The Effect of Peony and Licorice Decoction on the Voltage-Gated Sodium Channel Subtype 1.4 Based on Standard Decoction

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Abstract

Objective: The objective of this study is to investigate the inhibitory effect of peony and licorice decoction and its compatibility components on the Nav1.4 voltage-gated sodium channels (VGSCs). Materials and Methods: Writhing test was carried out with ICR mice. Paeonia lactiflora and Glycyrrhiza uralensis group were administrated 0.2 ml of solution of freeze-dried powder dissolved in normal saline with the concentration of 2.94 mg/ml, 1.47 mg/ml, and 0.74 mg/ml using intragastric administration, respectively. Peony and licorice decoction groups were administrated 0.2 ml of solution of freeze-dried powder dissolved in normal saline with the concentration of 5.89 mg/ml, 2.94 mg/ml, and 1.47 mg/ml using intragastric administration, respectively. For electrophysiology studies, each freeze-dried powder was dissolved in DMSO to make 10 mg/ml and 50 mg/ml stock solutions. The electrophysiological recordings were obtained under visual control of a microscope. For UPLC analysis, the freeze-dried powder was dissolved in methanol and then determines the contents of the nine marker compounds.

Results: The effect of G. uralensis on incubation period and writhing frequency was significantly better than that of peony and licorice decoction group and P. lactiflora group. The inhibition rate of 50 mg/ml water extracts of the three samples was significantly higher than that of the 10 mg/ml group. Moreover, the water extract of G. uralensis at 50 mg/ml had the strongest inhibitory effect on I

Conclusion: The possible mechanism of peony and licorice decoction in relieving spasm and pain is most likely by inhibiting Voltage-Gated Sodium Channel Subtype 1.4.

Keywords: Nav1.4 voltage-gated sodium channels, Paeonia lactiflora and Glycyrrhiza uralensis, peony and licorice decoction, spasm and pain relieving, standard decoction

INTRODUCTION

Peony and licorice decoction is one of the most classic traditional Chinese medicine prescription which was originally described in Treatise on Febrile Diseases, a medical classic written by Zhongjing Zhang in the 3rd century. It contains Paeonia lactiflora and Glycyrrhiza uralensis (stir-baked with honey) with the ratio of 1:1. In ancient China, it was used in clinical applications for thousands of years and was commonly used to relieve spasm and pain.

More and more studies have proved that the analgesic effect of peony and licorice decoction is mainly attributed to peony, and the basis of the analgesic substance is the total glucoside content of P. lactiflora. While the antispasmodic effect of peony and licorice decoction is mainly attributed to G. uralensis, and the basis of the spasmylytic substance is the total glucoside of G. uralensis.[1]

The analgesic effect of peony and licorice decoction is probably related to its inhibition on Nav1.4 voltage-gated sodium channel subtype 1.4 based on standard decoction.

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channels (VGSCs). The latest research shows that the inhibitory effects of the eleven compounds of *G. uralensis* on the I_{Nav1.4} at a concentration of 10 μmol/l.[5] Treatment with the eleven compounds have inhibitory effects on I_{Nav1.4} and decreased the I_{Nav1.4} by 39.98% ± 4.55%, 33.20% ± 1.61%, 22.62% ± 0.30%, 20.54% ± 4.82%, 19.89% ± 3.15%, 18.63% ± 0.53%, 15.02% ± 3.24%, and 14.90% ± 1.98%, respectively.

VGSCs are important since they are responsible for the initiation and propagation of action potentials in the nerve, heart, and muscles.[3] VGSCs are composed of an α-subunit, which forms a Na+ conducting pore, and a β-subunit, which acts as a modulator for the biophysical properties of the channel. So far, nine different α-subunits (Nav1.1–1.9) and four different β-subunits (β1–β4) have been identified in mammalian systems.[4] However, Nav1.4 can be primarily observed in skeletal muscles.[5] The expression of Nav1.4 is essential in the skeletal muscle. Mutations in the subtype 1.4 alpha subunit (SCN4A) gene encoding the human skeletal muscle Nav1.4 channel have been proven to cause five different skeletal muscle disorders, including potassium-aggravated myotonia, paramyotonia congenita, hyperkalemic periodic paralysis, and a form of congenital myasthenic syndrome.[6,7] Hence, this channel is considered a target for the treatment of abnormal muscle contractility, spasm, and paralysis.[8]

