NaCl 3.5 g/L, and CaCl₂ 0.1 g/L, pH 7.0-7.3). The zebrafish were fed freshly hatched brine shrimp twice daily. On the night before an experiment, a pair-wise spawning female and male zebrafish (1 year old) were transferred to a breeding tank with a sliding door. The next morning, the sliding door was removed to allow the male to pursue the female. Embryos (0 dpf) were collected 30 min later. Then normal fertilized eggs were raised in a standard system. The larvae (4 dpf) were used for experiments. All of the experimental protocols and procedures involving zebrafish were approved by the Committee for Animal Experimentation of the College of Life Science at Nankai University (No. 2008) and were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (No. 8023, revised in 1996).

Drug treatment and behavioral analyses
A capsule was opened and the contents ground into a dry powder. The powdered medicine was then dissolved in system water for 2 h to obtain a stock solution (1000 μg/mL). The stock solution was filtered to remove suspended material and to produce a homogeneous extract. The stock solution was diluted with system water to get the desired working concentrations (50-1000 μg/mL). VPA (valproic acid sodium salt) was dissolved in system water to obtain a stock solution (10 mmol/L). PTZ (Alfa Aesar) was dissolved in system water to 20 mmol/L. At 4 dpf, 12 larvae in each well and the desired solutions (Rongchang, Xifeng or 3 mmol/L VPA) were placed in 6-well plates and incubated away from light for 18 h. Larvae were then transferred to 48-well plates (one embryo per well). Ten min prior to trajectory tracking, the solution in each well was removed and PTZ (20 mmol/L) was added. In addition, at 9:00 am, larvae (4 dpf) were pipetted into each well of a 96-well plate in 360 μL of solution containing different dilutions of Rongchang and Xifeng. Zebrafish larval rest/wake behavior was observed beginning at 9:00 pm at 4 dpf and ending at 9:00 pm at 6 dpf.

Quantitative RT-PCR
Total RNA was extracted from zebrafish larvae using Trizol Reagent (CWBOI) according to the manufacturer’s instructions and stored at −80 °C. RNA was reverse-transcribed by reverse transcriptase (Promega) using oligo (dT) primers. Quantitative RT-PCR was performed using the SYBR Green Labeling System (Bio-Rad, Hercules, CA, USA). Conditions of qRT-PCR included a denaturing step at 95 °C for 2 min, 40 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s for real time monitoring, and a final extension at 72 °C for 5 min. Beta-actin was used for data normalization. Primer sequences were: beta-actin, Forward 5'-TGT-GGGCGTGGTGTGTTAGGATAC-3', Reverse 5'-CCGGATCTCGATGAGTCAGGGAA-3'; C-fos, Forward 5'-GAGAGGTGTTAGGTCTCATCC-3', Reverse 5'-GAAGGATCTCGATGATTGAT-3'. Each qRT-PCR was repeated three times.

Ultraprecision liquid chromatography (UPLC) and quadrupole time-of-flight mass spectrometry (Q-TOF-MS) analyses
The Rongchang capsule and Xifeng capsule were provided by the First Teaching Hospital of Tianjin University of Traditional Chinese Medicine (Tianjin, China). We opened the capsule and ground the contents into a dry powder. Then the powdered medicine was dissolved in 75% methanol for 2 h. The solution was then centrifuged (3913 g, 5 min) to remove suspended material and to yield a homogenous extract for UPLC and Q-TOF-MS analyses.

Data analyses
All experiments were repeated three times and had a control group (system water). The movement data were processed using Visual Studio 2005 (Microsoft) and Matlab R2011b (Math Works). The statistical analysis was conducted in Matlab R2011b (Math Works). All cluster diagrams were plotted with Microsoft Visio (Microsoft). A one-way analysis of variance was performed to analyze the effect of pharmacological concentration on activity level. Dunnert’s post hoc test was used to analyze multiple comparisons. Data are presented as the mean ± standard error of mean (SEM).

