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ARTICLE

HPLC Study on HPLC Characteristic Spectrum of Huoxiang (Herba Agastachis) Eliminating Summer-heat Soft Capsule

Jing Wang*

Wuhan Institute for Drug and Medical Device Control, Wuhan, Hubei, China

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ABSTRACT

To establish HPLC characteristic spectrum of Huoxiang (Herba Agastachis) Eliminating Summer-heat Soft Capsule. Chromatographic separation was performed on Tech Mate C18-ST (5 μm, 4.6×250 mm) column by Agilent 1260 high performance liquid chromatograph, eluted with acetonitrile and 0.05% phosphoric acid in a gradient elution at a flow rate of 1.0 mL/min. The detection wavelength was set at 270 nm. Hesperidin was selected as the reference peak to calculate the RSD of the relative retention time. Precision, stability and reproducibility were investigated as well. 80 different samples from 5 manufacturers were included in this study. The HPLC characteristic spectrum of Huoxiang Eliminating Summer-heat Soft Capsule was constructed with 11 specific chromatographic peaks in 80 samples. The method is accurate, reliable and with good reproducibility, and could overall control the quality of Huoxiang Eliminating Summer-heat Soft Capsule.

1. Introduction

Huoxiang (Herba Agastachis) Eliminating Summer-heat Soft Capsule is a commonly-used drug relieving summer-heat in summer, which can dispel summer heat and resolve dampness, relieve exterior syndrome and regulate the middle warmer and thus is mainly used for intrinsic wet hysteresis, fever with aversion to cold caused by heatstroke and cold, headache and adiaphaneustia, aching and tired limbs, nausea and vomiting, abdominal pain and diarrhea and other symptoms [1]. The prescription of Huoxiang Eliminating Summer-heat Soft Capsule includes 12 medicinal materials including Pogostemonis Herba; Moslae Herba; Angelicae Dahuricae Radix; Perillae Folium; Atractylodis Rhizoma; Caryophylli Flos; Citri Reticulatae Pericarpium; Arecae Pericarpium; Poria; Glycyrrhizae Radix Et Rhiza; Pinelliae Rhizoma Praeparatum and Zingiberis Rhizoma Recens [1]. At present, current standards only test content of hesperidin in the preparation [2,3], while quality standard evaluation of traditional Chinese medicine is developing from single component analysis to multi-index and multi-component analysis because single component of one medicinal material cannot effectively controls quality of preparations [4-7]. High performance liquid chromatography (HPLC) and high performance liquid chromatography/diode array detector (HPLC/DAD) are the most effective methods used in Characteristic Spectrum analysis and quality control of Chinese traditional medicine [8-10]. Hence, this project study characteristic chromatogram of Huoxiang Eliminating Summer-heat Soft Capsule by HPLC/DAD and provide methods and

*Corresponding Author:
Jing Wang,
Wuhan Institute for Drug and Medical Device Control, Wuhan, Hubei, China;
Email: whdxwj75@163.com
theoretical basis for comprehensive quality control of Huoxiang Eliminating Summer-heat Soft Capsule.

2. Experimental

2.1 Materials and Reagents

All experiments were performed using ‘A class’ volumetric glassware. Acetonitrile (HPLC grade / chromatographically pure, MERCK), and other analytical grade reagents (China National Pharmaceutical (Group) Shanghai Chemical Reagent Company) were used in the preparation of the mobile phase for gradient elution. HPLC grade Milli Q water was used in the preparation of the mobile phase for gradient elution. The mobile phase was filtered through a 0.45 µm PVDF filter (Welch Materials, Inc.) and degassed under vacuum, prior to use. For the preparation of the reference solution and reference crude herb solution, pharmaceutical grad reference standards were used.

Liquiritin (C21H22O9, lot number: 111610-201106); Ammonium Glycyrrhizinate (C42H65NO16∙5H2O, lot number:110731-201116); Hesperidin (C28H34O15, lot number: 110721-201316); Eugenol (C10H12O2, lot number: 110725-201112); Imperatorin (C16H14O4, lot number: 0826-9502) were provided by National institutes for food and drug control.

Pogostemonis Herba (lot number: 121135-201005); Moslae Herba (lot number: 121456-200401); Angelicae Dahuricae Radix (lot number: 120945-201309); Perillae Folium (lot number: 120914-201411); Atractylodis Rhizoma (lot number: 120932-201407); Caryophylli Flos (lot number: 1039-9701); Citri Reticulatae Pericarpium (lot number: 120969-201109); Arecae Pericarpium (lot number: 121584-201402); Poria (lot number: 12117-201308); Glycyrrhizae Radix Et Rhizoma (lot number: 120904-201519) were provided by National institutes for food and drug control. The other two medicinal materials, Pinelliae Rhizoma Praeparatum and Zingiberis Rhizoma Recens were provided by the manufacturer.

2.2 Analytical Solutions

Test Sample Solution

Took 2 g of drug, accurately weighed, placed in 100 ml stoppered conical flask, added 70% methanol 50 ml, sonicated for 30 min, shook, filtered through a 0.45 µm PVDF filter.

Reference Crude Herb Solution

The control medicinal materials extract was prepared by the preparation process of Huoxiang Eliminating Summer-heat Soft Capsule, which included Pogostemonis Herba; Moslae Herba; Angelicae Dahuricae Radix; Perillae Folium; Atractylodis Rhizoma; Caryophylli Flos; Citri Reticulatae Pericarpium; Arecae Pericarpium; Poria; Glycyrrhizae Radix Et Rhizoma; Pinelliae Rhizoma Praeparatum and Zingiberis Rhizoma Recens and each of them was prepared into control medicinal material solution according to the preparation method of sample solution.

Stock Solutions

Stock solution of each single reference substance (Liquiritin; Ammonium Glycyrrhizinate; Hesperidin; Eugenol; Imperatorin) was suitably diluted by 70% methanol to get concentrations of 0.8 mg ml\(^{-1}\) respectively.

Reference Substance Solution

A solution containing 40 µg ml\(^{-1}\) of mixed reference substances was used as a reference solution.

3. Results

3.1 The Choice of Measuring Wavelength

Huoxiang Eliminating Summer-heat Soft Capsule contains 12 medicinal materials, each of which has a great difference in ultraviolet absorption wavelength. For example, “Chinese Pharmacopoeia” 2015 Edition predetermined detection the wavelength of Liquirtin, Chenpi (Citri reticulatae pericarpium), Baizhi (Angelicae dahuricae radix), Cangzhu (Atractylodis rhizoma) are 237 nm, 283 nm, 300 nm, 340 nm respectively. In order to detect two or more different ingredients and reduce interference, five wavelengths were chosen for investigation (237 nm, 250 nm, 280 nm, 300 nm, 270 nm), through experimental comparison, the chromatographic peak information obtained at 270 nm is relatively rich, and its peak height is relatively homogenous, thus it selected as the most suitable wavelength for the test. (Figure 1, wavelength Selection investigation).

3.2 The Choice of Mobile Phase for Gradient Elution

Acetonitrile-0.05% phosphoric acid was chosen as flow phase system \([2,11]\), and three gradient of elution methods were investigated. The gradient elution programs are showed in Table 1 as follow. According to experimental results (Figure 2), chromatographic peaks of Elution Method 3 had good degree of separation, and its elution baseline was flat. Thus, the Elution Method 3 was chosen as formal elution method.
Figure 1. Wavelength Selection investigation

Figure 2. Chromatogram of three gradient elution methods(1 Gradient elution program ①, 2 Gradient elution program ②, 3 Gradient elution program ③) 

Figure 3. Chromatogram of three sample extraction method(1 sample extraction method ①, 2 sample extraction method ②, 3 sample extraction method ③)
### Table 1. Gradient elution program

<table>
<thead>
<tr>
<th>Gradient elution program ①</th>
<th>Time/min</th>
<th>Acetonitrile (%)</th>
<th>0.05% Phosphoric acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 ~ 33</td>
<td>5 → 40</td>
<td>95 → 60</td>
</tr>
<tr>
<td></td>
<td>33 ~ 50</td>
<td>40 → 75</td>
<td>60 → 25</td>
</tr>
<tr>
<td></td>
<td>50 ~ 62</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>62 ~ 64</td>
<td>75 → 20</td>
<td>25 → 80</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gradient elution program ②</th>
<th>Time/min</th>
<th>Acetonitrile (%)</th>
<th>0.05% Phosphoric acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 ~ 5</td>
<td>5 → 10</td>
<td>95 → 90</td>
</tr>
<tr>
<td></td>
<td>5 ~ 30</td>
<td>10 → 20</td>
<td>90 → 80</td>
</tr>
<tr>
<td></td>
<td>30 ~ 35</td>
<td>20 → 30</td>
<td>80 → 70</td>
</tr>
<tr>
<td></td>
<td>35 ~ 37</td>
<td>30 → 45</td>
<td>70 → 55</td>
</tr>
<tr>
<td></td>
<td>37 ~ 55</td>
<td>45 → 75</td>
<td>55 → 25</td>
</tr>
<tr>
<td></td>
<td>55 ~ 60</td>
<td>75 → 5</td>
<td>25 → 95</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gradient elution program ③</th>
<th>Time/min</th>
<th>Acetonitrile (%)</th>
<th>0.05% Phosphoric acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 ~ 30</td>
<td>5 → 30</td>
<td>95 → 70</td>
</tr>
<tr>
<td></td>
<td>30 ~ 35</td>
<td>30 → 40</td>
<td>70 → 60</td>
</tr>
<tr>
<td></td>
<td>35 ~ 55</td>
<td>40 → 75</td>
<td>60 → 25</td>
</tr>
<tr>
<td></td>
<td>55 ~ 60</td>
<td>75 → 5</td>
<td>25 → 95</td>
</tr>
</tbody>
</table>

#### 3.3 Suitable Sample Extraction Method

The aim of this study was to find a suitable sample extraction method. Three parallel samples (Pharmaceutical manufacturer A, batch number: 127160205) with 2 g each was taken for the follow three extraction methods. ① Add 25 ml petroleum ether, shake for 30 min, filter, discard the petroleum ether solution, evaporate the filter residue, add 50 ml methanol, and ultrasonic treatment for 30 min; ② 70% methanol ultrasonic treatment for 30 minutes; ③ 50 ml pure methanol ultrasonic treatment for 30 minutes [2,11,12]. According to experimental results shown in Figure 3, method ① has fewer chromatographic peaks and insufficient peak information. Although method ② and method ③ have little difference in liquid chromatograms, the baseline of chromatogram of method ② is more smoother than method ③. Thus, this method (method ②) was selected as extraction method of test sample solution.

#### 3.4 Investigation the Concentration Condition of Phosphoric Acid Solution in Mobile Phase

Since the concentration of phosphoric acid varies slightly in each actual operation, it is necessary to investigate whether the chromatographic peak information is different under the condition of different concentration of phosphoric acid when phosphoric acid is used as the mobile phase the concentrations of 0.04%, 0.05% and 0.06 phosphoric acid were investigated in the experiment. According to experimental results (Figure 4), it shows little difference in liquid chromatogram obtained from three phosphoric acid solution concentrations, which does not affect overall information of chromatographic peaks.

![Figure 4. Chromatogram of three concentrations of phosphoric acid (1, 0.04%, 2, 0.05%, 3, 0.06%)](https://doi.org/10.30564/jim.v10i1.2955)
Considering the simplicity of calculation, the 0.05% phosphoric acid was used in the subsequent experiments.

3.5 Identification and Attribution of Characteristic Peaks

Preparation of solution: according to analytical solutions preparation, test sample solution, reference crude herb solution and reference substance solution were prepared.

After successively prepared test sample solution, reference crude herb solution and reference substance solution, the solutions were tested under chromatographic conditions of characteristic chromatogram. By comparing the chromatogram of three solutions, that include test sample, reference crude herb and reference substance, and their DAD (Diode Array Detector) scanning (200 nm-400 nm) results, the characteristic peaks and each peak were affirmed. A total of 11 characteristic peaks were found in the chromatogram of the test sample (Figure 5) after comparing with chromatographic peaks of reference crude herb, which respectively represented *Caryophylli Flos* (Peak 1), *Glycyrrhizae Radix Et* (Peak 2, Liquiritin), *Caryophylli Flos* (Peak 3), *Moslae Herba* (Peak 4), *Citri Reticulatae Pericarpium* (Peak 5, Hesperidin), *Caryophylli Flos* (Peak 6, Eugenol), *Glycyrrhizae Radix Et Rhizoma* (Peak 7, Ammonium Glycyrrhizinate), *Angelicae Dahuricae Radix* (Peak 8, Imperatorin), *Angelicae Dahuricae Radix* (Peak 9), *Atractylodis Rhizoma* (Peak 10 and 11).

