Comparative studies on the toxicological, anti-inflammatory and analgesic properties of three sources of Xuedan in mice and their rapid identification by electronic tongue

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Abstract

Purpose: To compare the toxicological, anti-inflammatory and analgesic properties of three sources of Xuedan, viz, Hemsleya omeiensis (HO), Hemsleya giganta (HG) and Hemsleya dolichocarpa (HD) in mice, and to study their rapid identification based on electronic tongue (E-tongue).

Methods: After 7 days of administration, the median lethal doses (LD₅₀) of the three xuedan decoctions in mice were determined. In addition, the anti-inflammatory and analgesic effects of the three xuedans were evaluated in mice using xylene-induced ear edema and acetic acid-induced pain. Furthermore, E-tongue technology was used to identify HO, HG and HD. Principal component analysis (PCA) and discriminant factor analysis (DFA) were used to analyze the data acquired by E-tongue.

Results: The median lethal dose (LD₅₀) values of H. omeiensis, H. giganta and H. dolichocarpa were 32.3, 17.4 and 13.7g/kg, respectively. Compared with normal control group, the anti-inflammatory effects of Xuedan were obvious in xylene-induced ear edema (p < 0.05), and pain sensation was significantly inhibited in acetic acid-induced writhing test (p < 0.05). Furthermore, E-tongue technology effectively identified HO, HG and HD.

Conclusion: H. omeiensis exhibits the highest LD₅₀ value and best analgesic effect among the three sources of xuedan. E-tongue technology is effective and rapid in identifying HO, HG and HD.

Keywords: Xuedan, Hemsleya omeiensis, Hemsleya giganta, Hemsleya dolichocarpa, Anti-inflammation, Analgesia, Electronic tongue

INTRODUCTION

Xuedan is derived from Hemsleya (Cucurbitaceae) which is distributed in subtropical and temperate regions of Asia, especially in China [1]. In Chinese Traditional medicinal theory system, xuedan is used for clearing heat and removing toxins. Xuedan exerts antibacterial and anti-inflammatory effects. Consequently, xuedan is used for treating...
various diseases such as hepatitis, enteritis, bronchitis and coronary heart disease [2]. In China, xuedan comes from three varieties of Hemsleya viz. Hemsleya omeiensis, Hemsleya gigantha and Hemsleya dolichocarpa. Studies have revealed that there are wide differences in compositions and in bioactivities among the three sources of xuedan, especially with respect to their bitter taste. However, no studies have been carried out on effective and rapid strategy for discriminating between the three sources of xuedan. The present study was carried out to compare the toxicology and pharmacology of the three sources of xuedan, and to develop a rapid method based on electronic tongue for their identification.

EXPERIMENTAL

Plant materials

The three varieties of xuedan used in this study were provided by Sichuan Fuzheng Pharmaceutical Co., Ltd., and identified by Professor Xian-Ming Lu (College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, China).

Animal studies

A total of 200 mice (18 - 22 g) were obtained from the Chengdu DaShuo Experimental Animal Co., Ltd. (Chengdu, China). All animals received humane care according to the Declaration of Helsinki [3], and fed in accordance with standard laboratory protocols. All experimental protocols followed the principles and guidelines recommended by the Animal Care and Use Committee of Chengdu Municipal Hospital of Traditional Chinese Medicine, Chengdu, China [approval no. SCXK (chuan) 2015-3].

Acute toxicity studies

Acute toxicity studies were carried out in accordance with the procedure reported in previous studies, but with minor modifications [4-6]. First, the complete lethal dose (LD$_{100}$) and zero (0), i.e., lethal dose-0 (LD$_0$) were determined through oral administration of the test samples. The results showed that the LD$_{100}$ and LD$_0$ values for H. omeiensis were 48 and 24 g/kg, respectively; and LD$_{100}$ and LD$_0$ values for H. gigantha and H. dolichocarpa were 24 and 6 g/kg, respectively. Based on these results, mice were randomly divided into five groups (n = 8) for the testing of Xuedan samples.