In this study, we prepared samples by referring to the standard decoction of Traditional Chinese Medicinal Slices. Namely, the first decoction was added seven times the amount of water, and then, the raw materials first were soaked in water (as a solvent) for 30 min. The second decoction was added six times the amount of water. Medicinal slices were decocted for 30 min in the first decoction and 20 min of the second decoction. Concentration temperature should not be higher than 60°C, and the concentrated volume was five times the weight of slices.[9] The experimental model of acetic acid torsional body was established to research the effect of spasm and pain relieving of peony and licorice decoction, *P. lactiflora*, and *G. uralensis*. This study also researched the inhibitory effect of peony and licorice decoction and its compatibility components on Nav1.4 VGSCs to further explained the mechanism of peony and licorice decoction in relieving pain and pain.

**Materials and Methods**

**Drugs, chemicals, and reagents**

*P. lactiflora* and *G. uralensis* cultivated for 3 years were purchased from China Medico Corporation, and *G. uralensis* (stir-baked with honey) identified as *G. uralensis* Fisch.

Medicinal materials were separately immersed in deionized water and boiled twice for 30 min for the first time and 20 min for the second time. Then, the solutions were filtered with a screen mesh of 90 μm aperture and concentrated to 500 ml liquid extraction. Finally, they were converted to freeze-dried powder by freeze-drying. The compatibility of medicines was shown in Table 1.

The reference compounds of *oxypaeoniflorin* (purity ≥98%, No.150407), *albiflorin* (purity ≥98%, No.150407), *paenoniflorin* (purity ≥98%, No.150407), *liquiritin* (purity ≥98%, No. 160408), *isoliquiritin* (purity ≥98%, No.150714), *liquiritigenin* (purity ≥98%, No. 150511), benzylpaeonifloro-rin (purity ≥98%, No.150407), *isoliquiritigenin* (purity ≥98%, No. 141020), glycyrhrhizic acid (purity ≥98%, No.150407) were purchased from Chengdu Pufeide Biotech Co., Ltd. of China (Chengdu, China).

The extracellular solution was of the following composition: 140 mM NaCl, 2 mM CaCl$_2$, 40 mM tetraethylammonium-Cl, 4 mM KCl, 1 mM MgCl$_2$, 5 mM D-Glucose monohydrate, and 10 mM HEPES and was adjusted to pH = 7.4 with NaOH. The internal pipette solution was of the following composition: 145 mM CsCl, 0.1 mM CaCl$_2$, 2 mM MgCl$_2$, 10 mM NaCl, 0.5 mM Na2-GTP, 2 mM Mg-ATP, 1.1 mM EGTA and 10 mM HEPES and was adjusted to pH 7.2 with CsOH.

**Animals**

ICR mice weighing 18–22 g were purchased from the Beijing Vitalriver Pharmaceutical Co., Ltd and were divided into model group, *P. lactiflora* group, *G. uralensis* group and peony and licorice Decoction group. The animals were kept under constant conditions ([22°C ± 1°C], humidity [55% ± 1%]), 12 h light/dark cycle) and acclimatized for 3 days. Only water was provided to the animals within 12 h before the test. Experiments were performed in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigation of experimental pain, approved by the Experimental Animal Management Center of Beijing.

**Cell culture**

Recombinant VGSC SCN4A was expressed in Chinese hamster ovary (CHO) cells. The SCN4A cDNA was strictly similar to GenBank accession number NM_000334.4. Cells expressing channels were cultured in F12 medium supplemented with 10% fetal bovine serum and 0.8 mg/ml G418 in culture flasks.