The chromatographic peak of Hesperidin (Peak 5) was glaringly obvious in the chromatogram, so the Hesperidin peak (Peak 5) was chosen as the S peak. A solution containing 40 μg ml-1 of Hesperidin was used as a reference solution in identification experiment mentioned above.

![Figure 5. Chromatogram of test sample, reference crude herb and reference substance](image)

![Figure 6. Typical chromatogram of sample characteristic spectrum](image)
<table>
<thead>
<tr>
<th>Peak Number</th>
<th>relative retention time</th>
<th>The RSD of relative retention time</th>
<th>± 5% range of relative retention time</th>
<th>Identification of peak</th>
<th>Identification of crude herb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2351</td>
<td>1.75%(n=80)</td>
<td>0.2235-0.2471</td>
<td>Peak 1</td>
<td>Caryophylli Flos</td>
</tr>
<tr>
<td>2</td>
<td>0.8407</td>
<td>0.19%(n=75)</td>
<td>0.7987-0.8827</td>
<td>Peak 2 (Liquiritin)</td>
<td>Glycyrrhizae Radix Et Rhizoma</td>
</tr>
<tr>
<td>3</td>
<td>0.8668</td>
<td>0.26%(n=80)</td>
<td>0.8236-0.9102</td>
<td>Peak 3</td>
<td>Caryophylli Flos</td>
</tr>
<tr>
<td>4</td>
<td>0.9005</td>
<td>0.27%(n=80)</td>
<td>0.8556-0.9456</td>
<td>Peak 4</td>
<td>Moslae Herba</td>
</tr>
<tr>
<td>5</td>
<td>1.0000</td>
<td>0.00%(n=80)</td>
<td>0.9500-1.0500</td>
<td>Peak 5 (Hesperidin)</td>
<td>Citri Reticulatae Pericarpium</td>
</tr>
<tr>
<td>6</td>
<td>1.5399</td>
<td>0.40%(n=80)</td>
<td>1.4632-1.6172</td>
<td>Peak 6 (Eugenol)</td>
<td>Caryophylli Flos</td>
</tr>
<tr>
<td>7</td>
<td>1.6215</td>
<td>0.29%(n=80)</td>
<td>1.5408-1.7030</td>
<td>Peak 7 (Ammonium Glycyrrhizinate)</td>
<td>Glycyrrhizae Radix Et Rhizoma</td>
</tr>
<tr>
<td>8</td>
<td>1.8456</td>
<td>0.31%(n=75)</td>
<td>1.7533-1.9379</td>
<td>Peak 8 (Imperatorin)</td>
<td>Angelicae Dahuricae Radix</td>
</tr>
<tr>
<td>9</td>
<td>1.8959</td>
<td>0.23%(n=75)</td>
<td>1.8011-1.9907</td>
<td>Peak 9</td>
<td>Angelicae Dahuricae Radix</td>
</tr>
<tr>
<td>10</td>
<td>2.0300</td>
<td>0.21%(n=75)</td>
<td>1.9285-2.1315</td>
<td>Peak 10</td>
<td>Atractylodis Rhizoma</td>
</tr>
<tr>
<td>11</td>
<td>2.1836</td>
<td>0.30%(n=66)</td>
<td>2.0744-2.2928</td>
<td>Peak 11</td>
<td>Atractylodis Rhizoma</td>
</tr>
</tbody>
</table>

![Figure 7. DAD scan pattern of chromatographic peaks](https://doi.org/10.30564/jim.v10i1.2955)
The HPLC characteristic spectrum of the drug should be similar to the control characteristic map, and should show 11 chromatographic peaks. The retention time of peak 5 and the peak of hesperidin should be the same as well. Using the reference hesperidin peak as the S peak calculate the relative retention time of the 11 characteristic peaks. The relative retention time of each characteristic peak should be within ± 5% of the specified value. The specified value is: 0.2351 (Peak 1), 0.8407 (Peak 2), 0.8668 (Peak 3), 0.9005 (Peak 4), 1.0000 (Peak 5, hesperidin, S), 1.5399 (Peak 6), 1.6215 (Peak 7), 1.8456 (Peak 8), 1.8959 (Peak 9), 2.0300 (Peak 10), 2.1836 (Peak 11). And the DAD scan pattern of each characteristic peak is consistent with the following pattern.

The typical chromatogram of sample characteristic spectrum is shown in Figure 6, which contains 11 characteristic peaks marked from 1 to 11. Compared chromatographic peaks between the sample and reference, the attribution of 11 characteristic peaks were determined (Table 2), and the DAD scan pattern illustrates in Figure 7.

3.6 Stability of Analytical Solutions

Stability of analytical solutions: the aim of this study is to prove the stability of the sample solution and reference solution at room temperature\textsuperscript{13}. For the study, duplicate sets of spiked sample preparations and sample preparations as per the test method were prepared and kept on a bench top (25°C ± 2°C ) and analyzed initially (0day,0h), after 2h, 4h, 8h, 12h and 24h by a single injection of each sample preparations into chromatography column, and chromatograms were recorded. Using 5# Hesperidin Peak as referenced retention time to calculate relative retention time of different common peaks. According to experimental results (Table 3), relative standard deviation on relative retention time of different common peaks was less than 2%, indicating the test sample solution basically remains stable within 24h.

3.7 Repeatability Study

Method precision (Repeatability): a group of samples were taken again to conduct repetitive investigation. Six test sample solutions were prepared and tested according to set chromatographic conditions, and calculated relative retention time of different common peaks. According to experimental results (Table 4), relative standard deviation (RSD) on relative retention time of different common peaks was less than 2%, providing the experiment has good repeatability and could be repeated.

<table>
<thead>
<tr>
<th>Number</th>
<th>0h</th>
<th>2h</th>
<th>4h</th>
<th>8h</th>
<th>12h</th>
<th>24h</th>
<th>Average value</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak 1</td>
<td>0.2312</td>
<td>0.2314</td>
<td>0.2311</td>
<td>0.2313</td>
<td>0.2318</td>
<td>0.2321</td>
<td>0.2315</td>
<td>0.16%</td>
</tr>
<tr>
<td>Peak 2</td>
<td>0.8388</td>
<td>0.8388</td>
<td>0.8386</td>
<td>0.8386</td>
<td>0.8387</td>
<td>0.8298</td>
<td>0.8389</td>
<td>0.05%</td>
</tr>
<tr>
<td>Peak 3</td>
<td>0.8654</td>
<td>0.8654</td>
<td>0.8650</td>
<td>0.8647</td>
<td>0.8650</td>
<td>0.8659</td>
<td>0.8652</td>
<td>0.05%</td>
</tr>
<tr>
<td>Peak 4</td>
<td>0.8997</td>
<td>0.8998</td>
<td>0.8996</td>
<td>0.8994</td>
<td>0.8996</td>
<td>0.9001</td>
<td>0.8997</td>
<td>0.03%</td>
</tr>
<tr>
<td>Peak 5</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
<td>0.00%</td>
</tr>
<tr>
<td>Peak 6</td>
<td>1.5340</td>
<td>1.5339</td>
<td>1.5338</td>
<td>1.5358</td>
<td>1.5348</td>
<td>1.5351</td>
<td>1.5346</td>
<td>0.05%</td>
</tr>
<tr>
<td>Peak 7</td>
<td>1.6181</td>
<td>1.6181</td>
<td>1.6183</td>
<td>1.6208</td>
<td>1.6190</td>
<td>1.6177</td>
<td>1.6187</td>
<td>0.07%</td>
</tr>
<tr>
<td>Peak 8</td>
<td>1.8427</td>
<td>1.8422</td>
<td>1.8423</td>
<td>1.8448</td>
<td>1.8436</td>
<td>1.8429</td>
<td>1.8431</td>
<td>0.05%</td>
</tr>
<tr>
<td>Peak 9</td>
<td>1.8916</td>
<td>1.8910</td>
<td>1.8911</td>
<td>1.8938</td>
<td>1.8922</td>
<td>1.8914</td>
<td>1.8919</td>
<td>0.06%</td>
</tr>
<tr>
<td>Peak 10</td>
<td>2.0271</td>
<td>2.0264</td>
<td>2.0270</td>
<td>2.0299</td>
<td>2.0267</td>
<td>2.0260</td>
<td>2.0272</td>
<td>0.07%</td>
</tr>
<tr>
<td>Peak 11</td>
<td>2.1840</td>
<td>2.1829</td>
<td>2.1831</td>
<td>2.1860</td>
<td>2.1829</td>
<td>2.1819</td>
<td>2.1835</td>
<td>0.06%</td>
</tr>
</tbody>
</table>

DOI: https://doi.org/10.30564/jim.v10i1.2955
3.8 Instrument Precision Study

Instrument precision (suitability of the system): the suitability of the system was checked by a single injection of the resolution solution and five replication injections of the reference solution. The %RSD, theoretical plates, tailing factor and resolution were optimized as the system suitability parameters. Mixed reference substance solution was implemented as sample for six times during test according to set chromatographic conditions, and calculated relative retention time of different common peaks. According to experimental results (Table 5), relative standard deviation on relative retention time of different common peaks was also below 2%, indicating the experimental instrument had good accuracy and would not influence experimental results.

<table>
<thead>
<tr>
<th>Number</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Average value</th>
<th>RSD (%)</th>
</tr>
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<tbody>
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<td>0.2312</td>
<td>0.2311</td>
<td>0.2311</td>
<td>0.2309</td>
<td>0.2311</td>
<td>0.2311</td>
<td>0.2311</td>
<td>0.03%</td>
</tr>
<tr>
<td>Peak 2</td>
<td>0.8389</td>
<td>0.8388</td>
<td>0.8387</td>
<td>0.8384</td>
<td>0.8386</td>
<td>0.8287</td>
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<td>0.02%</td>
</tr>
<tr>
<td>Peak 3</td>
<td>0.8656</td>
<td>0.8654</td>
<td>0.8654</td>
<td>0.8651</td>
<td>0.8652</td>
<td>0.8653</td>
<td>0.8653</td>
<td>0.02%</td>
</tr>
<tr>
<td>Peak 4</td>
<td>0.8999</td>
<td>0.8998</td>
<td>0.8998</td>
<td>0.8996</td>
<td>0.8995</td>
<td>0.8996</td>
<td>0.8997</td>
<td>0.02%</td>
</tr>
<tr>
<td>Peak 5</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
<td>0.00%</td>
</tr>
<tr>
<td>Peak 6</td>
<td>1.5338</td>
<td>1.5336</td>
<td>1.5334</td>
<td>1.5336</td>
<td>1.5337</td>
<td>1.5341</td>
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</tr>
<tr>
<td>Peak 7</td>
<td>1.6178</td>
<td>1.6176</td>
<td>1.6172</td>
<td>1.6173</td>
<td>1.6178</td>
<td>1.6181</td>
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</tr>
<tr>
<td>Peak 8</td>
<td>1.8416</td>
<td>1.8417</td>
<td>1.8419</td>
<td>1.8422</td>
<td>1.8426</td>
<td>1.8428</td>
<td>1.8421</td>
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</tr>
<tr>
<td>Peak 9</td>
<td>1.8904</td>
<td>1.8906</td>
<td>1.8907</td>
<td>1.8909</td>
<td>1.8913</td>
<td>1.8917</td>
<td>1.8909</td>
<td>0.03%</td>
</tr>
<tr>
<td>Peak 10</td>
<td>2.0262</td>
<td>2.0264</td>
<td>2.0267</td>
<td>2.0267</td>
<td>2.0270</td>
<td>2.0275</td>
<td>2.0268</td>
<td>0.02%</td>
</tr>
<tr>
<td>Peak 11</td>
<td>2.1839</td>
<td>2.1839</td>
<td>2.1838</td>
<td>2.1838</td>
<td>2.1839</td>
<td>2.1843</td>
<td>2.1840</td>
<td>0.01%</td>
</tr>
</tbody>
</table>

Table 5. Relative retention time of different common peaks in Instrument precision study
3.9 The Universally of HPLC Instruments and Chromatograph Column

To test the universality of HPLC instruments and chromatograph column, two different instruments and three chromatograph columns were chosen in the test. Took 1 batch of this product (Pharmaceutical manufacturer A, batch number: 127160205), prepared the test solution according to the method in the test, and investigated the HPLC instruments and chromatographic columns according to the proposed mobile phase elution method: ① Agilent 1260 high performance liquid chromatograph, ② Diane Ultimate 3000 high performance liquid chromatograph; ① Tech Mate C18-ST, 5 um, 4.6 × 250 nm, ② Innova ODS-2, 5 um, 4.6 × 250 nm, ③ Alltima C18, 5 um, 4.6 × 250 nm. Shows in Figure 8 Chromatographic Column Investigation, Table 6 Chromatographic Column Investigation Results and Table 7 Different Instrument Investigation Results. The results indicated that the resolution of those three columns were the same. Each of the columns could be used in characteristic spectrum test of Huoxiang Eliminating Summer-heat Soft Capsule because of their well separation. Considered the peak shape and separation situation, the chromatographic column ① and HPLC instrument ① were finally selected for sample detection.