The mice were fasted overnight but were permitted free access to water, and were administered the designed dosages (Table 1) orally (intragastrically). The mice were observed for signs of poisoning or death for 14 days. Mortality was calculated from the number of mouse deaths in each group, and LD$_{50}$ was calculated using the weighted regression probability unit method (Bliss method) with LD$_{50}$ calculation version 2.0 software. The 95% confidence interval was calculated using the improved method of Käber [6].

Anti-inflammatory studies

Mice were randomly divided into four groups (n = 10): control group, H. omeiensis group, H. gigantha group and H. dolichocarpa group. The test samples were administered orally at a dose of 0.6 g/kg (equivalent to daily human adult dose) for 7 continuous days. Then, 30 min after the last administration, 20 µL of xylene was uniformly applied to both sides of the left auricle of each mouse, while the right ear was used as a control. After 30 min, the mice were euthanized. The ears were cut along the baseline of the auricle. The circular ear was punched in the same part of the left and right ears with an 8 mm diameter puncher [7]. The ears were weighed with an electronic balance, and the weight difference of the two ears (W$_l$ - W$_r$) was used as swelling index (Es), calculated as in Eq 1.

\[
Es = W_l - W_r \ldots \ldots \ldots \ldots \ldots (1)
\]

Inhibition of swelling (Pi) was calculated using Eq 2.

\[
Pi (%) = \{(Esc - Est)/Esc\}100 \ldots \ldots \ldots \ldots \ldots (2)
\]

where Esc and Est are the swelling index of control and test groups, respectively.

Analgesic studies

Mice were randomly divided into four groups (n = 10): control group, H. omeiensis group, H. gigantha group and H. dolichocarpa group. The mice in each group were orally administered corresponding test samples at a dose of 0.6 g/kg/day (equivalent to the daily adult human dose) continuously for 7 days. Then, one hour after the last administration, each mouse was treated with 0.6 % acetic acid (0.1 mL/kg) by intraperitoneal injection. The number of writhes (Nw) (abdominal contraction and extension of hind limbs) within 15 min after acetic acid injection in each group of mice was recorded, and the percentage inhibition (Pi) was calculated as in Eq 3 [8].

\[
H (%) = \{(NWc - Nwt)/NWc\}100 \ldots \ldots \ldots \ldots (3)
\]
where Nwc and Nwt are the number of writhes of control and test groups, respectively.

**Electronic tongue test**

The αAstree E-tongue, used to investigate the taste of the three varieties of Xuedan, had six cross-sensitive potential sensors (ZZ, AB, GA, CA, DA and JE), Alphasoft data acquisition system, signal processing system and pattern recognition software (Alpha M.O.S, Version 2012.45). The method [8] measures E-tongue under conditions that ensure that the acquired data are reliable and stable. Each test sample powder (1.0 g) was extracted with 200 mL boiled water in a conical flask for 3 h. Thereafter, it was filtered through a 0.22 µL microporous filter membrane, and 25 mL of the filtrate was placed in the auto-sampler tray of the E-tongue apparatus for analysis. Each sample was analyzed for 120 sec in triplicate. The data were recorded using Astree II software.

**Statistical analysis**

Data are expressed as mean ± standard deviation (SD), and were analyzed using SPSS 21.0 software with single factor analysis of variance between groups. Values of \( p < 0.05 \) were considered statistically significant. All E-tongue data were analyzed using Alpha M.O.S. statistical software. Principal component analysis (PCA) and discriminant factor analysis (DFA) were used for discrimination analysis of the samples.

**RESULTS**

**Acute toxicity**

As can be seen from the Table 1, LD\(_{50}\) values of the H. omeiensis, H. giganta, H. dolichocarpa were 32.3, 17.4 and 13.7 g/kg, respectively. All samples showed moderate toxicity, but the H. omeiensis showed the lowest toxicity in mice, when compared to the other two sources of xuedan (32.3 g/kg vs. 17.4 and 13.7 g/kg).