**Table 1: Compatibility of medicines**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Weight (g)</th>
<th>Water (ml)</th>
<th>Extract (the first time) (min)</th>
<th>Extract (the second time) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. lactiflora</em></td>
<td>55.2</td>
<td>600</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td><em>G. uralensis</em></td>
<td>55.2</td>
<td>600</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Peony and licorice decoction</td>
<td><em>P. lactiflora</em>: 55.2</td>
<td>600</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td><em>G. uralensis</em>: 55.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P. lactiflora*: Paeonia lactiflora, *G. uralensis*: Glycyrrhiza uralensis
Cells grew in a humidified incubator at 37°C under 5% carbon dioxide. For patch clamp experiment, seed $3 \times 10^5$ cells into 24-well plate (final medium volume: 500 μl) with 1 coverslip in each well and test 18 h after incubation at 37°C under 5% carbon dioxide.

### Stable expression of human Nav1.4 in Chinese hamster ovary cells

Nav1.4 cDNA (cDNA strictly similar to GenBank accession number: NM_000334.4) was subcloned into pCDNA3.1 vector (Thermo Fisher Scientific). This vector contains a CMV promoter and a SV40 promoter, which drive the expression of the inserted target cDNA and geneticin-resistant gene, respectively. The CHO-K1 cells were transfected with this construct using Lipofectamine 2000 Transfection Reagent purchased from Thermo Fisher Scientific. After transfection, which allow the cells to grow and express the protein for antibiotic resistance under nonselective conditions.

After 48 h of posttransfection, the medium was removed, and the cells were rinsed with phosphate-buffered saline, and the adherent cells were detached using TrypL Express solution (Gibco). The cells were harvested by HAM’S F12 culture medium (HyClone) supplemented with 10% fetal bovine serum (Gibco) and 1.6 mg/ml antibiotic pretested for the host cell. Plate cells in a 96-well plate with no more than two cells in each well. It is important to thoroughly suspend cells before seeding, but avoid harsh treatment by frequent pipetting. Incubate cells under standard conditions and feed cells after 2 weeks with fresh selection medium. The host cell was also prepared in parallel as null control. Cell clones were tested as soon as cells in the nontransfected control wells have completely died.

Once identified the resistant clones, expand the cells culture into 6-well plate and assay for the expression of gene of interest by using manual patch clamp test. Positive clones were expended and cryopreserved timely, stable cell line was maintained at half concentration of antibiotic routinely.

### Instrumentations

The instruments used in this study include electronic analytical balance (Sartorius-BS-210S 210 g/0.1 mg), electronic balance (LD510-2-500 g/0.1 g), micropipette puller (P97, Sutter Instruments, USA), centrifugal machine (H1650-W), capillary glass tube (BF150-86-10, Sutter Instruments, USA), microscope (IX71, Olympus, Japan), microelectrode manipulator (MP285, Sutter Instruments, USA), amplifier (EPC10, HEKA, Germany), and electronic and analytical balance (BP110S, Sartorius, Germany).

Liquid chromatographic analysis was performed on an Agilent UPLC-DAD system (1290 Infinity α, Agilent Technologies, USA) equipped with an Agilent DAD detector (G7117A, Agilent Technologies, USA), an Agilent 1290 autosampler (G7167B, Agilent Technologies, USA), an Agilent 1290 infinity column heater (G7166B, Agilent Technologies, USA) and a Thermo-C18 (1.5 mm × 210 mm, 2.6 μm) column (Thermo Fisher Scientific, USA).

### Writhing test

Writhing test was carried out as described by Perazzo et al.,[10] the control group was administrated 0.2 ml of physiological saline using intragastric administration. The P. lactiflora (P) and the G. uralensis (G) group were administrated 0.2 ml of solution of freeze-dried powder dissolved in normal saline with the concentration of 2.94 mg/ml, 1.47 mg/ml and 0.74 mg/ml using intragastric administration, respectively. Moreover, the peony and licorice decoction group (P&L) were administrated 0.2 ml of solution of freeze-dried powder dissolved in normal saline with the concentration of 5.89 mg/ml, 2.94 mg/ml and 1.47 mg/ml using intragastric administration, respectively. The animals were treated by intraperitoneal injection of 0.6% acetic acid solution (0.2 mL/mice) 60 min after the last administration.

The animals were then placed in a clear Plexiglas cage for observation. A writhe was defined as a wave of contraction of abdominal muscles followed by dorsiflexion and extension of the hind limbs. The pain activity was evaluated by the number of writhing in 15 min of observation immediately after acetic acid injection. All the experiments were carried out between 9:00 am and 12:00 am.