Figure 8. Chromatograms of Chromatographic Column Investigation

Table 6. Relative retention time of different common peaks in three columns

<table>
<thead>
<tr>
<th>Number</th>
<th>Column 1</th>
<th>Column 2</th>
<th>Column 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak 1</td>
<td>0.2312</td>
<td>0.2518</td>
<td>0.2717</td>
</tr>
<tr>
<td>Peak 2</td>
<td>0.8389</td>
<td>0.8650</td>
<td>0.8801</td>
</tr>
<tr>
<td>Peak 3</td>
<td>0.8656</td>
<td>0.8986</td>
<td>0.9007</td>
</tr>
<tr>
<td>Peak 4</td>
<td>0.8999</td>
<td>0.9248</td>
<td>0.9216</td>
</tr>
<tr>
<td>Peak 5</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
</tr>
<tr>
<td>Peak 6</td>
<td>1.5338</td>
<td>1.5593</td>
<td>1.5038</td>
</tr>
<tr>
<td>Peak 7</td>
<td>1.6178</td>
<td>1.5843</td>
<td>1.5790</td>
</tr>
<tr>
<td>Peak 8</td>
<td>1.8416</td>
<td>1.8760</td>
<td>1.8522</td>
</tr>
<tr>
<td>Peak 9</td>
<td>1.8904</td>
<td>1.9304</td>
<td>1.8992</td>
</tr>
<tr>
<td>Peak 10</td>
<td>2.0262</td>
<td>2.0597</td>
<td>2.0111</td>
</tr>
<tr>
<td>Peak 11</td>
<td>2.1839</td>
<td>2.1824</td>
<td>2.1582</td>
</tr>
</tbody>
</table>
3.10 Test Sample

All collected samples (80 different samples from 5 manufacturers) were tested by applying HPLC method. Tech Mate C18-ST Chromatographic Column and Agilent 1260 HPLC instrument were chosen to detect the samples. After detection of all samples, a quality conclusion about all those Huoxiang Eliminating Summer-heat Soft Capsules can be drawn from the five manufacturers. 11 characteristic peaks were shown out in products which came from 3 manufacturers, and the result indicated those three manufacturers’ Huoxiang Eliminating Summer-heat Soft Capsules have well quality. The remaining two manufacturers’ products were shown out 10 and 6 characteristic peaks respectively. The reason may be that the manufacturer did not use all 12 herbs specified on the prescription, or some herbs were of poor quality.

4. Discussions

A challenging, versatile HPLC method was developed for the simultaneous determination of multi-component in Huoxiang Eliminating Summer-heat Soft Capsule. The method was very simple and effective, and based on the validation data that can be concluded within an analysis time of 60 min, 11 characteristic peaks were determined accurately and precisely. Convenient operation, economy and high efficiency and providing method have been achieved to comprehensive quality control of Huoxiang Eliminating Summer-heat Soft Capsule.

This HPLC Characteristic Spectrum method compares the locations of the reference substance and different prescription medicines to determine 11 characteristic peaks with identification significance. By DAD scanning (200-400 nm) on the 11 peaks in the sample, the DAD scanning pattern of each characteristic peak is shown out. Via chromatogram contrast of three solutions included test sample, reference crude herb and reference substance, and their DAD scanning results, to affirm characteristic peaks and each peak.

The retention time of the mobile phase prepares at different times (0 h, 2 h, 4 h, 8 h, 12 h and 24 h), since these 11 characteristic peaks have little changes. Among these 11 characteristic peaks, hesperidin (peak 5) is relatively stable, and has a large peak area which is easy to identify. Therefore, hesperidin (peak 5) is selected to calculate the relative retention time. The relative retention time of each characteristic peak is basically stable. Meanwhile, different chromatographic columns are used to investigate the durability of the method. The HPLC Characteristic Spectrum can evaluate the quality of the products in more economical, efficient and scientific way.

Acknowledgements

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References


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ARTICLE
Comparative Analysis of Steel Plates and PLA Used for Joint Repair in Humans and Canines

Yi Zhu\textsuperscript{1*} Yibo Wang\textsuperscript{1} Fan Yang\textsuperscript{2} Hao Yue\textsuperscript{1}

\textsuperscript{1}Engineering Computing and Simulation Innovation Laboratory, North China University of Technology, Tangshan City, Hebei Province, 063210, China
\textsuperscript{2}School of Nursing and Rehabilitation, North China University of Technology, Tangshan City, Hebei Province, 063210, China

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ABSTRACT

The exploration of fracture internal fixation materials has been one of the research hotspots in the field of biomedical materials. The traditional internal fixation material for fracture is metal fixation. Although its mechanical strength is very large, it can not be degraded and absorbed in human body after implantation of human body or canine joint, which requires a secondary operation to remove, which not only brings pain to patients, but also causes economic pay. Therefore, the development of a biodegradable fracture internal fixation material has become the goal of many researchers. Polylactic acid (PLA) is nontoxic and harmless, has good biocompatibility and strong mechanical properties. It can be degraded in vivo after implantation. The degradation products are CO\textsubscript{2} and H\textsubscript{2}O. For the study of the feasibility of polylactic acid as a substitute for common fracture fixation materials, 18 northern Chinese pastoral dogs were randomly divided into blank group, PLA group and plate group. The data were recorded according to the Wakitani score from the first week to the fifteenth week after operation. First, all the indexes were divided into two categories by principal component analysis, then the blank group, steel plate group and PLA group were fitted and compared. Finally, it is concluded that PLA is more beneficial to joint repair than steel plate.

1. Introduction

Polylactic acid, as a substitute for metal fixation on bone repair, has a very broad market prospect. It can be degraded into CO\textsubscript{2} and H\textsubscript{2}O in vivo, so that patients do not need to secondary surgery. It also reduces economic efforts. Up to now, however, domestic PLA are generally used in human joint repair, and dogs, such as search and rescue dogs, need to reduce the suffering of secondary removal of metal solids and reduce the cost of treatment. Therefore, the possibility of PLA application in canine joint repair should be considered.

As an important method to achieve bone defect repair, bone tissue engineering is a scaffold with good biocompatibility and specific three-dimensional structure. At the same time, the scaffold can be gradually degraded and...
completely replaced by new bone after implantation. [3] At present, metal solids are usually used as scaffolds to help joint repair. However, metal solids are not compatible with human body in joint repair and need secondary surgery to remove them. In contrast, polylactic acid degrades in the body as the patient’s injury improves, thus alleviating the patient’s pain.

In this experiment, the evaluation of cartilage defect area repair tissue in each group of experimental animals combined with histological staining was performed with Wakitani cartilage repair score [4] histological score, double blind, and two orthopaedic physicians familiar with cartilage repair histology evaluation. Cell morphology, matrix staining, surface regularity, cartilage thickness, binding of repair tissue to surrounding cartilage and Col-II protein content were used as the evaluation basis to evaluate the experimental bodies in each group. Compared with the steel plate, the PLA can not only degrade in vivo, but also be better than the steel plate in joint repair. Therefore, PLA as a fixation material for canine joint fixation can not only reduce the cost and enhance the effect, which is also instructive for PLA joint repair for some other organisms.

2. Principal Component Analysis based on All Evaluation Indicators

2.1 Determination of parameters after principal component analysis

Because of too many original indexes, the difficulty and complexity of the analysis problem are increased, and in this experiment, there is a certain correlation between multiple variables. Accordingly, the six selected indexes, cell morphology, matrix staining, surface regularity, cartilage thickness, binding of repair tissue to peripheral cartilage and Col-II protein content were reduced.

There are 6 samples and 6 indexes, which can form a sample matrix x of n*p size:

\[
x = \begin{bmatrix}
x_{11} & x_{12} & \ldots & x_{16} \\
x_{21} & x_{22} & \ldots & x_{26} \\
\cdots & \cdots & \cdots & \cdots \\
x_{61} & x_{62} & \ldots & x_{66}
\end{bmatrix} = (X_1, X_2, \ldots, X_6)
\] (1)

It is standardized to calculate the mean value by column:

\[
\bar{x} = \frac{1}{n} \sum_{i=1}^{n} x_i
\] (2)

and standard deviation:

\[
S_j = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (x_{ij} - \bar{x}_j)^2}
\] (3)

Calculated standardized data Xij, the original sample matrix was standardized to:

\[
X = \begin{bmatrix}
X_{11} & X_{12} & \ldots & X_{16} \\
X_{21} & X_{22} & \ldots & X_{26} \\
\cdots & \cdots & \cdots & \cdots \\
X_{61} & X_{62} & \ldots & X_{66}
\end{bmatrix} = (X_1, X_2, \ldots, X_6)
\] (4)

Then the covariance matrix of the standardized sample is calculated:

\[
R = \begin{bmatrix}
r_{11} & r_{12} & \ldots & r_{16} \\
r_{21} & r_{22} & \ldots & r_{26} \\
\cdots & \cdots & \cdots & \cdots \\
r_{61} & r_{62} & \ldots & r_{66}
\end{bmatrix}
\] (5)

inside:

\[
t_i = \frac{1}{n-1} \sum_{j=1}^{n} (X_{ij} - \bar{X}_j)(X_{ij} - \bar{X}_j) = \frac{1}{n-1} \sum_{j=1}^{n} X_{ij}X_{ij}
\] (6)

Then the eigenvalues and Eigenvectors of the R are calculated, in which the blank group, the steel plate group and the PLA group obtain the Eigenvectors respectively:

| Blank group | 0.0001 | 0.0012 | 0.0022 | 0.0076 | 0.0175 | 5.9715 |
| Steel plate group | 0.0003 | 0.0006 | 0.0026 | 0.0240 | 0.0515 | 5.9210 |
| Group PLA | 0.0002 | 0.0012 | 0.0045 | 0.0100 | 0.0512 | 5.9329 |

Then calculate the contribution rate:

The contribution rate = \frac{\lambda_i}{\sum_{i=1}^{k} \lambda_i} \quad (i = 1, 2, \ldots, 6) (7)

Finally, the contribution rate of principal components of blank group, steel plate group and PLA group was obtained:

| Blank group | 0.0000 | 0.0002 | 0.0004 | 0.0013 | 0.029 | 0.9692 |
| Steel plate group | 0.0000 | 0.0001 | 0.0004 | 0.0040 | 0.086 | 0.9094 |
| PLA group | 0.0000 | 0.0002 | 0.0008 | 0.0017 | 0.085 | 0.9124 |

In order to ensure that the final data has more than 95% integrity, the latter two principal component indexes are taken, so that the data loss rate is kept below 5%. Finally, the winner’s composition coefficient is shown in Table 1:
2.2 Interpretation of the Principal Component Parameters and the Finding of the Principal Component Indexes

As can be seen from Table 1, all the indexes of blank group, steel plate group and PLA group have similar positive loads in principal component 1, and the proportion of item 1 and item 6 is higher. Therefore, principal component 1 can be interpreted as the recovery of surrounding tissues.

As can be seen from Table 2, the second principal component has different types of indexes compared with the three groups of experiments, but the main comparison of the steel plate group and the PLA group of principal component 2, the fourth and the fifth index occupy a high proportion. Therefore, principal component 2 can be interpreted as recovery of damaged matrix and cartilage.

Finally, the principal components of the blank group and the PLA group are shown in Table 2:

### Table 1. Principal Component Coefficient

<table>
<thead>
<tr>
<th>Component 1</th>
<th>Component 2</th>
<th>Component 1</th>
<th>Component 2</th>
<th>Component 1</th>
<th>Component 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>blank group</td>
<td>steel group</td>
<td>PLA group</td>
<td>blank group</td>
<td>steel group</td>
<td>PLA group</td>
</tr>
<tr>
<td>0.4089</td>
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<td>0.4087</td>
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<tr>
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<td>-0.1814</td>
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<td>-0.2059</td>
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</tbody>
</table>

### Table 2. Principal Component Score

<table>
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<tr>
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<th>Component 2</th>
<th>Component 1</th>
<th>Component 2</th>
<th>Component 1</th>
<th>Component 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>blank group</td>
<td>steel group</td>
<td>PLA group</td>
<td>blank group</td>
<td>steel group</td>
<td>PLA group</td>
</tr>
<tr>
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<td>0.240</td>
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<td>14.286</td>
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<td>0.020</td>
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<td>-0.006</td>
<td>18.478</td>
<td>0.052</td>
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</tbody>
</table>

3. Fitting Curve Analysis of Experimental Indexes based on Principal Component Analysis

#### 3.1 Curve Fitting Analysis of Principal Component 1

Using the sum of sine \(^3\) in the Matlab to fit the three groups of data, the blank group fitting results are shown in Figure 1:

![Figure 1. Fitting results of blank group](image)

Fitting function:

\[
f(x) = a_1\sin(b_1x+c_1)
\]

Inside \(a_1 = 15.64,\ b_1 = 0.1004,\ c_1 = 0.05465\)

The sum of square error is 0.1072, the goodness of fit is 0.9996, and the mean square error is 0.0945. Because the sum of square errors is SSE less than 1, the fitting effect is better.

