Effect of Baizhu (Rhizoma Atractylodis Macrocephalae) extract on intestinal absorption of brucine and strychnine in vitro and in situ

Lü Dan, Jiang Qieying, Zhang Jing, Zeng Ronggui, Liao Zhenggen, Liang Xinli

Lü Dan, Key Laboratory of Modern Preparation of Traditional Chinese Medicine of Ministry of Education, Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, China; Jiangsu College of Nursing, Huai'an 223001, China
Jiang Qieying, Zhang Jing, Zeng Ronggui, Key Laboratory of Modern Preparation of Traditional Chinese Medicine of Ministry of Education, Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, China

Supported by the National Natural Science Foundation of China (No. 81660757 and No. 81303237); the Academic and Technological Foregoer Funds of Jiangxi Province, China (No. 20162CB22015); the Project on Cultivation of Medical Elite (Gan Po Ying Cai 555) (2013296); the Youth Science Funds of Jiangxi Province, China (No. 20153CB23019); the National Natural Science Foundation of Jiangxi Province (No. 20161ACB21020); and the Natural Science Research Project of Huai'an (No. HAB201716)

Correspondence to: Prof. Liao Zhenggen and Liang Xinli, Key Laboratory of Modern Preparation of Traditional Chinese Medicine of Ministry of Education, Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, China. lyzyg@163.com; paln7@163.com
Telephone: +86-791-87118786; +86-13870974167.
Accepted: January 18, 2020

Abstract

OBJECTIVE: To investigate the antagonistic effect of the extract of Baizhu (Rhizoma Atractylodis Macrocephalae) (RAM) on the intestinal absorption of brucine and strychnine in Strychnos nux-vomica (NUX) and propose the mechanism of these effects.

METHODS: The apparent permeability value ($P_{app}$) and absorption rate constant ($K_a$) were chosen as indices. The everted intestinal sac model and in situ single-pass intestinal perfusion model were used to study the effects of the RAM extract on the absorption of brucine and strychnine. To confirm the results, the brucine and strychnine concentrations in hepatic portal venous blood were determined. Western blotting was used to study P-glycoprotein (P-gp) expression in the Caco-2 cell line.

RESULTS: $P_{app}$ and $K_a$ of brucine and strychnine were significantly increased in the presence of a P-gp inhibitor, but no significant increase was noted in the presence of a tight junction regulator. The RAM extract inhibited the absorption of brucine and strychnine and enhanced P-gp expression.

CONCLUSION: The primary absorption mechanism for brucine and strychnine is passive transport, which is affected by P-gp.

INTRODUCTION

Strychnos nux-vomica (NUX) is the mature dry seed of Strychnos nux-vomica L., which belongs to the Loganiaceae family.1 In China, NUX was first recorded in the Compendium of Materia Medica, and it is widely used in Chinese folk medicine. Many alkaloids have been separated from NUX and identified; among them, strychnine and brucine (Figure 1) are the chief active constituents.2,3 At low doses, NUX is used as an analgesic, anti-inflammatory agent,4 anti-tumour agent,5 and anti-diabetic drug,6 in addition to uses to treat arthromyodynia, relax the stomach and bowels,7 and im-
The spleen, which governs transport and transformation of Qi, can be enriched to prevent the invasion, can efficiently spread the essence of grain and water, and increase efficacy. In addition, the RAM-NUX formula has been applied in patients of all ages. In ancient China, this formula was recorded in Yi Xue Zhong Zhong Can Xi Lu by Zhang Xichun for the treatment of arthralgia. Clinical verification has indicated a favorable effect as well as the consistency of the intestinal absorption. Therefore, methods to reduce the toxicity of NUX and expand its applications are necessary. Compatibility, which refers to a relatively fixed composition of two or more herbs, is a characteristic of Traditional Chinese Medicine and is advantageous for attenuation.

Baizhu (Rhizoma Atractylodis Macrocephalae) (RAM)-NUX is a frequently used compatible formula that we have previously studied. RAM-NUX has significant positive effects on arthralgia syndrome and spleen and stomach pain, and the combination is associated with reduced toxicity and increased efficacy. In addition, the RAM-NUX formula has been applied in patients of all ages. In ancient China, this formula was recorded in Yi Xue Zhong Zhong Can Xi Lu by Zhang Xichun for the treatment of gastrosis.