### Statistical analysis

The data were expressed as the $\bar{X} \pm S$ and analyzed with SPSS 19.0 (statistical product and service solutions, IBM, New York, USA) statistical software. Statistical analysis was performed using one-way ANOVA Results. The level of significance was set at $P < 0.05$.

### Electrophysiology

#### Sample preparation

For electrophysiology studies, each freeze-dried powder was dissolved in DMSO to make 10 mg/ml and 50 mg/ml stock solutions.

### Electrophysiological recordings

The electrophysiological recordings were obtained under visual control of a microscope. The amplifier EPC10 was used for recording the electrophysiological signal. Offset potentials were nulled directly before the formation of a seal. No leak subtraction was performed. Fast capacitance (in pF) compensation was performed after a high seal was achieved. Cell capacitance (in pF) compensation was performed from whole-cell capacitance compensation after the whole cell mode was achieved. All experiments were performed at room temperature.

To measure the Nav1.4 channels, the membrane potential was held at-90 mV, and then depolarized to −10 mV for 50 ms to activate Nav1.4 currents. An interpulse interval of 10 s allows recovery from inactivation. The depolarized plus was confirmed by IV test.

Cells were incubated with the test article for 5 min, or until the current reached a steady-state level. Two concentrations of the test article were tested. The test and control solutions flowed...
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The data stored and analyzed with Patch master and Igor Pro were represented by \( X \pm S \) and analyzed by paired t-test. \( P < 0.05 \) indicates a significant difference. Sodium currents from activation were converted to sodium conductance \( (G = I/[V-V_{rev}]) \) and plotted as a function of test potential using the Boltzmann equation \( (G/G_{\text{max}} = 1/[1 + \exp (Vh-V)/K]) \) to give values for \( Vh \) (potential causing half-maximal activation) and \( K \) (slope factor). Similarly, currents from steady-state inactivation were also plotted as a function of prepulse potential and fitted with the Boltzmann equation \( (I/I_{\text{max}} = 1/[1 + \exp (Vh-V)/K]) \) to give values for \( Vh \) (potential causing half-maximal activation) and \( K \) (slope factor). Currents from steady-state recovery process curves were plotted as a function of prepulse potential and fitted with the Boltzmann equation \( (I = A_h \exp [-t/\tau] + A_d) \) to give values for \( \tau \) (recovery time).

**UPLC-DAD determination**

**Sample preparation**

For UPLC analysis, the solution was dissolved in methanol (v/v, 1:1), with sufficient mixing. Before injection, it was centrifuged at 12,000 rpm for 5 min at 4°C and filtered through a 0.22 μm filter.

**Separation conditions**

The binary gradient elution system consisted of solvent A (0.1% formic acid water) and solvent B (acetonitrile). Optimum separation was achieved using the gradient program described as follows: 0–10 min, 5%–15% (B); 10–18 min, 15%–20% (B); 18–25 min, 20%–25% (B); 25–35 min, 25%–40% (B); 35–36 min, and 40%–95% (B). The column temperature was maintained at 30°C. The autosampler was conditioned at 25°C, and the injection volume was 3 μl. The flow rate was 0.4 ml/min. The raw data were detected by a UPLC-DAD (Agilent1290 Infinity, USA), and the wavelengths were 230 nm for albiziflorin, paconinflorin, liquiritin, liquiritigenin, and benzoylpaconinflorin, 254 nm for oxypaconinflorin and glycyrrhizic acid, 360 nm for isoliquiritin and isoliquiritigenin.

**Preparation of standard solutions**

The appropriate amounts of oxypaconinflorin, albiziflorin, paconinflorin, liquiritin, isoliquiritin, liquiritigenin, benzoylpaconinflorin, isoliquiritigenin, and glycyrrhizic acid were separately weighed and dissolved together in methanol to achieve a standard working solution of nine different concentrations; the concentrations of the nine standard substances were 62 μg/ml, 40 μg/ml, 86 μg/m, 66 μg/ml, 52 μg/ml, 80 μg/ml, 44 μg/ml, 76 μg/ml, and 88 μg/ml. The calibration curves were constructed by analyzing the mixed solution; all solutions were prepared in dark brown calibrated flasks and stored at 4°C.