The fitting results of the steel plate group are shown in Figure 2:

![Figure 2. Fitting results of steel plate group](image)
The fitting function is:
\[ f(x) = a_2 \sin(b_2 x + c_2) \]  \hspace{1cm} (9)
Inside \( a_2 = 17.68 \), \( b_2 = 0.1096 \), \( c_2 = 0.06934 \)
The sum of square error is 1.339, the goodness of fit is 0.9961, and the mean square error is 0.334. Because the sum of error square SSE=1.339 is small, the fitting effect is better.

PLA group of fitting results are shown in Figure 3:

The fitting function is:
\[ f(x) = a_3 \sin(b_3 x + c_3) \]  \hspace{1cm} (10)
Inside \( a_3 = 18.25 \), \( b_3 = 0.1047 \), \( c_3 = 0.05545 \)
The sum of square error is 0.7661, the goodness of fit is 0.998, and the mean square error is 0.2527. Because the sum of square error SSE=0.7661 is small, the fitting effect is better.

The fitting results show that the \( a_3 \) is greater than that of \( a_2 \). Therefore, in the same time, the upper limit of the recovery of the surrounding tissue of the PLA group is better than that of the steel plate group, because the \( b_2 \)= is 0.1096 and the \( b_3 \)= is 0.1047. As a result, the fitting function analysis of principal component 1 shows that steel plate can be replaced by PLA in the recovery of tissue around joint damage.

3.2 Curve Fitting Analysis of Principal Component 2

Using the smooth spline fitting (Smoothing spline) \([6]\) in the Matlab, the fitting results of the three groups of data are as follows:

The fitting results show that the recovery curve of damaged matrix and cartilage in PLA group was smaller than that in the plate group at the 6th week to the 8th week. On the contrary, the slope of the recovery curve of damaged matrix and cartilage in the 8th to 12th week was larger than that in the plate group. This indicates that the recovery of damaged matrix and cartilage in the PLA group was weaker than that in the plate group in the early postoperative period, but the effect in the later PLA group was better than that in the plate group. Finally, the recovery of damaged matrix and cartilage in the two groups tended to be 0 at the 14th week, indicating that the damaged matrix and cartilage in the two groups returned to normal level at the 14th week.

4. Cluster Analysis of Experimental Indicators Based on Principal Component Analysis

4.1 Hierarchical Cluster Analysis

Clustering analysis \([7]\) is to classify the studied objects according to the characteristics of things themselves, so that the individuals in the same class have greater similarity and the individuals in different classes have greater differences. Is to classify subjects without prior knowl-
The distance between six experimental samples is calculated first as 
\[ D = (d_{ij}) \], and the distance matrix between samples is obtained by using Euclidean distance as distance measurement method.

\[ d(x_1, x_2) = \sum_{k=1}^{n} |x_{1k} - x_{2k}| \]

(11)

Take each sample as a class and \( G_1, G_2, G_3, G_4, G_5, G_6 \), get the minimum distance of two or two of the six samples, and gather them into a class as \( G_7 \). Compute the distance between the new class and the rest, and get the new distance matrix \( D_2 \).

\[
\begin{align*}
D(G_2, G_3) &= \min \{ D(G_1, G_1), D(G_1, G_2) \} \\
D(G_6, G_5) &= \min \{ D(G_1, G_1), D(G_5, G_3) \} \\
D(G_5, G_4) &= \min \{ D(G_4, G_3), D(G_1, G_3) \} \\
D(G_6, G_6) &= \min \{ D(G_4, G_3), D(G_5, G_5) \}
\end{align*}
\]

(12)

Find out the minimum of two distances in five samples, gather them into a class, and record them as \( G_8 \). Compute the distance between the new class and the rest, and get the new distance matrix \( D_2 \). By analogy, all experimental group samples are finally grouped into three categories.

### 4.2 Clustering Results and Category Determination

The experimental data of blank group, steel plate group, and PLA group were systematically clustered to obtain the PLA spectral system diagram of steel plate group as shown in Figure 6 and Figure 7:

**Figure 6. Spectra of steel plate**

**Figure 7. PLA Histogram**

It can be seen from the pedigree diagram that the clustering results can be divided into three categories. According to the experimental results, the first category is the joint damage period, the second is the joint repair period, and the third is the joint recovery period.

### 4.3 Analysis of Cluster Effect Maps

Analysis of cluster effect maps:

**Figure 8. Effect of Steel Plate Cohesion**

**Figure 9. PLA Aggregation effects**
The damage period of the steel plate group was shorter than that of the PLA group, and the repair period was slightly longer than that of the PLA group. The results showed that the effect of PLA on joint repair was slightly slower than that of steel plate in week 4 to week 7. Moreover, the distribution of sample points in the 6th to 8th weeks of the plate genealogy rapidly compared with the PLA group, which indicated that the plate group had faster effect on joint repair than the PLA group in the 6th to 8th weeks, and the sample point distribution of the steel plate in the 9th to 12th weeks of the PLA; pedigree was more dense than that in the PLA group. This is the same conclusion as the previous section by fitting.

5. Conclusions

In this paper, the principal component analysis method is used to evaluate the joint repair, which simplifies the problem under the condition of minimizing the data loss rate. At the same time, two fitting methods and systematic clustering method are used to analyze the blank group. Effects of plate group and PLA group on canine bone repair. The results showed that PLA, like metal solids, had a significant effect on the repair of damaged joints compared with the blank group, and compared with the steel plate, the PLA had a slightly smaller effect on the early repair of canine joints than the steel plate. But the repair effect of PLA is stronger than that of steel plate. According to this result PLA the repair effect of the joint tends to converge earlier than that of the metal solid, and the patient recovers early and reduces the pain.

While the current PLA is mainly used for the repair of human joints, the analysis of experimental data shows that PLA can also replace metal solids such as steel plates for canine joint repair, and its effect as fracture fixation is better than that of metal solids. This provides a guiding idea for the study of whether PLA can be used as fracture fixation for joint repair in other organisms.

References

CASE STUDY

Urticaria Successfully Treated with Mindfulness and Nutraceutical Supplements

Jozelio Freire de Carvalho *
Institute for Health Sciences from Federal University of Bahia, Salvador, BA, Brazil

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ABSTRACT

The study is to describe a patient with urticarial lesions which was successfully treated with mindfulness and nutraceuticals. A 20-year-old female patient with an unmarked past medical history presented with diffuse pruritic urticarial lesions associated with breathless and severe fatigue, lasting for 5 months. The lesions covered all body, except palms and plants. Hospitalized for three days, she received dexamethasone, promethazine, and subcutaneous adrenalin and discharged with fexofenadine, prednisone, and ranitidine. Mindfulness and relaxation practice were instructed and some nutraceuticals. After two months, she returned asymptomatic, without any new episodes of urticaria or fatigue. Most of the chronic urticaria patients are treated with antihistamines and/or steroids. This case illustrates an alternative holistic approach, combining mindfulness and relaxation practices with nutraceuticals. This alternative therapeutical strategy should be explored by back to back comparison to the conventional pharmaceutical approach.

1. Introduction

Urticaria is a complex allergic disorder characterized by pruritic eruption over the skin. It is commonly caused by allergies to food, drugs or other environmental agents and it may affect 15% to 20% of the population at least once in the lifetime [1]. It has been estimated that up to 25% of patients with acute urticaria progress to chronic urticaria. Chronic urticaria is defined by the occurrence of frequent, recurrent skin lesions for at least six weeks in the absence of any causative physical or environmental trigger. In half of the chronic urticarial symptoms resolve in about one year. Conversely, 20% experienced urticarial attacks for more than 20 years [1].

Regarding chronic urticaria pathophysiology, it is unclear yet. It may be related to immunological mediators such as IgE, and IgG antibodies directed to IgE or even other factors that may activate mast cell degranulation of histamine. Some authors hypothesize that one of the possible causative factors is psychological distress, including anxiety [19]. Chronic conditions may be associated with anxiety or depression, and they occur in urticaria. Several potential underlying shared mechanisms can be offered to explain the joint presentation of chronic urticaria with anxiety and depression. One potential mechanism is the existence. The immune system abnormalities occurring in CSU, anxiety, and depression may indicate a systemic link between the three disorders of shared immune-mediated mechanisms, as a systemic immune malfunction is prevalent in these conditions [20].

*Corresponding Author:
Jozélio Jozélio De Carvalho,
Institute for Health Sciences from Federal University of Bahia, Salvador, BA, Brazil;
Email: jotafc@gmail.com
Treatment modalities for urticaria involve the use of anti-histaminic, H2 antagonists, and glucocorticoids. More than 50% of patients with urticaria do not respond to antihistamines doses that have been approved by the US Food and Drug Administration. For these patients, higher than recommended doses are usually used in the clinical practice, and this approach may lead to a higher incidence of drug side effects, limiting the use of these medications.

New modalities of treatment of chronic urticaria are definitely welcomed. In this regard, the inclusion of behavioral practices combined by a non-pharmacological nutraceutical approach, with lower risk of side effects, is highly desired.

The present article aims to report a case of a patient with recurrent chronic urticarial that was successfully and quickly treated with mindful and behavioral therapies combined with nutraceutical additives.

2. Case Report

A 20-year-old female patient with a negative past medical history presented with a 5-month history of diffuse pruritic and erythematous skin eruption compatible with urticaria, associated with breathless difficulties and severe fatigue. The lesions covered all body, except palms and plants. She was hospitalized in May 2018 and received dexamethasone, promethazine, and subcutaneous adrenaline. One month later the disease relapsed and was controlled with antihistamines (fexofenadine 120 mg), prednisone, and ranitidine only during acute attacks. In August 2018, a new relapse with a more diffuse and severe clinical picture of urticaria, but less pruritic appeared. Coming to our clinical facility, the woman denied any background diseases, previous use of long term medications, allergies, asthma, and food intolerance. On physical examination she demonstrated urticarial plaques on arms and face (Figure 1A), trunk, and legs (Figure 1B), and she was very anxious. A Beck anxiety inventory of 21 (normal: < 8) was calculated. Laboratory tests showed white blood count of 25,900/mm³ (neutrophils 5,160/mm³, lymphocytes 1,672/mm³, eosinophils 76/mm³), negative HIV 1 and 2 and syphilis serologies, total IgG 1264 mg/L (730-1620 mg/dL), IgM 172 mg/dL (50-300 mg/dL), IgA 217 mg/dL (40-350 mg/dL), IgE 355 mg/dL (<100 mg/dL), CH50-158 U (60-265 U), 25-OH-vitamin D 20.4 ng/mL (> 30 ng/mL), cortisol 13.1 mcg/dL, vitamin B12 402 pg/mL, C-reactive protein < 0.03 mg/L, Antinuclear antibodies, anti-Ro/SS-A, anti-La/SS-B, anti-RNP, anti-Sm lupus anticoagulant, anticytadilopin, rheumatoid factor, IgA and IgG anti-gliadin, anti-endomysium, anti-tissue transglutaminase, anti-mitochondrial, anti-myeloperoxidase, anti-proteinase 3, anti-insulin, anti-GAD were all negative. Serology for infectious diseases (syphilis, hepatitis B and C, cytomegalovirus, Epstein-Barr, rubella, HIV 1 and 2, HTLV I and II, and dengue) were all negative. Since she was symptomatic with low quality of life, it was offered a practice of mindfulness and an intake of the following supplements twice a day for 30 days: 5-hydroxytryptamine 100 mg, vitamin C 500 mg, magnesium 100mg, vitamin D3 10,000IU, vitamin A 10,000IU, quercetin 120 mg, and L-methionine 500mg. Omega-3 2 g per day, glutamine 5 g/day for microbiome reinforcement and valerian extract 1g in the morning and 2 g at night for anxiety, were also included. After two months, she returned asymptomatic, without any new episodes of urticaria or fatigue. No antihistamines, ranitidine, or glucocorticoids were consumed, during this period. Beck anxiety inventory returned back to normal in 3. Valerian was then reduced to 1 g twice a day, and the additives were reduced to 5-hydroxytryptamine 100 mg, vitamin C 500 mg, magnesium 100 mg, and vitamin D3 10,000IU once a day, and methyl cobalamin 200 mcg. Currently, two years after, the patient is asymptomatic, without fatigue, she had one or two episodes of mild urticarial per year, still practicing mindfulness and relaxation and no need more supplements for urticarial.

3. Discussion

This article reports a female patient who suffered recurrent urticaria and was successfully treated with several supplements and mindfulness exercises.