In modern times, the RAM-NUX combination has been used in some commonly prescribed Chinese herbal remedies, such as Bi-qì capsules, Maqianzi tablets and Busheng Tongluo pills for the treatment of arthralgia. Clinical verification has indicated a favorable effect of the two compatible herbs and no toxicity. A previous study reported that extracting RAM and NUX together could result in antagonistic effects. However, the compatibility of RAM and NUX from the perspective of intestinal absorption has not yet been reported. Therefore, this study sought to explore the transport properties of brucine and strychnine in NUX and the effects of a RAM extract on the absorption of these compounds using in vitro and in vivo models to explore the compatibility of RAM-NUX.

Modern pharmacology has revealed that the oral bioavailability of a drug is closely related to its biological effect as well as the consistency of the intestinal absorption of the active components. According to Traditional Chinese Medicine theory, defensive Qi is the most active Qi in the Qi dynamic system, and it can prevent the invasion of exogenous pathogenic factors. The spleen, which governs transport and transformation, can efficiently spread the essence of grain and water. Defensive Qi can be enriched to prevent the invasion of exogenous pathogenic factors via strengthening the spleen. Furthermore, RAM is a typical strengthening herb. Thus, regarding the compatibility of NUX and RAM, RAM prevents the invasion of exogenous pathogenic factors, whereas NUX may be regarded as an exogenous pathogen that is inhibited by RAM. More specifically, RAM can inhibit the absorption of NUX. Therefore, modern pharmacology and Traditional Chinese Medicine theory have led to the conclusion that absorption is an important factor of compatibility. Moreover, an increasing number of studies on the role of intestinal absorption in compatibility have been reported, and considerable progress has been made. For example, the compatibility mechanisms of radix angelicae dahuricae-radix scutellariae, flavones-rasagiline mesylate and radix angelicae dahuricae-puerarin root have been elucidated. In this study, we investigated the antagonistic effects of a RAM extract on the intestinal absorption of brucine and strychnine in NUX and provided a mechanism of action.

**MATERIALS AND METHODS**

All animal studies were performed according to approved protocols and the guidelines of the Institutional Animal Ethical Care Committee (NIPER) and were approved by the local ethics committee for animal experimentation. All efforts were made to minimise the number of animals used and their suffering. The Minimum Standards of Reporting Checklist contains details of the experimental design, statistics and resources used in this study.

**Materials**

NUX was purchased from the Happy People Pharmaceutical Co., Ltd. (Nanchang, China). RAM was purchased from Huang Qingren Pharmaceutical Co., Ltd. (Nanchang, China). Standards of strychnine, brucine and matrine were purchased from the Jiangxi Natural Herbs Technological Co., Ltd. (batch No. BCTG-0612, BCTG-0442 and BCTG-0359, respectively, Nanchang, China). Verapamil hydrochloride was purchased from the National Institutes for Food and Drug Control (batch No. 100223-200102, Beijing, China). Disodium ethylenediaminetetra-acetic acid (EDTA-Na2) was purchased from Solarbio Co., Ltd. (batch No. 20150203 and 20140320, respectively, Nanchang, China). Anti-P-glycoprotein (P-gp) antibody F4 and GAPDH were purchased from Sigma. Goat anti-mouse IgG, HRP was obtained from CW-BIO. Sodium heptanesulfonate was purchased from Shanghai Zhanyun Chemical Co., Ltd. (batch No. 150405, Shanghai, China). HPLC-grade solvents from BIO. Sodium heptanesulfonate was purchased from Shanghai Zhanyun Chemical Co., Ltd. (batch No. 150405, Shanghai, China). HPLC-grade solvents from TEDIA (Columbus, OH, USA) were used for drug analyses. The water used in this study was purified using a Milli-Q water system (Bedford, MA, USA).

**Figure 1** Chemical structures of strychnine and brucine

A: strychnine; B: brucine.
Animals and cells
Male Sprague-Dawley rats ([250 ± 20] g, 8 weeks), which were procured from the Hunan Slack Jingda Experimental Animal Co., Ltd. (No. SYXK 2014-0008, Hunan, China), were housed under standard conditions, fed a commercial diet and given water ad libitum. All animal studies were performed according to approved protocols and the guidelines of the Institutional Animal Ethical Care Committee (NIPER) and were approved by the local ethics committee for animal experimentation. All efforts were made to minimise the number of animals used and their suffering. The human colon adenocarcinoma cell line Caco-2 was purchased from American Type Culture Collection (Manassas, VA, USA).