**Method validation**

The UPLC-DAD method was validated regarding linearity, precision, stability, repeatability, and recovery. The validation was performed based on the relative peak areas. Linear regression analysis was employed to construct calibration curves. The data were expressed as mean ± standard deviation, and the relative standard deviation (RSD) was used to evaluate precision, stability, repeatability, and recovery.

**Results**

**Writhing test**

The writhing frequency of the control group increased, and the incubation period became shorter after intraperitoneal injection of 0.6% acetic acid solution.

The peony and licorice decoction group and the *G. uralensis* group exhibited more significant prolongation of the incubation period, which were 4.81 ± 2.17 min, 2.13 ± 0.77 min, 5.38 ± 2.97 min and 9.71 ± 1.87 min, 7.39 ± 2.91 min, 5.15 ± 1.87 min respectively. Moreover, the writhing frequency significantly decreased compared with the control (**\( P < 0.01, *P < 0.05, n = 6 \)**) in a dose-dependent manner which were 17.67 ± 4.26, 26.50 ± 3.65, 27.00 ± 2.74 and 6.50 ± 2.77, 15.33 ± 2.75, and 21.33 ± 3.64.

The *P. lactiflora* group significantly prolonged the incubation period only when the drug concentration is 2.94 mg/ml and 1.47 mg/ml. The incubation period was 6.55 ± 2.54 min and 5.44 ± 1.20 min. Moreover, the writhing frequency significantly decreased compared with the control (\( *P < 0.05, n = 6 \) which were 25.50 ± 2.10 and 25.83 ± 2.99. However, it did not significantly change the incubation period and the writhing frequency at the concentration of 0.74 mg/ml compared with the control (**\( ***P > 0.05, n = 6 \)***).

The research showed that the effect of *G. uralensis* on incubation period and writhing frequency was significantly better than that of peony and licorice decoction group and *P. lactiflora* group.

Therefore, we can infer that *G. uralensis* has a better effect in relieving spasm and pain than that of peony and licorice decoction and *P. lactiflora* by this experiment. The results are shown in Figure 1.

![Figure 1: The effect of relieving spasm and pain.](image)

**Figure 1**: The effect of relieving spasm and pain. “P” and “L” stands for Peony and Licorice Decoction group; “P” stands for *Paeonia lactiflora*; group “G” stands for *Glycyrrhiza uralensis* group.
Effect of peony and licorice decoction, *Paeonia lactiflora* and *Glycyrrhiza uralensis* on Nav1.4

In this study, whole-cell patch clamp technique was performed to investigate the effect of water extracts of peony and licorice decoction, *P. lactiflora* and *G. uralensis* on the $I_{Nav1.4}$.

The peak current decreased significantly after treatment with 50 mg/ml water extracts of peony and licorice decoction, *P. lactiflora* and *G. uralensis* compared with the control ($P<0.05, n=3$) [Figure 2A2-1 and A2-2, 2A4-1 and A4-2, 2A6-1 and A6-2]. The $I_{Nav1.4}$ decreased from $(-4.83E-09 \pm 1.49E-09)$ pA, $(-3.59E-09 \pm 1.53E-09)$ pA and $(-4.14E-09 \pm 1.22E-09)$ pA to $(-4.10E-09 \pm 1.21E-09)$ pA, $(-2.46E-09 \pm 4.90E-10)$ pA and $(-9.33E-10 \pm 6.62E-11)$ pA, respectively. Moreover, the inhibitory effect on $I_{Nav1.4}$ were $29.79\% \pm 2.83\%$, $29.57\% \pm 4.21\%$, and $77.72\% \pm 0.72\%$, respectively.