When a physician deals with chronic urticaria, the history of contacts, ingestions, inhalations, prescriptive and over the counter drug intake is mandatory. In fact, the leading cause of urticarial is idiopathic, comprising 75% of the cases. Drugs are responsible for only 9% of the
urticaria etiology, and food additives, preservatives, and pseudo allergens are the most common offenders[4].

Therapeutic strategies are wide. If the causative agent is identified, its elimination is most rewarding and if no etiology is identified, most probably, the patient will get anti-allergic medications. In the present woman, the antihistamines and steroids were effective only in the acute phase during hospitalization. Since the urticaria relapsed and the drugs’ side-effects were a concern, an alternative therapeutic strategy was searched.

A combination of strategies was employed to rescue the young woman from her complaints and low quality of life. Hence, the patient was supplemented with vitamin D due to her deficiency, supported by the literature on vitamin D in urticaria. A systematic review of 17 studies supported vitamin supplementation[5]. Furthermore, a randomized, prospective, blinded trial demonstrating symptom improvement when a high dose of vitamin D3 supplementation is added to the conventional treatment[6].

Regarding magnesium, a study evaluating magnesium kinetics in acute urticaria showed low levels of this mineral in urticaria[8].

Stress is a crucial factor related to urticarial pathophysiology. The present patient got some supplements intending to reduce stress, including valerian and 5-hydroxytryptamine[9-11]. And more interesting, 5-hydroxytriptamine seems to have a role in reducing the allergic inflammation[11]. Notably, stress and dysbiosis are vital factors in the urticarial pathophysiology[9,12]. Likewise, quercetin, vitamin A, and glutamine were added to the patient to improve dysbiosis and reduce stress[13,14]. A study in HIV patients using supplementation with glutamine for six weeks was able to show that this short-term dietary supplementation attenuated HIV-associated intestinal dysbiosis[14,15]. It should be stressed that their effects on skin microbiome are unknown.

Mindfulness and relaxation, are mind-body techniques with beneficial results for anxiety therapy. However, no study on mindfulness in urticarial was found in the literature. Briefly, in our patient, we asked her to practice this technique deliberately focusing awareness on everyday activities and savouring pleasant experiences, to stay in a suggested posture with the eyes closed and to observe her attitude, feelings and how attention is directed.

Concerning omega-3, an experimental study suggested that omega-6 and omega-3 series of polyunsaturated fatty acids may be one of the mediators in chronic urticaria[16]. Reinforcing omega-3 over omega-6 is known to improve tissue injury and wound healing[17].

Ascorbic acid or vitamin C has an anti-histaminic action[18]. In this line, it is logical to supplement vitamin C in patients suffering from chronic urticaria.

Future randomized and controlled studies using vitamin D, omega-3, magnesium, and other supplements are desired to confirm the efficacy and quick response observed in the herein described patient.

Other possible explanations for the patient improvement may be due to the natural history of the disease, changes in exposures, changes in life circumstances, residual effects of pharmacological interventions, neuropathic interventions. Indeed, the patient changed her life style including mindfulness practice and diet changes with gluten-free diet. Regarding natural history of the disease, the general consensus is that about one-third to one-half of patients with chronic urticaria will have remission of their disease within 1 year. More recent studies that have looked at remission rates in children, estimate that remission rates are low and are only about 10.3% per year [Netchiporouk E, Sasseville D, Moreau L, Habel Y, Rahme E, Ben-Shoshan M. Evaluating comorbidities, natural history, and predictors of early resolution in a cohort of children with chronic urticaria. JAMA Dermatol. 2017;153(12):1236–42.]. In conclusion, the present successfully treated woman brings up an alternative, safe and efficacious treatment of chronic urticaria using a holistic approach of nutraceutical supplements and behavioral protocol of mindfulness and relaxation.

Conflict of Interest

None of the authors have conflict of interest.

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Ethical Statement

The authors declare that he followed the World Medical Association Declaration of Helsinki in this study. An informed consent was obtained from the patient for publication of her case.

References


REVIEW
Analysis on the Special Prescriptions from the Book of Chengfang Qieyong

Kaiping Jiang  Xiaoyun Jian*

Department of Internal Medicine, Foshan Hospital of Traditional Chinese Medicine, Affiliated to Guangzhou University of Chinese Medicine, Foshan, 528000, China

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ABSTRACT

The kinds of special prescriptions in the book of Chengfang Qieyong cover a wide range of subjects such as internal medicine, gynecology, pediatrics and surgery, etc. These prescriptions have gathered the original discussions of doctors of all ages, condensed the author's own in-depth insights, and displayed the profound connotation of traditional Chinese medicine prescriptions or clinical efficacy characteristics. Guided by the function and categories of the original monograph, 26 prescriptions were integrated into seven categories, i.e. treating Qi and regulating Blood, nourishing and eliminating, exterior dispersing and dispelling cold, compromising and purging fire, drying dampness and expelling phlegm, fetal birth and baby, and carbuncle. Based on the characteristics of clinical efficacy, the connotation of legislative prescriptions, the modification of prescriptions and medicines, and the degree of decoction and prescription, etc, this paper analyzes the connotation of traditional Chinese medicine of these special prescriptions, and provides important reference for the research of modern prescriptions, the expansion of clinical application and the development of new drugs of traditional Chinese medicine.

Keywords: Chengfang Qieyong, Prescriptions, Chinese pharmacy, Clinic effect, Drug development.

1. Introduction

The Chengfang Qieyong, [1] written by Wu Yiluo in Qing Dynasty of China based on modification of the Textual Research on Prescriptions and the Collected Exegesis of Prescriptions, is a famous prescription book. This book has a wide collection of 1102 effective prescriptions in the past dynasties, which are divided into 23 categories according to the efficacy. The notes and citations in the book are very detailed. It is an important reference book for learning the formulaology.

Taken “in recent history, the fashion doctors accept and teach prescriptions one after another while there is no one to seek for classics discuss” as mirror, guided by “comply with all classics to observe the integration, revise them in accordance with syndromes to explore the subtleties”, and based on “suit the use of times” and “suit the patient’s condition”, the Chengfang Qieyong gathered all kinds of prescriptions. Tracing the principle with syndrome, demonstrating the method with theory, clarifying the prescription with treatment, seeking medicine with prescription, and achieving change with medicine, the contents of

*Corresponding Author:
Xiaoyun Jian,
Department of Internal Medicine, Foshan Hospital of Traditional Chinese Medicine, Affiliated to Guangzhou University of Chinese Medicine, Foshan, 528000, China;
Email: xyxy1100@126.com
prescriptions and syndromes in this book correspond to each other. Therefore, it is a collection of principles, treatment, prescriptions and medicines, as well as the wisdom and application of traditional Chinese medicine thinking, which is an important and indispensable book for the clinical practice of traditional Chinese medicine.

In an overview of Chengfang Qieyong, some prescriptions that are not commonly used in clinical practice are explained in terms of their clinical efficacy, connotation of legislative formulae, adaptations of prescriptions and formulas, and methods of decocting, taking and dispensing. From time to time, it is described by such words as “magical”, “wonderful”or “all”, “the most”, “the most appropriate”, “immediately”, “but with”, “especially”, “disappeared completely”, “enough”, etc., while such special description is rare in the explanation of a large number of other commonly used prescriptions, which deserves our attention. In this paper, 26 prescriptions of this kind are analyzed to provide reference for improving the level of clinical prescription of TCM or drug research and development.

2. Treating Qi and Regulating Blood

2.1 Tiaozhong Yiqi Decoction

There are two kinds of Tiaozhong Yiqi Decoction. One is composed of Aucklandia, Atractyloides lancea and Buzhong Yiqi Decoction without Radix Angelicae sinensis and Rhizoma atractylodis macrocephalae. It is used to treat disarray of the spleen and stomach, chest fullness and limb fatigue, less food and shortness of breath, ignorance of taste in the mouth and vomit after eating. Xiong Jibai, a master of traditional Chinese medicine, summed up it as “treating abdominal distention caused by dampness blocked in Middle-jiao based on the treatment of tonifying the Middle-Qi “. [3] The another one is composed of Buzhong Yiqi Decoction with Radix Paeoniae alba and Fructus schisandrae, which can treat Qi deficiency and sweating, …… Added the tart flavour of Radix Paeoniae Alba and Fructus Schisandræ, with the function of promoting and astringency by collecting the dissipative Qi for the Tiaozhong Yiqi Decoction, Li Dongyuan, a famous physician in the Jin-Yuan Dynasties of China, opened another way to broaden the “wonderful”of tonifying the middle Qi. Therefore, clinical attention should be paid to the overall utility characteristics of Buzhong Yiqi Decoction and the different application of Tiaozhong Yiqi Decoction after addition and subtraction.

2.2 Shenxiang Powder

Shenxiang powder is composed of Syzygium aromatium and Amomum cardamom or Amomi fructus grinded into fine powder with the proportion of 1:1, which show the most “wonderful”effective in the treatment of severe adverse Qi and pain in chest diaphragm and epigastric cavity, vomiting and abdominal fullness, phlegm retention, choking, etc. A wonderful decoction as it is, but it belongs to the category of pungent heat and divergence, so it should be used with caution if there is no cold phenomenon.

2.3 Mahuang Renshen Shaoyao Decoction

Mahuang Renshen Shaoyao Decoction, originated from Li Dongyuan’s “ Secret Book of Orchid Chamber” in the Jin and Yuan Dynasties, is composed of Ephedra, Astragalus membranaceus, Baked Licorice, Radix Paeoniae alba, Ginseng, Ophiopogon japonicus, Schisandra chinesis, Radix Angelicae sinensis, and Cassia twig. It can treat hematemesis, exogenous cold evil, internal deficiency accumulation of heat. All those who take Zhongjing prescription should take it as a rule. Compendium of Materia Medica praises: Looking at this decoction, it is “enough”to be a model for all generations. Based on a comprehensive study of the ancient treatment and the patient’s combination of physical condition and syndrome, Xie Yinglu in Qing dynasty treated a case of cough and hematemesis with Dongyuan Mahuang Renshen Shaoyao Decoction and one dose was effective, which was overwhelmed with admiration for the unique curative effect. [3]

2.4 Renshen Yangying Decoction

Renshen Yangying Decoction is composed of Shiquan Dabu Decoction without Ligusticum wallichii and adding Fructus schisandrae, Dried Orange Peel and Polygala tenuifolia. Xue Lizhai of Ming Dynasty discussed when there are various syndromes of deficiency of Qi and Blood and can not be described, regardless of the disease or the pulse conditions, only using this decoction, all the syndromes will subside, which corresponding to Shiquan Dabu Decoction. Shiquan Dabu Decoction focuses on the characteristics of Tonifying Qi and Blood, while Renshen Yangrong Decoction tonify all the five internal organs, everywhere. The clinical application research showed that this prescription could not only promote the hematopoietic function and improve the immune function, but also alleviate the complications of coronary heart disease, diabetes, malignant tumor and brain injury, and had satisfactory curative effect on sleep disorders and fatigue. [4]

2.5 Juejin Decoction

Composed of Angelica sinensis, Alisma orientalis,
Cinnamomum cassia, Radix rehmanniae and Radix lingderae, Juejin Decoction, a “magical” prescription that uses tonifying treatment as purgation treatment, treat the severe dysmenorrhea caused by deficiency of blood and stagnation of meridians in the female patients. This prescription contains the treatment connotation of water helping water, just like a river burst its dam and flush away all scale deposit, which is an example of “treating obstruction with tonics”. Clinically, Juejin decoction combined with Shixiao powder is effective in treating dysmenorrhea of cold coagulation and blood stasis type caused by adenomyosis.\[5\]

3. Nourishing Healthy Qi

3.1 Yougui Decoction

Yougui Decoction is a fire tonic prescription created by Zhang Jingyue in Ming Dynasty. It is composed of Radix rehmanniae, Rhizoma dioscoreae, Eucommia ulmoides, Wolfberry fruit, Liquorice root, Cinnamon, Radyx aconiti lateralis preparata and Cornus officinalis. All the patients’ Mingmen Yang failing and Yin winning are treated with this prescription plus Rhizoma Alismatis soaking in cold water and then take it. This kind of contrary treatment of taking hot medicine with a low temperature is a wonderful way to take prescriptions.

3.2 Renshen Buqi Decoction

Renshen Buqi Decoction is composed of Liuwei Dihuang Pill without Alisma orientalis and with Ginseng, Angelica sinensis, Atractylodes macrocephala, Dried Tangerine Peel and is used to treat cold-induced diseases, pestilence and malaria. Those who Yin-cold Qi prevails and evil can not penetrate can use the prescription plus Rhizoma Alismatis soaking in cold water and then take it. This kind of contrary treatment of taking hot medicine with a low temperature is a wonderful way to take prescriptions.