Preparation of RAM and NUX extract and composition of the drug systems
Preparation of RAM and NUX extract: RAM or NUX was extracted twice for 1 h using a 10-fold volume of water after soaking for 30 min. After combining two rounds of extract solution, the extract was obtained. Composition of the drug systems for rats: the drugs used for perfusion were dissolved in Krebs-Ringer (K-R) buffer. The perfusion dose was converted to a physiologic animal dose according to the recommended daily dose for humans in Chinese pharmacopoeia 1. Composition of the drug systems for cells: preparation of NUX: the appropriate NUX extract was dissolved in DMEM (containing 4.5 g/L D-glucose) containing 10% heat-inactivated foetal bovine serum (FBS) which had inactivated at 56 °C for 30 min), 1% non-essential amino acids, 1% L-glutamine, penicillin (100 μg/mL) and streptomycin (100 μg/mL). The herb concentration of NUX was 10 mg/L.
Preparation of NUX-RAM: the appropriate NUX and RAM extracts were dissolved in DMEM (containing 4.5 g/L D-glucose) containing 10% heat-inactivated FBS, 1% non-essential amino acids, 1% L-glutamine, penicillin (100 μg/mL) and streptomycin (100 μg/mL). The compatibility ratio was 1:4 or 1:6.
Preparation of NUX-Ver: the appropriate NUX and RAM extracts were dissolved in DMEM (containing 4.5 g/L D-glucose) containing 10% heat-inactivated FBS, 1% non-essential amino acids, 1% L-glutamine, penicillin (100 μg/mL) and streptomycin (100 μg/mL). The herb concentration of NUX was 10 mg/L, and the concentrations of Ver were 100, 200 or 300 μmol/L.

Effect of RAM on the intestinal transport of brucine and strychnine from NUX in an everted gut sac model and an in situ single-pass perfusion model
Everted gut sac model: rats were anaesthetised with 10% chloral hydrate (0.3 mL/100 g) via intraperitoneal injection after an overnight fast, with water provided ad libitum. After laparotomy, the duodenum, jejunum, ileum and colon were rapidly removed and excised into 8-10-cm segments. The segments were washed with cold K-R buffer, the redundant fat and mesenterium were removed and the segments were gently everted with a capillary. One end of an everted segment was tied with a silk-braded suture and filled with 2 mL of K-R buffer, and then the other end was tied. The sealed segments were transferred to a drug-containing 20-mL cuvette in an atmosphere of 95% air and 5% CO2 at 37 °C. After 1 h, the sacs were washed four times with K-R buffer, the serosal fluid was placed in a volumetric flask with a constant volume of methanol and the area of the segment was measured. The samples were conserved for subsequent testing. The permeability value (Pm) in the everted gut sac model was calculated as follows:

\[ P_{m} = \frac{dQ/dt}{A \times C_{m}} \]

where dQ/dt is the amount of drug transported per unit time, \( C_{m} \) (mg/L) is the concentration of the drug and A (cm²) is the area of the segment.
The effects of RAM (25.8 g/L) on the intestinal transport of brucine and strychnine from NUX (3.03 g/L) were analysed using the everted gut sac model.

In situ single-pass perfusion model
The rats were anaesthetised with 10% chloral hydrate (0.3 mL/100 g) via intraperitoneal injection after an overnight fast, with water provided ad libitum. After laparotomy, the duodenum (8-10 cm) was exposed via a midline incision and gently cannulated at both ends. To remove residual debris, K-R buffer at 37 °C was infused at a flow rate of 2 mL/min for 30 min using a constant-flow pump, and the drug was then perfused at a flow rate of 0.2 mL/min for 60 min until the entire system was at equilibrium. After steady state was achieved, perfusate samples were collected in 15-min intervals (0-15, 15-30, 30-45, 45-60, 60-75, 75-90, 90-105 and 105-120 min) in weighed vials, which were then weighed again. After the experiment, hepatic portal venous blood was taken at 120 min and centrifuged to separate the plasma, which was frozen at −20 °C until analysis. An intestinal segment was cut, and the area was measured. The samples were conserved for subsequent testing. The absorption rate constant (K) in the in situ single-pass perfusion model was calculated as follows:

\[ K_{a} = \left(1 - \frac{C_{m} Q_{\text{in}}}{C_{o} Q_{\text{out}}}\right) \frac{Q}{V} \]