![Figure 2](image)

Figure 2: The effect of the water extract on Nav1.4. (A1-1, A2-1, A3-1, A4-1, A5-1 and A6-1): $I_{Nav1.4}$ recorded from CHO cells expressing human Nav1.4 VGSCs before the water extract treatment (control), under treatment, and after washing out the water extract. (A1-2, A2-2, A3-2, A4-2, A5-2, and A6-2): Time course of inhibition of the $I_{Nav1.4}$ evoked by depolarization from a holding potential of $-120 \text{ mV}$ to a test potential of $+10 \text{ mV}$ by sequential exposure to the water extract. (A1-1, A1-2, A3-1, A3-2, A5-1, and A5-2): 10 mg/ml water extract of peony and licorice Decoction, *Paeonia lactiflora* and *Glycyrrhiza uralensis*. (A2-1, A2-2, A4-1, A4-2, A6-1, and A6-2): 50 mg/ml water extract of peony and licorice Decoction, *Paeonia lactiflora* and *Glycyrrhiza uralensis*.
The peak current decreased significantly after treatment with 10 mg/ml water extracts of peony and licorice decoction and *G. uralensis* compared with the control (*P* < 0.05, *n* = 3) [Figure 2A1-1 and A1-2, 2A5-1, A5-2], the *I*\textsubscript{Nav} 1.4 decreased from (−4.83E-09 ± 1.49E-09) pA and (−4.14E-09 ± 1.22E-09) pA to (−3.78E-09 ± 1.20E-09) pA and (−3.84E-09 ± 1.43E-09) pA, respectively. Moreover, the inhibitory effect on *I*\textsubscript{Nav} 1.4 was 13.37% ± 2.40% and 15.27% ± 1.51%, respectively.

The inhibition rate of 50 mg/ml water extracts of peony and licorice decoction, *P. lactiflora* and *G. uralensis* was significantly higher than that of the 10 mg/ml group (*P* < 0.05, *n* = 3) [Figure 2]. This inhibitory effect of 50 mg/ml water extracts on the *I*\textsubscript{Nav} 1.4 appeared within 4 min, 4 min, and 3 min after the start of perfusion of the bath solution containing 50 mg/ml water extracts of peony and licorice Decoction, *P. lactiflora* and *G. uralensis* [Figure 2].

The peak current decreased after treatment with 10 mg/ml water extracts of *P. lactiflora* from (−3.59E-09 ± 1.53E-09) pA to (−3.32E-09 ± 1.87E-09) pA. There was no significant difference between the two groups compared with the control (*P* > 0.05, *n* = 3) [Figure 2A3-1 and A3-2]. The inhibitory effects of 10 mg/ml *P. lactiflora* on *I*\textsubscript{Nav} 1.4 were 7.74% ± 4.00%.

After washing out water extracts of peony and licorice decoction, *P. lactiflora* and *G. uralensis*, the inhibitory effect on *I*\textsubscript{Nav} 1.4 was abolished quickly. The inhibitory effect of the water extracts on *I*\textsubscript{Nav} 1.4 is shown in Figure 2.

**Contents of the nine components of peony and licorice decoction and its compatibility components**

**Calibration curves**

Linearity was evaluated by analyzing six injection quantities of standard solutions, and the calibration curves were constructed by plotting the peak areas and the injection quantity of 5 μl of each compound with six different concentrations, the results are shown in Table 2.

**Precision, stability, repeatability, and recovery**

Precision was tested by six replicate determinations of the standard working solution. Stability was evaluated by analyzing the solutions stored at room temperature (about 25°C) at different time points (0, 4, 8, 12, and 24 h after preparation). The solutions used in the stability test included mixed solutions of reference standard and sample solutions. Six replicates were performed for the test. The obtained data confirmed that the nine compounds were stable within 24 h at 25°C and their RSD values were between 1.23% and 1.86%. Repeatability was examined by six replications of a sample. In the recovery test, samples were prepared at three concentration levels in triplicate by spiking known quantities of each of the nine standards into the sample, after which extraction and analysis were applied according to the described procedures. The validation data are shown in Table 3.

**Content**

The UPLC-DAD data demonstrated that oxypaeoniflorin, albiflorin, paeoniflorin, liquiritin, isoliquiritin, liquiritigenin, benzoylpaeoniflorin, isoliquiritigenin, and glycyrrhizic acid are present in the water extracts of the sample [Figures 3-5]. The contents of the nine marker compounds in the water extracts are shown in Table 4.