3.3 Wufu Decoction

Wufuyin Decoction was the representative prescription of Zhang Jingyue in Ming Dynasty for the treatment of all the deficiency of Qi and Blood in five internal organs.\[6\] It is composed of Ginseng, Radix rehmanniae, Angelica sinensis, fried Atractylodes macrocephala, Baked Licorice, ginger and jujube. This prescription can give consideration to the treatment of all the deficiency of Qi and Blood in five internal organs and is the most one. Experimental study confirmed that Wufu Decoction could delay the apoptosis of rat chondrocytes induced by TNF-α and the mechanism may be related to the inhibition of MMP-3, MMP-9 and MMP-13 expression.\[7\]

3.4 Liangyi Paste

Liangyi Paste is composed of Ginseng and Radix rehmanniae. It is used to treat the deficiency of essence and Qi which is not effective by many other medicines, or those who consume the genuine-Yin by attacking treatment too much. If the deficiency lies in Yin and the essence does not transform Qi, there is nothing better than using this prescription. When you feel Yin deficiency due to a serious disease that has not yet come, it’s wonderful to use this prescription to recuperate vitality. The compound Ejiao paste, based on Liangyi Paste, has been proved to be effective in the treatment of anemia, leukopenia and so on, as well as in the prevention of anemia in sub-health population.\[8\]

3.5 Zhenyuan Decoction

Zhenyuan Decoction is composed of Radix rehmanniae, Angelica sinensis and Baked Licorice. It is used to treat the critically ill patients with shortness of breath, inability to lift, inability to swallow and choking airway,… which is the most common syndrome especially when a woman’s blood-sea is abnormal. So it is appropriate to use Zhenyuan Decoction, which can be boldly called as a “magic” prescription, to help and relieve the above symptoms related to woman’s blood-sea. The above-mentioned syndromes are closely related to the deficiency of Pectoral Qi and the Pectoral Qi, which is insufficient due to rising, is also related to deficiency of kidney-essence and inability to absorb. The Zhenyuan decoction, created by Zhang Jingyue in Ming Dynasty, can relieve palpitation by tonifying kidney-essence and invigorating Pectoral Qi, which was a pioneering work and has a guiding role in the clinical treatment of atrial fibrillation in palpitation.\[9\]

4. Relieving Exterior and Dispelling Cold

4.1 Magui Decoction

Magui Decoction is composed of Cinnamon, Angelica sinensis, Baked Licorice, Ephedra and Dried Tangerine Peel and is used to treat cold-induced diseases, pestilence and malaria. Those who Yin-cold Qi prevails and evil can
not be dispersed, it is necessary. Regardless of the meridians of the four seasons, if you have these symptoms, you should use this kind of prescription, not to say that you can’t use it in summer. This is the changing prescription of Mahuang Decoction and Guizhi Decoction, and its magical effect is far beyond the two prescriptions, so we must observe it carefully. The clinical application showed that this prescription has a good curative effect in cervical spondylosis of cervical type of wind-cold-dampness arthralgia. [10]

4.2 Da Wenzhong Decoction

Da Wenzhong Decoction is composed of Radix rehmanniae, Atractyloides macrocephala, Angelica sinensis, Ginseng, Liquorice, Ephedria, Radix Bupleuri, Rhizoma zingiberis and so on. Those with weak physical endowment, or those who are affected by Yin-cold and pestilence evil and show fever and drowsiness, though they have not seen the Yin Syndrome as before and the heat evil is not serious, just at the beginning of its suffering evil, immediately use this Decoction, take two or three doses and then make a rapid recovery. What a wonderful prescription! The patients who catch cold with a poor health or got an epidemic with the symptoms of influenza are more common. Among them, deficiency of both Qi and Blood, Yang deficiency and cold-pestilence are not uncommon. In the treatment of this kind of patients, Yupingfeng Powder, Shensu Decoction, Buzhong Yiqi Decoction or Mahuang Fuzi Xixin Decoction, Zaizao Powder and so on have been paid more attention, while little attention has been paid to Da Wenzhong Decoction. [11,12,13]

Why don’t we use the way to verify this wonderful prescription?

4.3 Zhishi Lizhong pill

Zhishi Lizhong pill is composed of Lizhong Pill, Fructus Aurantii Immaturus and Poria cocos. It is used for the treatment of diseases with poor curative effect of Daxianxiong Pill, such as cold-excess in the chest and a high rising of chest and diaphragm which cannot bear the slight touch of the hand. Cui Xinggong, a doctor in the Tang Dynasty, said: This is the deficiency of middle Qi and upward inversion after being treated with the purgative therapy, the effect of regulating Qi is not good, pathogenic toxin returns to attack upside, as a result that Qi and pathogenic toxin are combined in the chest. At this time, this pill is used to regulate the Qi first, and then to treat other diseases and the curative effect is magical. The experimental study found that Zhishi Lizhong Pill has obvious therapeutic effect on chronic gastritis model rats of spleen deficiency type, and has certain anti-inflammatory and analgesic effect. [14]

4.4 Liyin Decoction

Liyin Decoction consists of Radix rehmanniae, Angelica sinensis, Rhizoma zingiberis, Baked Licorice or add Cinnamomum cassia is used to treat deficiency of genuine-Yin, abdominal fullness and vomiting, nausea of phlegm and retained fluid, vomiting and diarrhea, abdominal pain, delayed menstruation and blood stagnation of women. If the invasion degree of exogenous pathogenic wind-cold is not deep, as long as you see fever, the pulse is fast but not strong, and there is no fire syndrome in the body and deficiency in natural endowment, you only need to take this prescription, or add Radix Bupleuri for one and a half qian or two qian, and take one or two doses continuously, a magical curative effect will be showed. In view of the above, the main functions of Liyin decoction is to warm Middle-jiao and dispel cold, benefit Yin and nourish Blood. In case of Yin deficiency and exogenous wind-cold, the clinical application can be effective as long as grasping the three major indications of aversion to cold, white tongue, floating and thin and fast pulse or deep and powerful pulse. [15]

4.5 Qide Pill

Qide pill is composed of stir fried Psoralea corylifolia, Rhizoma Zingiberis, Parched Atractyloides rhizome, Radix Linderae, Evodia rutaecarpa, Radix Aucklandia, Poria cocos and Medicated leaven. It is used to treat raw cold injury of spleen, diarrhea and dysentery at the beginning, and Abdominal pain. All young patients without deficiency of Qi and Blood and with stagnation of cold-dampness-food should be treated with this magical prescription. The application points of this prescription are “cold”, “dampness” and “food”. Therefore, it is suitable for patients with excess syndrome and should be used with caution for patients with deficiency syndrome.

4.6 Shoupi Decoction

Shoupi Decoction, also known as Sheying Decoction, is composed of Atractyloides macrocephala, Angelica sinensis, Chinese yam, Jujube kernel, Baked Licorice, Polygonal tenuifolia, roasted Ginger, Lotus seed and Ginseng. It is used for the treatment of blood that cannot be controlled due to spleen deficiency. All the symptoms of melancholy, depression, anger, fatigue and misuse of attacking drugs to damage spleen-yin, such as depression of middle Qi, restlessness of spirit and soul, bloody stools, or women’s uterine bleeding without fire, especially the critical syndrome.

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of nausea and vomiting occurred at the same time, Shoupi Decoction should be used quickly to save the spleen-Qi and then the control function of the spleen is firm and the blood naturally returns to its source. This is the changed prescription of Guipi Decoction, which has a very magical effect. According to this, there are two prescriptions for spleen failing to control blood syndrome: Guipi Decoction is used for spleen-Qi deficiency, while Shoupi Decoction is used for spleen-yin deficiency.

4.7 Shenzhu Decoction

Shengzhu Decoction consists of fried Atractylodes macrocephala, Rhizoma zingiberis, Cinnamomum cassia and Dried Tangerine Peel, which can be used quickly to treat improper diet, vomiting or diarrhea, chest and diaphragm stuffiness or rib pain, or, weakness, faint breath and mental fatigue caused by damaged viscera-Qi due to overuse of attacking drugs. If the distension pain is serious, it is the best to take Shengzhu Decoction along with Shenxiang Powder. If it is used to treat diarrhea due to cold dampness and vomiting, it is especially the holy medicine. Prescriptions such as Huoxiang Zhengqi Powder, Lizhong Decoction and Weiling Decoction are used to treat cold-dampness type of vomiting and diarrhea. In Shengzhu Decoction, Cinnamomum cassia has its own characteristics, if the Syzygium aromaticum and Angelica sinensis are added, a great curative effect is worth to be expected.

5. Reconciling and Purging Fire

5.1 Qingpi Decoction

Qingpi Decoction is composed of pericarpium citri reticulatae viride, vinegar Magnolia officinalis, Radix Bupleuri, fried Scutellaria baicalensis, Rhizoma pinelliae, Puria cocos, fried Atractylodes macrocephala, Baked Licorice, and Amomum Tsao-ko. Gao Gufeng, a doctor in the Ming Dynasty, said: there are many ways to treat malaria, none of which reaches the main point. This is the only prescription to treat malaria. I often use this prescription to treat malaria, and there is no one who can’t be cured. In case of the heat is more than the cold, a little Peucedanum is added to diffuse the stagnated heat, it is easy to be verified without a hitch.” Qingpi Decoction comes from Jisheng Fang written by Yan Yonghe in Song Dynasty, combined fragrancing to avoid filth, drying dampness with warmth and bitterness and clearing heat with cold and bitterness, and is an effective prescription for the treatment of stagnation-fever due to phlegm-dampness obstruction caused by damp-warm disease, damp-heat syndrome and heat malaria, etc.\[16\]

5.2 Five Chaihu Decoction

Five Chaihu Decoction is composed of Herbaceous peony, Atractylodes macrocephala, Angelica sinensis, Radix Bupleuri, Radix rehmanniae, Baked Licorice and Dried Orange Peel. Because the spleen-earth of Middle-jiao provides the material basis for the five internal organs, those who are deficient in the middle-Qi but do not disperse the exogenous evil must be treated with this prescription. This prescription and Four Chaihu Decoction are interior-exteriorly related. However, the Four Chaihu Decoction can only regulate Qi, while the Five Chaihu Decoction can also tonic Blood-Qi to expel the exogenous evil, especially when it is at the right time, and its magical effect cannot be fully described. Five Chaihu Decoction is suitable for typhoid, malaria and variola. In Ming Dynasty, Zhang Jingyue created six “Chaihu Decoction” including Zhengchaihu Decoction and One, Two, Three, Four and Five Chaihu Decoction. Among them, Zhengchaihu Decoction was mostly used because of its obvious antipyretic and antiallergic effects.\[17\] Only experimental studies have confirmed that Five Chaihu Decoction has significant antitussive and analgesic effects on exterior wind-cold syndrome.\[18\] There is no report on clinical application research about it, which is worthy of attention.

5.3 Guichai Decoction

Guichai Decoction is composed of Angelica sinensis, Radix Bupleuri and Baked Licorice, which can be added with Ginger or Dried Orange Peel or Ginseng. It’s can be used to treat no sweating due to Yingfen deficiency. This is the divine prescription for those who are lack of genuine-Yin and can’t get rid of exogenous cold-evil. In Guichai Decoction, the dosage of Angelica is the largest\[19\]. In Ming Dynasty, Zhang Jingyue had a deep understanding of this: Angelica sinensis can not only replenish blood, but also promote blood circulation. There is activity in tonifying as well as tonic in promoting. Angelica sinensis, Supplemented with Radix Bupleuri, Radix puerariae, Ephedra, Cassia Twig, etc, has a great chance of dispersing exogenous evil in the treatment of Yingfen deficiency and the undispersed exogenous evil, and its essentials in the two words of “activiting” and “sliding”.

6. Drying Dampness and Removing Phlegm

6.1 Jiawei Shenqi Decoction Pill

Jiawei Shenqi Pill comes from Jisheng Prescription by Yan Yonghe in Song Dynasty. It is also called Jisheng Shenqi Pill by adding Plantain Seed and Achyranthes bidentata to Zhongjing Bawei pill, in which Cassia Twig
is exchanged for Cinnamomum cassia and Dried Rehmannia glutinosa is exchanged for Radix Rehmanniae Praeparata. Zhang Jingyue said: edema syndrome is a disease in which the spleen, lung and kidney interfere with each other. At this time, deficiency of the lung leads to Qi not turn into essence but into water, the spleen-earth is controlled on the contrary due to spleen-deficiency unable to control the movement of water, and the water is disorder due to vacuous kidney unable to govern water. Seeking ancient treatment, only the modified Shenci Decoction in the Golden Chamber was used for the suitable prescription.

It was effective to use it repeatedly. On the basis of warming kidney and helping Yang, this prescription is more effective in promoting diuresis and detumescence. It is mainly used to treat kidney-yang deficiency and Yin-water stagnation. Generally, Jiawei Shenqi Pill is widely used in kidney and heart related edema or water metabolism disorders, with high recognition of curative effect.