Pm in the in situ single-pass perfusion model was calculated as follows:

\[ P_{m} = \frac{-Q \ln \left(\frac{C_{w} Q_{\text{in}}}{C_{o} Q_{\text{out}}}\right)}{2 \pi l} \]

where K is the intestinal absorption rate constant, Q (mL/min) is the flow rate through the intestine, Cw (μg/mL) is the inlet drug concentration in the perfusion buffer, Cm (μg/mL) is the outlet drug concentra-
tion in the perfusion buffer when the density of the perfusion buffer at the inlet and outlet was 1.0 g/mL, \( Q_i \) (mL) is the volume of the perfusion buffer at the inlet, \( Q_o \) (mL) is the volume of perfusion buffer at the outlet, \( V \) (cm\(^3\)) is the volume of the perfused segment, \( r \) is the luminal radius of the duodenum segment and \( l \) is the mean length of the perfused duodenum segment.

The effects of RAM (25.8 g/L) on the intestinal transport of brucine and strychnine from NUX (3.03 g/L) were analysed using the in situ single-pass perfusion model.

In addition, plasma was analysed. Hepatic portal venous blood, which was taken from the in situ single-pass perfusion model, was purified, concentrated, transferred to an auto-sampler vial and injected (5 \( \mu L \)) into an LC-MS system.

**Transport properties of brucine and strychnine in NUX in an in situ single-pass perfusion model**

Effect of the NUX extract concentration on the intestinal transport of brucine and strychnine: the NUX extract was dissolved in K-R buffer to prepare a series of crude drug solutions at concentrations of 1.52, 2.28, 3.03, 4.55 and 6.07 g/L. These solutions were applied in the in situ single-pass intestinal perfusion model to study the effects of the NUX extract concentration on the intestinal transport of brucine and strychnine.

Effect of pH on the intestinal transport of brucine and strychnine: the in situ single-pass intestinal perfusion model was applied to study the effects of the NUX extract at various pH values (pH 5.0, 6.8 and 7.4) on the intestinal transport of brucine and strychnine. The crude drug concentration was 3.03 g/L.

Effect of P-gp on the intestinal transport of brucine and strychnine: the in situ single-pass intestinal perfusion model was applied to study the effects of P-gp on the intestinal transport of brucine and strychnine. The P-gp inhibitor verapamil (200 \( \mu \text{mol/L} \)) was dissolved in the prepared NUX extract. The crude drug concentration of NUX was 3.03 g/L, and the pH was 7.4.

Effects of tight junctions (TJs) on the intestinal transport of brucine and strychnine: the in situ single-pass intestinal perfusion model was applied to study the effects of TJs on the intestinal transport of brucine and strychnine. The TJ regulator EDTA-Na\( _2 \) (1 mmol/L) was dissolved in the prepared NUX extract. The crude drug concentration of NUX was 3.03 g/L, and the pH was 7.4.

**Western blot analysis of the effects of brucine and strychnine on P-gp expression**

Caco-2 cells were cultured at 37 °C in an atmosphere of 95% air and 5% \( \text{CO}_2 \) at 95% relative humidity in DMEM (containing D-glucose 4.5 g/L) containing 10% heat-inactivated FBS, 1% NEAA, 1% L-glutamine, penicillin (100 U/mL) and streptomycin (100 \( \mu \text{g/mL} \)). The medium was changed every other day during cell growth and differentiation. The cells were grown in a T-75 culture bottle for 5 d.
Effect of RAM on the intestinal transport of brucine and strychnine from NUX in the in situ single-pass intestinal perfusion model

*K* and *P* app of brucine from NUX decreased by 2.10- and 3.50-fold, respectively, after the RAM extract was added, and *K* and *P* app of strychnine from NUX decreased by 2.18- and 2.12-fold, respectively, after the addition of the extract (Table 1). Moreover, the antagonistic effects of the RAM extract on the intestinal absorption of brucine and strychnine were further confirmed by the plasma concentration results (Figure 4).

Effect of the NUX extract concentration on the intestinal transport of brucine and strychnine

In this experiment, five crude drug concentrations of NUX were selected for evaluation using the in situ single-pass intestinal perfusion model. *P* app and *K* of brucine and strychnine were not significantly different at crude drug concentrations of 1.52-6.07 g/L (Table 2). We also investigated the plasma concentrations of brucine and strychnine in the rat portal vein after perfusion. Figure 5 shows that the plasma concentrations of brucine and strychnine were significantly higher at higher concentrations, indicating that saturated absorption did not occur over the crude NUX concentration range of 1.52-6.07 g/L.