For correlation analysis among different capsule concentrations and known compounds, we calculated the behavioral parameters in Schier’s hierarchical diagram from the colors. In Schier’s hierarchical diagram, different colors represent different parameter values. The color value (Red, Green, Blue [RGB]) was (0, 0, 0) for the parameter of 0 in the color bar. We set the RGB values of parameters 3 (the yellow block on the right) and -3 (the blue block on the left) to (R₃, G₃, B₃) and (R₋₃, G₋₃, B₋₃), respectively, in the color bar. If the RGB value of the color block was (r, g, b) in the dendrogram, the corresponding parameter could be calculated by the following equation:  

\[
\text{para} = \begin{cases} 
1.5 \times \left( \frac{r}{R_{3}} + \frac{g}{G_{3}} \right) B_{3}, & B_{3} = 0 \\
-1 \times \left( \frac{r}{R_{3}} + \frac{g}{G_{3}} + \frac{b}{B_{3}} \right) B_{3}, & B_{3} \neq 0 
\end{cases}
\]

With these parameters, we performed correlation analysis and the correlation matrix was visualized using Matlab (Math Works).
RESULTS

Establishment of the experimental procedure and analysis of Rongchang and Xifeng capsules’ constituents

We utilized extracted solutions of Rongchang and Xifeng capsules contents to conduct anti-epileptiform assays and a rest/wake behavioral experiment. UPLC and Q-TOF-MS analyses were conducted for each capsule. The protonated [M+H]^+ or deprotonated [M-H] ions were obtained with as much characteristic fragment information as possible to deduce the molecular and elemental compositions of every constituent. The inferred chemical structures were compared with published data and reported natural product information. The electrospray ionisation-mass spectrometry (ESI-MS) spectra were acquired in both the positive and negative ion modes for each capsule (Figure 1B-E). Thirty compounds were identified in Rongchang capsule, including valine, 4-hydroxy-3-methoxybenzoic acid, citric acid, xanthine, L-pyrogulatamic acid, p-hydroxybenzoic acid, hyperoside, astragalin, hesperidin, 2,4,5-trimethoxybenzaldehyde, neohesperidin, chrysophanol, natsaudaidain, quercetin, neocuscutoside C, narin-3-hydroxybenzaldehyde, citric acid, methoxybenzoic acid, citric acid, xanthine, L-pyrogulatamic acid, p-hydroxybenzoic acid, hyperoside, astragalin, hesperidin, 2,4,5-trimethoxybenzaldehyde, neohesperidin, chrysophanol, natsaudaidain, quercetin, neocuscutoside C, narin-3-hydroxybenzaldehyde, citric acid, methoxybenzoic acid, citric acid, xanthine, L-pyrogulatamic acid, p-hydroxybenzoic acid, hyperoside, astragalin, hesperidin, 2,4,5-trimethoxybenzaldehyde, neohesperidin, chrysophanol, natsaudaidain, quercetin, neocuscutoside C, narin

Certain concentrations of Xifeng and Rongchang produce antiepileptic effects comparable to VPA

Five-dpf zebrafish larvae were pre-incubated with different treatments (system water, 3 mM VPA and Xifeng or Rongchang concentration gradient solutions) and then acute seizures were induced by 20 mM PTZ. Concentration of Xifeng and Rongchang ranged from 50 to 800 μg/ml. Due to the capsules’ bioactive properties, the effective concentration was different between the two. Too high or too low concentrations caused hyperactive movements. Representative 3D traces demonstrated the locomotor activity of one zebrafish larva (randomly selected from one treatment group) through the 10-min recording, where the x-axis represents the time-line of the recording and the x-y plane represents