6.2 Jinshui Liujun Decoction

Jinshui Liujun Decoction is composed of Erchen Decoction adding Angelica sinensis and Radix rehmanniae, which is the change prescription of Liujunzi Decoction. It is a magical prescription for the treatment of water being used for phlegm due to lung-kidney deficiency-cold, or Yin-deficiency and deficiency of Qi in blood in the elderly, or cough, vomiting, phlegm and asthma caused by exogenous wind-cold. Jinshui Liujun Decoction has been widely used in the treatment of patients with asthma or chronic obstructive pulmonary disease with Yin-deficiency phlegm retention syndrome. The clinical and experimental data have accumulated a lot, and the name of “the magical or wonderful decoction” is not unreal, but the clinical application is detailed in the theory of Yin-deficiency and phlegm-dampness, which is slightly used in the “lung-kidney deficiency-cold, water is used for phlegm”. In clinical practice, when Xiao Qinglong’s original prescription has no obvious effect, or the body is deficient-fat and catches the exogenous cold, and cough and phlegm are not removed, we often choose Jinshui Liujun decoction to treat it, which is more effective. Therefore, the prescription should not be limited to treating Yin-deficiency and phlegm, but focus on “deficiency” and “phlegm”.

7. Prescription for Fetal Birth and Baby

7.1 Taiyuan Decoction

Taiyuan Decoction is composed of Ginseng, Angelica sinensis, Eucommia ulmoides, Herbaceous peony, Radix rehmanniae, Atractylodes macrocephala, Baked Licorice and Dried Orange Peel. In the treatment of women who have lost Chong-ren, and whose fetal-yuan is restless and unstable due to deficiency of spleen and kidney, it should be added or subtracted according to the different syndromes. This prescription is the most appropriate. The study confirmed that Taiyuan Decoction may play a therapeutic role by regulating the expression of IL-2, IFN-γ, IL-10 and TGF-β1 in peripheral blood of threatened abortion patients with Qi and Blood deficiency.

7.2 Shuxie Decoction

Shuxie Decoction is composed of fried Herbaceous peony, Radix Bupleurum, Based Licorice, Perilla leaves and Schizonepeta tenuifolia. It is used to treat fever at the beginning of a pox and rash. Where Qi and Blood are strong without nourishing, it is only appropriate to dispel pathogenic evil and Shuxie Decoction is the most stable one to replace Shengma Gegen Decoction and so on.

It should be noted that whether Shengma Gegen Decoction is a prescription for resolving exterior and dispelling rash is questionable. When the pox and rash first appear, infants and young children should make the best use of the situation that their body are full of blood-Qi. Therefore, it is wise to use only Shuxie Decoction to dispel dispel pathogenic evil.

8. Prescription for Carbuncle and Abscess

8.1 Shaoyao Jili Decoction

Shaoyao Jili Decoction is composed of Gentiana, Scutellaria baicalensis, Akebia, Alisma orientalis, Herbaceous peony, Rehmannia and Tribulus terrestris. It can treat the sores and rashes caused by dampness-heat all over the body and those sores with redness, swelling, heat and pain in the lower part, and the curative effect is amazing. The prescription follows the structure of Longdan Xiegan Decoction, adding Herbaceous peony and Tribulus terrestris to treat carbuncle better than Longdan Xiegan Decoction. It has been reported for a long time that this prescription has characteristics in the treatment of generalized eczema, vulvar ulcer, flat wart, erythema nodosum, acute gonorrhea and other diseases, which is worthy of further exploration.

8.2 Lianqiao Jinbei Decoction

Lianqiao Jinbei Decoction is composed of Honeysuckle, Fritillaria thunbergii, Dandelion, Prunella vulgaris, Red vine and Forsythia. It is the best Prescription to treat carbuncle-toxin in Yang-fen, or between viscera, breast, lung and diaphragm. More serious patients, take several
doses of this prescription quickly, there is no one who is not be cured. There have been clinical research reports on the treatment of peptic ulcer with this prescription [27], and the understanding and application are profound.

9. Conclusions

Chengfang Qieyong is an important carrier of the classic prescriptions of traditional Chinese medicine. It is of great inheritance significance to deeply explore its characteristic content. The special prescriptions in Chengfang Qieyong not only gather the original expositions of physicians of past dynasties, but also condense the author’s own in-depth views. It is an integrated representation of the profound connotation of traditional Chinese medicine prescription and the characteristic advantages of clinical efficacy. It has important reference value for modern prescription research, clinical application expansion or new drug development of traditional Chinese medicine, quite a few of them have not been included or quoted in the current textbooks of prescriptions, and there is still room for improvement in clinical application. At present, the state is vigorously promoting the development of ancient classic famous prescription and new drugs, and the inheritance and innovation of traditional Chinese medicine compound preparations have ushered in unprecedented opportunities and challenges. In the process of selecting classic famous prescriptions, the special prescriptions mentioned in “Chengfang cutting use” should not be or missing. These special prescriptions in Chengfang Qieyong can be further verified by real world research and other means, taking the special prescriptions in Chengfang Qieyong as the foundation, further verifying its application value, collecting the superior varieties, and adding wings to the treasure house of traditional Chinese medicine.

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REVIEW
Science in Qi

C.P. Ong*
Independent Researcher, Potomac, USA.

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ABSTRACT

Qi-nurturing exercises like Taijiquan and Qigong produce significant changes in the body's emissions of electromagnetic energy, which evidence the science in Qi. Reviewing Qi in its functional forms of bioenergy brings scientific familiarity of the abstruse concept. The complexity of Qi transformations in the function of food breakdown and the formation of vital Qi essences reflect the biochemistry of metabolism and the production of ATPs.

The review leads to the basic Qi form of Zhen Qi as a representation of ATP and to a new interpretation of the premise of prenatal Qi, relating it to the transmission of mitochondrial DNA. While the basis of Qi and Chinese Medicine is the Taiji Philosophy of Yin and Yang, the regulation of Qi flow in accord with the Principle of Yin-Yang Balance translates to the discipline of the dynamics of bioenergy in homeostasis. The trained cognition of Qi allows for the manipulation of Qi energy at functional pathways to reduce the errors of excessive or deficient Qi levels, that leads to Qi harmony and homeostasis. This opens up a role for Artificial Intelligence via the cognitive perception of Qi to establish the science of Qi.

1. Background

Qi is pervasive in the Chinese culture. It is used in all things of “Heaven, Man, and Earth” from food, health, medicine, to fengshui-geomancy and cosmogony. Colloquially, Qi means “air, breath, or vapor,” but it takes on the meaning of a “refined substance” or energy in various forms in technical use, that connotes something fundamental in nature with universal applicability. More specifically, it distills in essence to “vital energy” that animates all things—no Qi, no life. Just as the cosmic Qi moves galaxies in the universe, Qi energizes all physiological activities in the microcosm of the human body. This ancient tenet of a universal Qi energy is a philosophical idealism of the theory of Yin and Yang, used quintessentially by Chinese thinkers as a grand overarching theory to explain all things from the smallest of the unseeable to the largest of the cosmos.

Qi is both simple and complex—simple in usage but complex in theory. A quick answer to the question of Qi is to think of it as a bioenergy, namely, energy in the biological processes of life. This was also the pragmatic view taken by the ancient thinkers:

Human beings are born by the accumulation of Qi. When Qi accumulates there is life, when it dissipates, there is death. There is one Qi that connects and pervades everything in the world. (Zhuangzi 22:11/84)

On a cosmic scale, the old classical texts describe Qi as permeating the universe from the beginnings of time. The

*Corresponding Author:
C.P. Ong,
Independent Researcher Potomac, USA;
Email: cpTaiji@gmail.com
role of Qi in cosmogony was formalized in Huainanzi, a compilation of the works by the eight venerated masters of Huainan, in the second century BC:

Qi originated in the primordial void and became differentiated; the purer and clearer. Yang Qi, ascended to form heaven, and the turbid and grosser Yin Qi descended to congeal and form earth. The intercourse of heaven and earth gave rise to the four seasons and the myriad things and creatures. (Huainanzi 3:1)

Confucius used the term xue Qi (literally, blood Qi) to refer to Qi, and injected spiritual and moral aspects into the concept of Qi:

The noble man guards himself against three things. When he is young his xue Qi has not yet stabilized, so he guards himself against sexual passion. When he reaches his prime, his xue Qi is not easily subdued, so he guards himself against combativeness. When he reaches old age, his xue Qi is already depleted, so he guards himself against acquisitiveness. (Confucius—Analects 16:7)

The Chinese culture of yangsheng (“nurturing life”) encompasses all matters of health wellbeing, and underlying it is Qi. Not surprising then the ancient concept of Qi is still of practical and functional relevance, like the written Chinese characters. Common concerns about health and food nutrition are denominated in Qi values. Good health and the body’s self-healing ability are characterized by a robust Qi coursing through the meridian network interconnecting the system of internal organs in harmony. The theory of Qi forms the bedrock upon which Traditional Chinese Medicine (TCM) rests.

However, despite Qi’s pervasive presence in TCM physiology, its existence is still in question in Medical Science. Chinese Medicine is dismissed as unscientific, despite its rich store of knowledge and materia medica accumulated over two millennia from case studies and clinical practice.

TCM did not come to the fore challenging Western Medicine, but it nevertheless posed a threat to the healthcare marketplace. The National Center for Complementary and Integrative Health (NCCIH) reports a government survey that about 38% of adults use some form of complementary or alternative medicine (CAM), of which TCM is one, to treat a variety of chronic disease syndromes. The impact of alternative medicine had been noted in another earlier survey which found that 80% of family practice physicians expressed an interest in receiving training in “acupuncture, hypnotherapy, and massage therapy.” Despite scientific skepticism, public demand has led U.S. hospitals to open clinics that offer CAM treatments. This led to the formation of The Consortium of Academies Health Centers for Integrative Medicine, which now counts as members the major U.S. medical schools and others numbering over a hundred.

However, Qi, the central concept in TCM, thus far, has escaped scientific definition and measurements. Also problematic is the conspicuous absence of the study of anatomy in TCM, in lieu of which is an elaborate chart of the system of meridians and acupuncture points. The underlying theory of Qi, based on the Principles of Yin and Yang and Wuxing (The Five Phases), may not transcribe in science, but it does not mean that there is no science there.

While herbal medicines may be prescribed for their healing Qi against evil Qi attacking the body, science can study the pathogenic agents and investigate the therapeutic compounds in the herbs. Indeed, this occurred in 1969 when a young 39 year old scientist, also trained in TCM, Tu Youyou, was tasked to lead an effort to find a cure for a new strain of malaria parasite (Plasmodium falciparum) that had become resistant to the existing quinine drugs. She combed through the Chinese materia medica treating malarial symptoms, and succeeded in extracting the artemisinin compound from the qinghao herbal plant. The discovery became a game-changer for the cure of the new malaria strain, for which she received the Nobel Prize for Medicine in 2015.

The Nobel recognition might have shone a bright light on TCM but it seemed to have engendered only a passing interest in Medical Science. Lacking in science, the West could not apply the Qingfei Paidu decoction (QFPD) or the other herbal combinations deployed in China that successfully contained the Covid-19 pandemic. With no cure on hand, TCM could point to a “dampness toxin” ("Shidu") as the pathogenic agent of the pestilential Qi causing the infectious pneumonia disease. Though unscientific, TCM could turn immediately to the herbal and physical therapies that invigorate the Spleen Qi to remove dampness.

It takes time to develop medicine in science, a luxury not available in an epidemic outbreak. The medical establishment would perhaps be less dismissive if it had a better comprehension of Qi and TCM. Thus, it is imperative that medical science gains more familiarity of the Qi concepts, and that TCM adopts more science in clinical practice. Meanwhile, the advent of “network pharmacology” as a field of research has proven to be more conducive to examining how TCM drugs might work on multiple therapeutic targets through multiple pathways.

Qi may seem esoteric by its singular nature of life-force vitality, but it does not have to entail a new force-energy outside of known science. Though the life-force vitality may draw a parallel to Bergson’s élan vital, our review of Qi is grounded in bioenergy in its multiple functional
forms, as well as its sensations and perceptions, towards unraveling its philosophical underpinnings.

Qi’s key function in the practice of Taijiquan lies in the discipline of the art, which relies significantly on the role of the fascia. So the review of the science in Taijiquan will be presented in a separate forthcoming paper, “Qi, Fascia, and Taijiquan.”

2. Biomarkers of Qi

The pioneering work of Dr. Shin Lin took the basic science approach to investigate the effects of Qi nurturing practices that produce consistent responses in bioenergy. With hi-tech sensitive instruments, Dr. Lin’s research team was able to measure changes in bioenergy levels associated with the traditional practices of Taijiquan (Tai Chi Chuan) and Qigong (Qi Energetics). The measurements showed a significant increase in peripheral blood flow and elevation of bioenergy in the form of heat, light photons, and electrical charges which are referred to as biomarkers of Qi.