**Anti-inflammatory effects**

The results of anti-inflammatory studies of the three xuedan sources are shown in Table 2. Compared with the normal group, the three aqueous extracts of samples significantly inhibited xylene-induced ear swelling in mice (\( p < 0.01 \)). There was no significant difference in percent inhibition of swelling between the three samples.

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**Table 1**: LD\(_{50}\) values of the three sources of xuedan (n = 8)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (g/kg)</th>
<th>Mortality (%)</th>
<th>LD(_{50}) (g·(kg·day(^{-1}))</th>
<th>95 % confidence limit (g·(kg·day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. omeiensis</td>
<td>20.48</td>
<td>0</td>
<td>32.3</td>
<td>28.8-36.1</td>
</tr>
<tr>
<td></td>
<td>25.6</td>
<td>0</td>
<td>32</td>
<td>28.8</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>87.5</td>
<td>30</td>
<td>25.6</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>100</td>
<td>25</td>
<td>17.4</td>
</tr>
<tr>
<td>H. giganta</td>
<td>10.24</td>
<td>0</td>
<td>17.4</td>
<td>15.6-19.4</td>
</tr>
<tr>
<td></td>
<td>12.8</td>
<td>0</td>
<td>16</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>87.5</td>
<td>25</td>
<td>13.7</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>100</td>
<td>20</td>
<td>13.7</td>
</tr>
<tr>
<td>H. dolichocarpa</td>
<td>10.24</td>
<td>0</td>
<td>13.7</td>
<td>11.8-16.0</td>
</tr>
<tr>
<td></td>
<td>12.8</td>
<td>62.5</td>
<td>16</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>62.5</td>
<td>20</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>100</td>
<td>25</td>
<td>11.8</td>
</tr>
</tbody>
</table>

**Table 2**: Effect of the three sources of xuedan on ear edema induced by xylene in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dosage (g/kg)</th>
<th>Swelling (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>--</td>
<td>76.43 ± 6.05</td>
<td>--</td>
</tr>
<tr>
<td>H. omeiensis</td>
<td>0.6</td>
<td>54.85 ± 7.17</td>
<td>23.93</td>
</tr>
<tr>
<td>H. giganta</td>
<td>0.6</td>
<td>62.88 ± 6.08</td>
<td>25.36</td>
</tr>
<tr>
<td>H. dolichocarpa</td>
<td>0.6</td>
<td>59.43 ± 7.26</td>
<td>27.58</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD (n =10); * \( p < 0.05 \), ** \( p < 0.01 \), compared with normal
Analgesic activity

Compared with the normal group, the three varieties of *xuedan* significantly reduced the number of acetic acid-induced writhes in mice \((p < 0.05)\), and the effect of HO was the most significant \((p < 0.01)\). There were significant differences in the percentage inhibition of writhes among HO, HG and HD \((p < 0.05)\) (Table 3).

E-tongue results

Typical sensor responses recorded by E-tongue apparatus are shown in Figure 1. An average value was acquired based on the stable sensor responses of E-tongue analyses from 100 to 120 sec, and was used as the output data. The repeatability of the E-tongue results using this method of study is shown in Table 4.

![Figure 1: Typical sensor responses of E-tongue during measurement](image)

In this study, 9 batches of samples were collected for each source of *xuedan*. Each sample was measured and analyzed three times. From the signals acquired through E-tongue measurements, 81 data points were used for further PCA and DFA. The raw data was projected onto the PC axis, and each sample will a set on the PCA three-dimensional map. The distance between sample sets is usually used to indicate similarities in taste. The three-dimensional map of PCA is shown in Figure 2, and the three principal components were PC1 (82.83 %), PC2 (16.21%), and PC3 (0.66 %). The total degree of contribution of the three principal components was 99 %.