Figure 1 Schematic diagram of the experimental procedure and ESI-MS spectra of Rongchang capsule and Xifeng capsule. A: capsule contents were ground to obtain an extracted solution. Zebrafish larvae (4 dpf) were co-incubated with different extracted solution dilutions for 18 h, and then individually pipetted into wells of a 48-well plate (in ~1.7 ml solution) for PTZ seizure induction and video tracking at 5 dpf. In addition, larvae (4 dpf) were pipetted into each well of a 96-well plate (in ~360 μl solution) with different dilutions of Rongchang and Xifeng at 9:00 am. Zebrafish larvae rest/wake behavior was observed beginning at 9:00 pm at 4 dpf. B: ESI-MS spectra of Rongchang capsule in the positive; C: negative ion voltage mode; D: ESI-MS spectra of Xifeng capsule in the positive; E: negative ion voltage mode.
the vertical view of a well. In the representative 3D traces (Figure 2A-E), the control group only displayed a few spontaneous movements during the recording period (Figure 2A), while PTZ-treated larvae exhibited constant hyperactive movements and swam in both the top and bottom halves of the well (Figure 2B). The application of VPA, 200 μg/mL Xifeng and 500 μg/mL Rongchang differentially alleviated PTZ-induced hyperactivities; 500 μg/mL Rongchang produced the best rescue effect (Figure 2C-E). Consistently, statistical analysis of the tracking results indicated an increase in locomotor behaviors caused by PTZ. During the 30-min recording period, the larval movement times and total distance traveled were significantly increased compared to the control. Treatment with VPA rescued the hyperactive locomotor behaviors caused by PTZ. Different Rongchang and Xifeng concentrations induce behavioral changes in larvae

We conducted a high-throughput, quantifiable approach to record larval locomotor activity after Rongchang and Xifeng capsule treatment. Preliminary

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<th>Movement times</th>
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<td>Control PTZ</td>
<td>VPA X50 X100 X200</td>
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<td>Control PTZ</td>
<td>VPA X50 X100 X200</td>
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Figure 2 Antiepileptic effects of Rongchang and Xifeng on PTZ-induced seizures

A-E: representative 3D traces (red lines) and locomotor traces (the top right corner: red lines) of larvae (5 dpf) during a 10-min recording period immediately after PTZ induction. Individual larvae were in wells of 48-well plates. F-G: locomotor behaviors in control, PTZ (20 mmol/L), VPA (3 mmol/L) + PTZ and Xifeng capsule + PTZ-treated larvae. Left: the number of spontaneous movements; Right: total swimming distances (cm). H-I: locomotor behaviors in control, PTZ (20 mmol/L), VPA (3 mmol/L) + PTZ and Rongchang capsule + PTZ-treated larvae. Left: the number of spontaneous movements; Right: total swimming distances (cm). Data represent the mean ± standard error of mean (SEM), n = 18; a represents significant differences from control (P < 0.05). b represents significant differences from PTZ (P < 0.05). J) Relative c-fos gene expression in treated zebrafish larvae. Data are the mean ± SEM, n = 40, a represents significant differences from control (P < 0.05). b represents significant differences from PTZ (P < 0.05). “R” and “X” represent “Rongchang capsule” and “Xifeng capsule”, respectively. For example, “X200” represents 200 μg/mL of the Xifeng capsule and “R500” represents 500 μg/mL of the Rongchang capsule.
experiments were performed to determine the appropriate concentrations of 50, 100, 200, 400, 600, 800 and 1000 µg/mL. The two capsules contain traditional Chinese medicines that influence sleep. Therefore, the total amount of rest, the number of rest bouts, rest bout length, total activity, and waking activity were used to assess the effects of the two capsules. The test was run from 96 hpf (h post fertilization) (4 dpf) to 144 hpf (6 dpf) and we obtained locomotor activity data of larval diel activity for 48 continuous hours. We applied clustering algorithms to perform hierarchical clustering and k-means clustering according to the shared behavior of the concentrations. Based on the concentration effects and the correlation between the concentration pairs, the clustering was performed using k values of 4. The clustering analysis revealed that the two capsules induced four different behavioral phenotypes (Figure 3A). Rongchang 600, 800 and 1000 µg/mL co-clustered into one category. These concentrations induced disturbances in sleep, increased activity on the first day and second night, and decreased activity on the second day (Figure 3B, C). Xifeng 1000 µg/mL increased wakefulness at night, especially the first night, and slightly decreased activities on the second day (Figure 3D, E). Rongchang and Xifeng at low concentrations (Rongchang 50-400 µg/mL and Xifeng 50-400 µg/mL) co-clustered into one class and induced similar behavioral changes. The waking activity and rest didn’t change significantly throughout the experimental period (Figure 3F, G). Xifeng 600 and 800 µg/mL selectively increased wakefulness and decreased rest during the first night and day, and it had little effect on the second night and day (Figure 3H, I).