**Discussion**

Peony and licorice decoction is a Traditional Chinese Prescription containing *P. lactiflora* and *G. uralensis* (stir-baked with honey) with the ratio of 1:1. It is commonly used to relieve spasm and pain and originally described in Treatise on Febrile Diseases, a medical classic written by Zhongjing Zhang in the 3rd century. Today, it is used clinically for its antispasmodic and muscle relaxation effects in the treatment of leg cramps, stomachache, and menstrual colic pain.[11-14]

In this research, an experimental model of acetic acid torsional body was established to study the effect of peony and licorice decoction, *P. lactiflora* and *G. uralensis* (stir-baked with honey) in relieving spasm and pain. The results showed that peony and licorice decoction, *P. lactiflora* and *G. uralensis* could prolong the incubation period of the experimental mice, which can significantly reduce the number of twisting times in experimental mice. Moreover, the *G. uralensis* group has the best inhibitory effect of these three.

The results showed that the effect of relieving spasm and pain of peony and licorice decoction was related to the inhibition of VGSC subtype 1.4. Moreover, *G. uralensis*

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**Table 2: Linear regression equation, linear range, and correlation coefficient for nine standard substances**

<table>
<thead>
<tr>
<th>Standard substance</th>
<th>Linear regression equation (y=ax+b)</th>
<th>Correlation (r²)</th>
<th>Linear range (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxypaeoniflorin</td>
<td>Y = 1681887.5x – 13.4</td>
<td>0.9992</td>
<td>1.24-34.40</td>
</tr>
<tr>
<td>Albiflorin</td>
<td>Y = 1106081.1x – 2.5</td>
<td>1.0000</td>
<td>0.80-16.00</td>
</tr>
<tr>
<td>Paeoniflorin</td>
<td>Y = 1909635.8x + 4.6</td>
<td>0.9991</td>
<td>1.72-38.20</td>
</tr>
<tr>
<td>Liquiritin</td>
<td>Y = 3697762.5x + 2.2</td>
<td>1.0000</td>
<td>1.32-26.40</td>
</tr>
<tr>
<td>Isoliquiritin</td>
<td>Y = 6317717.7x + 2.2</td>
<td>0.9992</td>
<td>2.04-20.40</td>
</tr>
<tr>
<td>Liquiritigenin</td>
<td>Y = 6012645.3x + 7.1</td>
<td>1.0000</td>
<td>1.04-20.8</td>
</tr>
<tr>
<td>Benzoylpaeoniflorin</td>
<td>Y = 2752598.7x + 0.5</td>
<td>0.9997</td>
<td>0.88-17.60</td>
</tr>
<tr>
<td>Isoliquiritigenin</td>
<td>Y = 8823821.9x + 5.9</td>
<td>1.0000</td>
<td>1.60-32.00</td>
</tr>
<tr>
<td>Glycyrrhizic acid</td>
<td>Y = 1112094.8x – 1.9</td>
<td>1.0000</td>
<td>1.76-35.2</td>
</tr>
</tbody>
</table>

**Figure 3: UPLC-DAD chromatogram of the water extract of *Glycyrrhiza uralensis* at a wavelength of 254 nm. (1) oxypaeoniflorin, (2) albiflorin, (3) paeoniflorin, (7) benzoylpaeoniflorin**
does a better job in the inhibition of water extract of G. uralensis on VGSC subtype 1.4 than P. lactiflora and G. uralensis.

In this study, we also investigated the inhibitory effect of the water extract of peony and licorice decoction, P. lactiflora and G. uralensis on I_{Nav}1.4. The experiment found that 50 mg/ml water extracts of the three samples can significantly inhibit I_{Nav}1.4. Moreover, the inhibitory effect on I_{Nav}1.4 was 29.79% ± 2.83%, 29.57% ± 4.21%, and 77.72% ± 0.72%, respectively. The water extract of G. uralensis at 50 mg/ml had a stronger inhibitory effect on I_{Nav}1.4, with inhibitory rate of 77.72% ± 0.72%. Treatment with 10 mg/ml water extract of P. lactiflora showed a lower inhibitory effect on I_{Nav}1.4, which decreased to 7.47% ± 4.00%. After washing out water extracts of the sample, the inhibitory effect on I_{Nav}1.4 was abolished quickly and restore to normal level.