![Figure 2: PCA score plots of E-tongue measurements of three varieties of *Hemsleya chinensis* (n = 9). Each sample was measured and analyzed three times. HO, HG and HG refer to *H. omeiensis*, *H. Giganta*, *H. dolichocarpa*, respectively](image)

Similar to PCA, a total 81 data points were projected onto the DFA score plots. As can be seen in Figure 3, the two principal components for E-tongue were DF1 = 92.36 % and DF2 = 7.64 %, and their total contribution was 100 %. The DFA model effectively classified the three sources *xuedan*. In addition, DFA is often used to establish a sample database for qualitative discrimination between unknown samples and determination of grouping information of unknown samples. This model can be used to identify samples from unknown base sources of *xuedan*.

**Table 3:** Effects of the three sources of *xuedan* on number of writhes in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (g/kg)</th>
<th>Number of writhes</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>--</td>
<td>37.3 ± 6.86</td>
<td>--</td>
</tr>
<tr>
<td><em>H. omeiensis</em></td>
<td>0.6</td>
<td>19.2 ± 5.53</td>
<td>48.53</td>
</tr>
<tr>
<td><em>H. Giganta</em></td>
<td>0.6</td>
<td>28.2 ± 6.05</td>
<td>26.66</td>
</tr>
<tr>
<td><em>H. dolichocarpa</em></td>
<td>0.6</td>
<td>30.6 ± 5.54</td>
<td>17.96</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD \((n =10)\); * \(p < 0.05\), ** \(p < 0.01\), compared with normal.

**Table 4:** Repeatability of E-tongue determination method \((n = 6)\)

<table>
<thead>
<tr>
<th>Sensor</th>
<th>CA</th>
<th>ZZ</th>
<th>AB</th>
<th>GA</th>
<th>DA</th>
<th>JE</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSD (%)</td>
<td>1.24</td>
<td>0.61</td>
<td>0.19</td>
<td>0.30</td>
<td>0.50</td>
<td>0.28</td>
</tr>
</tbody>
</table>
which have moderate bitter taste instead of extreme bitter taste. In addition, it is produces better bioactive effects, relative to the other two sources. In view of these desirable qualities, a proposal to distinguish *H. omeiensis* from the other two sources of *xuedan* was designed in the present study. The bitter taste is the key point for quick and effective discrimination of the 3 sources of *xuedan*.

Electronic tongue technology is an objective quantitative evaluation method for sensory indicators. By collecting objective and accurate data, the results of the taste characteristics of samples are obtained, and the differences in the taste of different samples are scientifically reflected. It is increasingly used in the Chinese medicine industry. These results suggest that E-tongue combined with PCA can effectively discriminate between the three varieties of *Xuedan*, especially *H. omeiensis* which differs in taste from the other two sources of *Xuedan*.

DFA is a classification technique that optimizes differentiation by recombining sensor data and is another classical analysis model in addition to PCA. It can be used to establish a sample database for qualitative discrimination between samples from unknown base sources of *xuedan*. This study has demonstrated that electronic tongue technology, when combined with PCA and DFA can effectively distinguish among *Xuedans* from the three varieties of *Hemsleya*.

**CONCLUSION**

*Hemsleya omeiensis* possesses the highest LD$_{50}$ value and best analgesic effect among the three varieties of *Hemsleya*. In addition, E-tongue, when combined with PCA and DFA, can effectively and rapidly discriminate between the three sources of *xuedan*.

**DECLARATIONS**

**Acknowledgement**

This study was supported by Science and Technology Plan Projects in Sichuan Province-Technical Standards and Technical Specifications and Quality Standards for Innovative Processing of *xuedan* (no. 18ZDZX0063).

**Conflict of interest**

No conflict of interest is associated with this work.
Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. All authors read and approved the manuscript for publication.

REFERENCES