Figure 2 Effect of RAM on the transport of brucine by intestinal absorption in different intestinal segments

NUX: Strychnos nux-vomica; RAM: Baizhu (*Rhizoma Atractylodis Macrocephalae*). Differences between groups were statistically analysed using Student’s *t*-test. The data were expressed as the mean ± standard deviation (*n* = 6). *P* < 0.05, *P* < 0.01, compared with the NUX (3.03 g/L) group.

Figure 3 Effect of RAM on the transport of strychnine by intestinal absorption in different intestinal segments

NUX: Strychnos nux-vomica; RAM: Baizhu (*Rhizoma Atractylodis Macrocephalae*). Differences between groups were statistically analysed using Student’s *t*-test. The data were expressed as the mean ± standard deviation (*n* = 6). *P* < 0.05, *P* < 0.01, compared with the NUX (3.03 g/L) group.

Table 1 Effect of RAM on the transport of brucine and strychnine (*±* s)

<table>
<thead>
<tr>
<th>Group</th>
<th><em>n</em></th>
<th><em>K</em> app/× 10^-5·s^-1</th>
<th><em>P</em> app/× 10^-5·cm·s^-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Brucine</td>
<td>Strychnine</td>
</tr>
<tr>
<td>NUX (3.03 g/L)</td>
<td>6</td>
<td>15.32±4.60</td>
<td>49.03±15.24</td>
</tr>
<tr>
<td>RAM (25.8 g/L)-NUX (3.03 g/L)</td>
<td>6</td>
<td>7.29±2.93</td>
<td>22.54±5.76</td>
</tr>
</tbody>
</table>

Notes: NUX: Strychnos nux-vomica; RAM: Baizhu (*Rhizoma Atractylodis Macrocephalae*). NUX (3.03 g/L) means the herb concentration of NUX was 3.03 g/L. RAM (25.8 g/L)-NUX (3.03 g/L) means the herb concentration of NUX was 3.03 g/L and the herb concentration of RAM was 25.8 g/L. Differences between groups were statistically analysed using Student’s *t*-test. *P* < 0.05, *P* < 0.01, compared with the NUX (3.03 g/L) group.
using Student’s t-test. 0, 601 t < 0.1 group; < 8 group.

5.0, 601, 03

Notes: NUX: Strychnos nux-vomica. The herb concentration of NUX was 0.3 g/L. Differences between groups were statistically analysed using Student’s t-test. *P < 0.05, **P < 0.01, compared with the pH 5.0 group; *P < 0.05, **P < 0.01, compared with the pH 6.8 group.

### DISCUSSION

Compatibility plays a vital role in the development of Traditional Chinese Medicine, the original formulae of Chinese herbal medicine.30 The intestinal tract is an indispensable protective barrier that can affect the intestinal absorption of drugs.31 Therefore, intestinal absorption interactions could represent a possible pathway for the intestinal absorption of herbal pairs. In this study, we confirmed the antagonistic effects of the RAM extract on the intestinal absorption of brucine and strychnine. To increase the reliability of the experimental results, we adopted three intestinal absorption models: the everted gut sac model, the in situ single-pass perfusion animal model and portal vein blood measure-

<table>
<thead>
<tr>
<th>Crude drug concentration (g/L)</th>
<th>K&lt;sub&gt;j&lt;/sub&gt;/10&lt;sup&gt;-3&lt;/sup&gt; cm·s&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>P&lt;sub&gt;j&lt;/sub&gt;/10&lt;sup&gt;-3&lt;/sup&gt; cm·s&lt;sup&gt;-1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brucine</td>
<td>Strychnine</td>
</tr>
<tr>
<td>1.52</td>
<td>11.3e±4.95</td>
<td>34.3e±4.66</td>
</tr>
<tr>
<td>2.28</td>
<td>8.6e±2.54</td>
<td>35.2e±3.99</td>
</tr>
<tr>
<td>3.03</td>
<td>10.77±1.42</td>
<td>31.6±6.22</td>
</tr>
<tr>
<td>4.55</td>
<td>8.00±3.37</td>
<td>30.1±7.26</td>
</tr>
<tr>
<td>6.07</td>
<td>11.5±0.96</td>
<td>36.1±8.10</td>
</tr>
</tbody>
</table>

Note: NUX: Strychnos nux-vomica.