To compare the difference for each capsule between the treated groups and the controls more clearly, we analyzed the behavioral fingerprints (Figure 4A, B). In addition, total rest, waking activity, number of rest bouts and rest bout length were quantitatively analyzed. After Xifeng capsule treatment, the total rest was significantly decreased at night and not changed during the day (Figure 4C). Waking activity and the number of rest bouts were significantly increased (Figure 4D, E). Xifeng decreased rest bout length during the rest time (Figure 4F). After Rongchang capsule treatment, the total rest was decreased at all concentrations except 1000 µg/mL on the last day (Figure 4G). Waking activity did not significantly change except during the first night (Figure 4H). The number of rest bouts was significantly increased for 100 µg/mL at night time and 600 µg/mL during the day time (Figure 4I). Except for the second day, rest bout length was significantly decreased during the rest time (Figure 4J).

Figure 3 Clustering analysis revealed the behavioral similarities between Rongchang and Xifeng capsule
A: K-means clustering analysis. The clustergram was divided into four sectors, each of which represents a similar behavioral phenotype. Each row represents a unique concentration of a certain capsule, and each column indicates a behavioral measurement. The black bars indicate the night measurements, and the white bars indicate the day measurements. The measurements were normalized as standard deviations from the controls. The red and green colors indicate that the values are higher and lower relative to controls, respectively. In this clustergram, “R” and “X” represent “Rongchang capsule” and “Xifeng capsule”, respectively. For example, “R600” represents 600 µg/mL of the Rongchang capsule and “X200” represents 200 µg/mL of the Xifeng capsule. B-I: waking activity and rest normalized to control values (system water treated). In the time series analysis, the red trace indicates the treated group, and the blue trace indicates the control group. The black and white bars represent the night and day measurements, respectively.
**Prediction of neural signaling pathways affected by Xifeng and Rongchang capsules based on behavioral changes**

To reveal the probable mechanisms associated with the behavioral changes induced by Xifeng and Rongchang capsules, we selected 76 compounds for correlation analysis according to Rihel et al.\(^{10}\) and Wang et al.\(^{24}\) Rihel et al.\(^{10}\) screened hundreds of psychoactive compounds and predicted the biological targets of some poorly characterized compounds. Wang et al.\(^{24}\) predicted the biological targets for seven antidepressant TCM prescriptions. Based on the correlation analysis of different concentrations of the two capsules and 76 compounds that have clear biological targets, we found that the two capsules had a high correlation with compounds that have known biological targets (Figure 5). The two capsules are likely to be associated with the serotonin (5-HT), adrenergic, histamine, GABA (gamma-aminobutyric acid) and glutamate signaling pathways. This correlation may be related to the capsule’s concentration. For example, high concentrations of Rongchang (600-1000 µg/mL) and Xifeng (600-1000 µg/mL) had a low correlation with SDZ220-581, 7-chlorokynurenic acid and L-689560, which affect the glutamate signaling pathway. Rongchang 400-600 µg/mL had a high correlation with UK14304 and guanfacine hydrochloride, which affect adrenergic signaling. In contrast, other Xifeng and Rongchang concentrations had low correlations with them.