In the research, nine chemical compounds of the sample were selected to clarify their different degrees of inhibition with respect to I_{Nav}1.4. They were oxypaeoniflorin, albiflorin, paeoniflorin, liquiritin, isoliquiritin, liquiritigenin, benzoylpaeoniflorin, isoliquiritigenin, and glycyrrhizic acid. The results showed that all the nine chemical compounds are present in the water extracts of the sample. The contents of the nine compounds are 0.063, 1.202, 3.812, 0.103, 0.735, 0.054, 0.031, 0.011, and 1.928 mg/ml, respectively in peony and licorice decoction. The contents of the four compounds are 0.049, 1.191, 3.607, and 0.082 mg/ml respectively in the water extract of P. lactiflora. The contents of the five compounds are 0.686, 0.068, 0.023, 0.003, and 1.852 mg/ml, respectively, in the water extract of G. uralensis. There was no obvious correlation between the concentration of the marker substances of the water extracts of the sample and the inhibitory rate on I_{Nav}1.4. The basic theory of Traditional Chinese Medicine considers that there is a synergistic effect between the chemical compounds of this Chinese medicine, which results in a therapeutic effect. The nine compounds may have synergistic effects and eventually play a role in the inhibition of VGSCs currents, or there may be many other compounds besides these nine chemical compounds that have been studied previously, one or more of them may have an inhibition effect on I_{Nav}1.4 and work in coordination with each other to play a therapeutic role. Therefore, it is difficult to determine which compound is most responsible for the inhibitory effect of water extract of peony and licorice decoction on Nav1.4 VGSCs currents. Further research is in progress to provide scientific explanation of such phenomenon.

<table>
<thead>
<tr>
<th>Standard substance</th>
<th>Precision RSD (n=6) (%)</th>
<th>Repeatability RSD (n=6) (%)</th>
<th>Stability RSD (n=5) (%)</th>
<th>Recoveries (%)</th>
<th>RSD (%) (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxypaeoniflorin</td>
<td>1.34</td>
<td>1.10</td>
<td>1.86</td>
<td>98.00</td>
<td>1.21</td>
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<tr>
<td>Albiflorin</td>
<td>1.24</td>
<td>1.33</td>
<td>1.23</td>
<td>99.00</td>
<td>1.30</td>
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<tr>
<td>Paeoniflorin</td>
<td>1.14</td>
<td>1.44</td>
<td>1.38</td>
<td>99.00</td>
<td>2.22</td>
</tr>
<tr>
<td>Liquiritin</td>
<td>1.64</td>
<td>1.65</td>
<td>1.57</td>
<td>101.00</td>
<td>1.04</td>
</tr>
<tr>
<td>Isoliquiritin</td>
<td>1.10</td>
<td>1.56</td>
<td>1.42</td>
<td>99.00</td>
<td>2.12</td>
</tr>
<tr>
<td>Liquiritigenin</td>
<td>1.22</td>
<td>0.89</td>
<td>1.46</td>
<td>99.00</td>
<td>1.79</td>
</tr>
<tr>
<td>Benzoylpaeoniflorin</td>
<td>1.41</td>
<td>2.02</td>
<td>1.45</td>
<td>102.00</td>
<td>2.23</td>
</tr>
<tr>
<td>Isoliquiritigenin</td>
<td>1.29</td>
<td>1.36</td>
<td>1.47</td>
<td>98.00</td>
<td>1.57</td>
</tr>
<tr>
<td>Glycyrrhizic acid</td>
<td>1.23</td>
<td>0.99</td>
<td>1.39</td>
<td>99.00</td>
<td>2.25</td>
</tr>
</tbody>
</table>

Recovery (%) = 100 × (amount found − original amount)/amount spiked. RSD: Relative standard deviation.

Table 3: Precision, repeatability, stability, and recovery of nine standard substances.
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Conflicts of interest

There are no conflicts of interest.

REFERENCES