As shown in Figure 7, the plasma concentrations of brucine and strychnine were significantly changed by the addition of the P-gp inhibitor, indicating that brucine and strychnine might be substrates for P-gp.

### Effects of TJs on the intestinal transport of brucine and strychnine

A possible mechanism of intestinal absorption is the loosening or blocking of TJs of the intestinal epithelium by drugs, which consequently increases intestinal absorption via the paracellular pathway. EDTA-Na2 is a TJ regulator that can promote paracellular permeability for the intestinal absorption of drugs by opening cellular TJs. To confirm this possibility, Papp and K<sub>a</sub> of brucine and strychnine in the presence of EDTA-Na2 (1 mmol/L) were measured using the in situ single-pass intestinal perfusion model (Table 5).

### Expression of P-gp

Figure 8 shows the effects of different types of drug systems (A-F) on P-gp expression. The relative quantification of P-gp was analysed using the ChemiDoc™ XR system, as shown in Figure 8.

<table>
<thead>
<tr>
<th>pH</th>
<th>K&lt;sub&gt;j&lt;/sub&gt;/10&lt;sup&gt;-3&lt;/sup&gt; cm·s&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>P&lt;sub&gt;j&lt;/sub&gt;/10&lt;sup&gt;-3&lt;/sup&gt; cm·s&lt;sup&gt;-1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brucine</td>
<td>Strychnine</td>
</tr>
<tr>
<td>5.0</td>
<td>10.2±2.69</td>
<td>8.9±3.07</td>
</tr>
<tr>
<td>6.8</td>
<td>15.5±4.06</td>
<td>30.6±8.76</td>
</tr>
<tr>
<td>7.4</td>
<td>26.8±5.94</td>
<td>52.6±12.92</td>
</tr>
</tbody>
</table>

Notes: NUX: Strychnos nux-vomica. The herb concentration of NUX was 3.03 g/L. Differences between groups were statistically analysed using Student’s t-test. *P < 0.05, **P < 0.01, compared with the pH 5.0 group; *P < 0.05, **P < 0.01, compared with the pH 6.8 group.
ment. The everted sac model is a useful and simple in vitro model for assessing drug absorption, and it is often applied to choose an intestinal segment for further study. The in situ single-pass perfusion animal model can simulate the human intestinal absorption process for oral drugs and maintain the integrity of the blood vessels and tissues in rats. \(^{26,54}\) We also determined portal vein blood drug concentrations using the in situ single-pass perfusion animal model to overcome the limitations of the everted sac and in situ single-pass perfusion animal models. The results for the plasma concentrations of brucine and strychnine all correlated with the results of the everted gut sac and in situ single-pass intestinal perfusion models, which demonstrates the reliability of these results.

We also studied factors that might affect the intestinal absorption of brucine and strychnine, including the stability of brucine and strychnine and intestinal membrane damage, in the basic study. The results allowed us to exclude the interference of these three factors with the experimental results.

To explore the compatibility of RAM and NUX, we investigated the absorptive mechanism of NUX from the following four perspectives: concentration, pH, P-gp and TJs.

pH affects the intestinal absorption of a drug. In the fasting state, the pH is approximately 4.6 in the duodenum, and it then gradually increases from 6.0 to 8.0 from the jejunum to the ileum. The pH of the colon ranges between 7 and 8.35. We investigated the intestinal absorption of brucine and strychnine at pH 5.0, 6.8 and 7.4, and the results indicated that pH 7.4 was optimal. According to the pH partition hypothesis, 36 the degree of the dissociation of a drug is different at various pH values. Brucine and strychnine are alkaloids, and their degree of dissociation is low; thus, brucine and strychnine dissociate to their molecular forms more readily under alkaline conditions.

Five crude NUX concentrations were studied. The results indicated that Papp and Ka of brucine and strychnine did not significantly differ over a crude drug concentration range of 1.52-6.07 g/L, which illustrated that concentration saturation or dependency of the absorption of brucine and strychnine did not occur. These results were in accordance with Fick’s diffusion principle. These findings suggested that passive diffusion occurs during intestinal transport. Moreover, two channels exist for passive transport: the paracellular and transcellular routes. The paracellular route is more suited for transporting hydrophilic drugs. Because brucine and strychnine are lipophilic and rapidly absorbed drugs, their transport could therefore occur via the transcellular route. EDTA-Na\(_2\), a TJ regulator, can promote paracellular permeability and reduce the cellular area by opening cellular TJs, thereby decreasing the intestinal absorption of drugs transported via the transcellular route. Therefore, the absorption of brucine and strychnine was slightly reduced after the addition of EDTA-Na\(_2\).