**DISCUSSION**

At present, Western Medicine is the best treatment to control epileptic seizures, but the side effects of long-term medication and the dangers of epilepsy itself can cause serious damage to the development and cog-
nitive function of a child’s brain. However, Chinese medicine offers an alternative treatment for epilepsy. The dosage of Chinese medicine is important in epilepsy treatment. TCMs that have an antiepileptic effect can cause seizures at inappropriate concentrations. c-fos mRNA levels within the embryonic zebrafish brain are a robust in vivo indicator of the neural response to convulsant treatment and lends itself well to high-throughput chemical screening applications. In addition, suppression of PTZ-induced c-fos expression provides a sensitive approach of identifying compounds with antiepileptic activities. Rongchang capsule and Xifeng capsule exhibited concentration-related inhibition of seizure-like activity and PTZ-induced c-fos expression, confirming their antiepileptic effects. Rongchang capsule can effectively control the attack frequency and level of the epilepsy, and can improve learning and memory of epilepsy rats. It might inhibit mossy fiber sprouting and protect hippocampal neurons to improve learning and memory capacity. A clinical pediatric epilepsy study confirmed that Rongchang capsule, based on tonifying kidney and essence replenishment, has dual functions as an anti-epileptic and as a medicine that can increase cognitive function. In this study, 100 children with tonic-clonic seizures were randomly divided into groups and treated with Rongchang capsule and carbamazepine, respectively, for one year. In the respect of improving disease symptoms, the two treatments had similar effects. Rongchang capsule was superior to carbamazepine in improving syndrome of TCM. With respect to improving cognitive function, Rongchang capsule was superior to carbamazepine. Clinical testing of Xifeng capsule on 19 cases of benign childhood epilepsy with centro-temporal spikes showed that Xifeng capsule is as effective as carbamazepine in controlling seizures and improving electroencephalograms. In addition, the reduction of seizure frequency, duration and syndrome of TCM in 200 children with tonic-clonic seizures treated with Xifeng capsule were better than with phenobarbital. These clinical studies and our experimental results provide reliable evidence for effective treat-
ment of pediatric epilepsy with Rongchang capsule and Xifeng capsule. The relationship between epilepsy and sleep is complicated and bidirectional. Lack of sound sleep can induce seizures. In turn, seizures and antiepileptics likely disturb sleep architecture. Some AEDs can cause drowsiness or sleepiness while others may lead to insomnia. For example, first-generation AEDs, such as phenobarbital, phenytoin, barbiturates and benzodiazepines, tend to cause broken-night time sleep by increasing the number of arousals and stage shifts, and at the same time promote daytime sleepiness. VPA mainly increases waking activity and total activity in the daytime. In our study, Rongchang capsule and Xifeng capsule also changed the larval rest/wake behaviors. They caused insomnia in the night-time and increased locomotor activities in the daytime by increasing the number of rest bouts and decreasing rest bout length. But the influences on increased daytime activities caused by the two capsules are different. Xifeng significantly promoted larval diurnal activities while Rongchang did not. In addition, we investigated the correlation between Rongchang capsule and Xifeng capsule-induced changes in rest/wake behavior and neural signaling pathways. We chose 76 compounds associated with eight signaling pathways (adrenergic, serotonin, dopamine, GABAergic, melatonin, histamine, adenosine, and glutamate signaling) for correlation analyses with experimental concentrations of capsules. The capsules had high correlation with some compounds in some pathways, such as adrenergic, serotonin, GABAergic, histamine, and glutamate signaling. AEDs are not fully responsible for disturbance to sleep architecture, but understanding their impacts can help optimize treatment plans to prevent unnecessary worsening of sleep quality. For children, improving sleep efficiency may contribute to suppression of seizures and recovery from epilepsy, and also to brain development. In conclusion, appropriate concentrations of Rongchang capsule and Xifeng capsule suppressed PTZ-induced seizures with down-regulation of c-fos expression in zebrafish larvae. They also changed larval rest/wake behaviors in a similar manner to AEDs, probably by affecting neural signaling pathways. In conclusion, the efficacy of Rongchang capsule and Xifeng capsule was confirmed experimentally and new insights into the clinical treatment of epilepsy are suggested. However, we are unsure by which signaling pathway the capsules change rest/wake behavior. Our findings will assist in confirming the mechanism of action of these TCMs.

REFERENCES