P-gp, an ATP-dependent multidrug efflux pump, can function as an absorption barrier to decrease the absorption efficiency of orally absorbed drugs by transporting them into the intestinal lumen.\(^{55}\) Our results illustrated that verapamil significantly increased the absorption of brucine and strychnine, suggesting the participation of active transport during the intestinal transport of brucine and strychnine.

In summary, active transport and passive diffusion both occur during the intestinal transport of brucine and strychnine, but transport is dominated by passive diffusion. We concluded from the study of intestinal transport that the RAM extract inhibited the absorption of brucine and strychnine, and the primary absorption mechanism for brucine and strychnine was passive transport, which was affected by P-gp. Western blotting was used to study P-gp expression in Caco-2 cells, and the results indicated that the compatibility of NUX and RAM enhanced the expression of P-gp and promoted the efflux of toxic components in NUX, thus reducing its toxicity.

The RAM extract inhibited the intestinal absorption of brucine and strychnine from NUX. The main absorp-

---

**Table 4 Effect of P-gp on the intestinal transport of brucine and strychnine ( \( \bar{x} \pm s \) )**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>( K_i \times 10^{-3} \cdot s^{-1} )</th>
<th>( P_{app} \times 10^{-6} \cdot cm \cdot s^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Brucine</td>
<td>Strychnine</td>
</tr>
<tr>
<td>NUX (3.03 g/L)</td>
<td>6</td>
<td>11.5±5.8</td>
<td>34.8±5.2</td>
</tr>
<tr>
<td>NUX (3.03 g/L)-Ver (200 μmol/L)</td>
<td>6</td>
<td>29.1±7.3</td>
<td>46.5±6.7</td>
</tr>
</tbody>
</table>

Notes: NUX: Strychnos nux-vomica; RAM: Baizhu (Rhizoma Atractylodis Macrocephalae); Ver: verapamil. NUX (3.03 g/L) means the herb concentration of NUX was 3.03 g/L, NUX (3.03 g/L)-Ver (200 μmol/L) means the herb concentration of NUX was 3.03 g/L and the concentration of Ver was 200 μmol/L. Differences between groups were statistically analysed using Student’s t-test. *\( P < 0.05 \), compared with the NUX (3.03 g/L) group.

---

**Figure 6 Effect of pH of the NUX extract on the plasma concentrations of brucine and strychnine**

NUX: Strychnos nux-vomica. The data were expressed as the mean ± standard deviation (n = 6).
Chen J, Bisset NG, Hylands PJ. Protostrychnine, a Chinese Pharmacopoeia Commission. Pharmacopoeia of complex formulae. The elucidated compatibility mechanism, and it can reflect the characteristics of commercial Chinese Medicine, compatibility plays an essential role, and it might be attributable to the activity of P-gp. In Traditional Chinese Medicine, compatibility plays an essential role, and it can reflect the characteristics of complex formulae. The elucidated compatibility mechanism could provide useful information for the further development of novel drugs and could serve as a simpler experimental starting point for studying more complex formulae.

REFERENCES


19 Liang XD. Experimentation of decreasing toxicity and increasing efficacy on compatibility of Strychnos nux vomica and Atractylodes macrocephala koidz. Shi Zhen Guo Yi Guo Yao 2014; 25(10): 2364-2366.


Table 5 Effects of cellular TJs on the intestinal transport of brucine and strychnine ( ± s )

<table>
<thead>
<tr>
<th>Group</th>
<th>K (×10⁻¹·s⁻¹)</th>
<th>P cm⁻²·s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>NUX (3.03 g/L)</td>
<td>15.3±3.5</td>
<td>4.9±1.4</td>
</tr>
<tr>
<td>NUX (3.03 g/L)-EDTA-Na₂ (1 mmol/L)</td>
<td>13.8±4.1</td>
<td>44.4±12.6</td>
</tr>
</tbody>
</table>

Notes: TJ: tight junctions; NUX: Strychnos nux-vomica.