In the lab studies, peripheral blood flow was measured by laser Doppler flowmetry, body temperature distribution by infrared thermography, electrical conductance and capacitance at acupuncture points by Motoyama’s single square voltage pulse, bioelectrical charges by gas discharge visualization, and biophoton emission by single-photon counting.

In addition, the studies also showed that the Qi exercises could induce a dual mental state of focus and relaxation (based on electroencephalography and variability analysis of instantaneous heart rate and blood pressure), boost muscle strength and endurance, and exercise the whole body as determined by electromyography.

The studies also showed that dozens of high-level practitioners of the Qi-energetic arts who were tested, could at will, bring about measurable changes in the biomarkers, including a decrease in sympathetic and an increase in parasympathetic tone in the autonomic nervous system [7].

2.1 Blood-Qi

Of the Qi biomarkers, the most readily relatable is that of blood perfusion. It is thus not surprising then that blood flow is traditionally regarded as a window to Qi. Qi and blood are treated inseparably as one, Xue-Qi (Blood-Qi), and presented as:

Qi is the commander of blood;
Blood is the mother of Qi.

The Lao-Gong acupoint (PC8 at the center of the palm) is considered a center of Qi flow. To validate the premise of the blood-and-Qi relationship, the research team took local blood perfusion and other Qi biomarker measurements at PC8 and compared them with other points on the palm. The measurements of blood perfusion, electrical conductance, temperature, and biophoton count, were highest at the Lao-Gong point, affirming the acupoint as a local center of Qi. Also, the high correlation of the bioenergy values validates them as biomarkers of Qi, in addition to confirming the relationship between the blood-flow biomarker and Qi, thus affirming the conflation of Blood-Qi and Qi [9].

“Silk-reeling” motion refers to the body’s rotational motions. Silk-reeling exercise is a core training component of Taijiquan to instill in the body the synchronization principle of the translational and rotational movements. (An object has two modes of motions, the overall motion of its center of mass and its rotation about an axis.) This basic exercise of nurturing Qi is more fully described in the author’s book [9]. It should be noted that the slow-motion methodology induces a mindful attentiveness that integrates the elements of body, mind and breath in the exercise, necessary for Qi nurturing.

In the experimental study of Qi in silk-reeling, the measurements showed not only the direct correlation between the biomarkers of electrical conductance and blood flow, but also brought out the external physical factor of gravity on blood flow. The gravitational pull due to the hand-level motion caused a corresponding cycle of increase/decrease in the blood flow measured at the Lao-Gong acupoint and similarly, in the electrical conductance at the 7 jing-well acupoints (close to the tips of the fingers).

This correspondence of the biomarker responses was also observed in the down/up motion simulated on a subject laying flat on an inversion exercise table with the arms stretched upwards, passively rocked down/up. This shows that gravity is a non-trivial external factor, representing a physical component in generating Qi. The TCM therapies of acupuncture, cupping, Guasha scraping, moxibustion and Tui-na massage all have a physical component in them [10]. Subsequent experiments showed that these TCM therapies, including topical herbal remedies, also brought about increases in blood flow and heat, as well as in some other biomarkers. These findings establish the relationship between Qi and blood as well as the evidence of Science in Qi, and give basis to healing therapies by the intervention of external energy in the form of heat, light, and electricity [11].

The innovations of enhancing acupuncture therapy with heat, electricity, and laser are thus consistent with the manipulation Qi by its biomarker representation. In electroacupuncture, a mild electrical current is sent through...
the acupuncture needle to stimulate the acupoints. In laser acupuncture, the acupoints are individually stimulated by low-intensity laser irradiation focused at the appropriate depth. For a more up-to-date review, the reader is referred to *Vital Energy, Health, and Medicine*, by Shin Lin and Gaetan Chevalier [12].

3. Qi, Metabolism and ATP

Qi takes on many forms, depending on its functional and regulatory roles, location, meridian or organ, such as Blood-Qi, Spleen-Qi, Lung-Qi, and so on. While that may exemplify its universal applicability, confusion will inevitably arise because the functions described in Qi theory often do not correspond well in physiology.

Nevertheless, in reviewing the life-force vitality of Qi in its multiple forms, we are led to the life-sustaining activities of metabolism. Rather than fight the abstruseness of Qi, we take the pragmatic approach to simply look for the science in the Qi forms as they relate to the bioenergy of metabolism. Where is ATP in the Qi forms?

3.1 Qi Forms in Traditional Theory

Traditional Qi theory posits that at birth one is bestowed a store of Original Qi (Yuan Qi 元气), which is also called Prenatal Qi. The theory holds that Yuan Qi is in the form of an innate essence (Jing) or energy, which sparks life in the embryo. This hereditary endowment comes in a fixed amount. Yuan Qi, stored in the Kidneys, cannot be replenished but it can be preserved by the regulation of its consumption. In old age, the supply dwindles, the body slows down and weakens, and finally, when depleted, life expires.

The Qi energy that we consume all the time is produced by the body, and is referred to as Postnatal Qi, to differentiate it from the inherited Prenatal Yuan Qi. The supply of Qi is replenished by the transformations of the many Qi forms from a basic Qi, called True Qi or Zhen Qi (真气). Thus the body’s energy production is about the production of Zhen Qi.

Three main sources of Qi-essences are involved in the production of Zhen Qi: 1) Gu-Qi 谷气, or Food-Qi, the Qi of food essence, which comes from the ingestion of food; 2) Air-Qi, the essence from natural air; 3) Yuan Qi, from the store of Prenatal Qi.

Air-Qi essence in the lungs comes from the air we breathe. The production of Gu-Qi is a process of transforming ingested food by the action of Spleen Qi to refine food essence and then to Gu-Qi. Air-Qi and Gu-Qi combine to transform to Zong-Qi (宗气) or Pectoral Qi. Yuan Qi enters next in the last step of the production process, promoting and transforming the Pectoral Qi to the final state of Zhen Qi (True Qi or more appropriately Realized Qi) (Figure 3).

Zhen Qi is deployed in the many functional Qi forms which fall into two main categories: Nutritive Qi (Ying Qi 血气) and Defensive Qi (Wei Qi 正气). Nutritive Qi supports the growth, development and maintenance of the body, including the Qi energy for acting functions, transportation and motion. In the form of Blood-Qi, Nutritive Qi nourishes the whole body. Defensive Qi protects and defends the body from pathogenic agents.

There are other subcategories, such as of Zhong Qi (Central Qi) and Zheng Qi (Upright Qi), which refer to Qi functions that cross over with those Pectoral Qi and Wei Qi. For example, Zheng Qi is described as protective Qi but is nuanced by its fortitude of Qi strength in guarding against attacks from external pathogens.

Sorting out the myriad Qi forms may prove to be intractable as the functions attributed to, say, the Zang-Fu organs often do not relate to their counterparts in anatomy. For example, Spleen Qi’s role in transforming ingested food to Food-Qi does not correspond to the function of the anatomical spleen. Similarly, the functions attributed to the Lungs of assisting the Spleen in sending Food-Qi to the Heart to form blood, do not correspond in physiology.

The most intriguing organ is TCM’s designation of a Fu organ, called the Sanjiao (三焦) or Triple Burner. This “organ” does not take on a physical form nor does it identify with any of the internal organs in anatomy, yet it plays a very significant role in Qi transformations. All the functional Qi forms, including Zhen Qi, undergo a complex of mechanisms involving the Triple Burner [13]. (TCM refers to Food-Qi being produced in the Middle Burner with Spleen Qi, transformed to Zong Qi with Air-Qi and further refined with Yuan Qi to Zhen Qi in the Upper Burner.)

The redoubtable Sanjiao, whether as a process or organ, highlights the complex nature of Qi transformations. For a more comprehensive account, the reader is referred to “An Approach to the Nature of Qi – Qi and Bioenergy” [14] As we shall see, these Qi processes parallel the complexity of biochemical processes of metabolism.

3.2 Energy in Metabolism

The body needs energy to run but it relates to only certain sources of energy. The whole range of processes, the replenishing and the consuming of energy, is summed up as metabolism.

An automobile gets its energy from gasoline. We cook with the heat energy from fire but we do not harness the heat from a car crash to cook. An electric car is engineered to run on electricity but we cannot bioengineer the body’s
metabolic processes to run on heat, electricity or light. At the macro level, the body can access energy only in the form of food and oxygen. TCM’s answer to the body’s energy is Blood-Qi, which implicitly includes oxygen (Air-Qi) and food nutrients (Food-Qi) in the blood circulation. The discussion here thus brings science right at Qi’s doorstep.

The micro activities of physiology are mainly about the metabolism of four macromolecules, proteins, fats, carbohydrates and nucleic acids, which we consume as food. These are broken down into their subunits of amino acids, fatty acids, glucose and nucleotides respectively, which are processed at the cellular level. The body is constantly consuming energy to power the muscle contractile actions and to synthesize macromolecules from their subunit building blocks, as well as to send nerve signals, move ions, regulate hormones, and others.

However, the only form of energy accepted in metabolism at the cellular level is not Air-Qi or Food-Qi, but the energy in the chemical bonds of adenosine triphosphate (ATP). At the micro level, ATP energy is the only energy currency that can be used in physiology.

A lot of ATP is needed and recycled. The ATPs that work the muscles are recharged more frequently, in seconds, while others may take minutes. The ATP energy is stored in the phosphate bonds, and released by hydrolysis:

\[
ATP + H_2O \rightarrow ADP + Pi + Energy
\]

where Pi represents an inorganic phosphate, and ADP, adenosine diphosphate (which is phosphorylated to ATP in the reverse reaction of recharging).

The energy for ATP comes from the burning the food-fuel in the body, which goes through a series of biochemical reactions. The basic chemistry of combustion is the same as that of fire in wood burning but the combustion process reflecting that of the Triple Burner. The review also brings out the highly controlled process where the protons H⁺ and electrons e⁻ released from the hydrocarbon are immediately harnessed by the coenzymes, NADH and FADH₂, also called electron carriers, to segregate them from reacting with oxygen as in an open burning.

This harnessing of protons and electrons begins in Stage 1, once glucose is transported into the cell. We see here the initial process of Spleen Qi transforming food essence to Gu-Qi. In the cytosol, glucose is broken down into pyruvates, by ATP energy and a series of enzyme actions, releasing high-energy protons and electrons, H⁺ and e⁻, which are secured by NADH:

\[
NAD^+ + 2e^- + H^+ \rightarrow NADH
\]

Before entering the Krebs Cycle, the pyruvates are further oxidized by coenzymes, forming acetyl-CoA and releasing more high-energy H⁺ and e⁻. The formation of acetyl-CoA initiates the Krebs Cycle, in which even more H⁺ and e⁻ are harvested in another series of enzyme and coenzyme actions. The Krebs Cycle stage takes place inside the inner matrix chamber of the mitochondrion protected by an inner membrane (Figure 2). The Qi footprint of the formation of Gu-Qi by the Spleen Q is found here, which continues on to the next stage.

One glucose molecule in glycolysis yields 2 NADH and nets 2 ATPs; in the acetyl-CoA formation stage, 2 FADH₂; and in the Krebs cycle, 6 NADHs and 2 FADH₂, as well as 2 ATPs. This represents a clean harvesting of all the 12 H⁺ protons in the glucose molecule, C₆H₁₂O₆, which are all securely carried in the NADH and FADH₂ for use in Stage 3.

In Stage 3, all the high energy H⁺ and e⁻ in NADH and FADH₂ are transported to four protein gate-complexes embedded in the cristae fold of the inner membrane. Here the protons and electrons, released from the coenzymes, are separated, with the protons pumped out through the gates, building up a high concentration of protons outside the inner membrane, and the electrons ferried along the complexes in an electron transport chain to the last complex. With the protons and electrons segregated along two...
paths, reaction with oxygen is forestalled until the final phase of the synthesis of ATPs (Figure 2). At the end of Stage 3, the Food-Qi essence is extracted in the Qi dynamics of the proton and electron flow with the formation of Zong Qi.

The phosphorylation of ATPs takes place at the ATP synthase, another complex protein transport structure, also embedded in the cristae fold, which incorporates a mechanical nano rotary device. The motive force of the proton concentration gradient drives the protons from outside the inner membrane through this transport structure in a stream, turning the rotary device, and assembling ADP and Pi into ATP, making three ATPs in each complete rotation. The phosphorylation phase represents the action of Yuan Qi that transforms Zong Qi to the final basic Qi form of Zhen Qi.

**Figure 2.** The mitochondrion ATP factory is well protected in the inner matrix compartment where the last two stages of the combustion reaction take place, with the last stage in protein structures embedded in the cristae folds of the inner membrane.

In doing the hard work of phosphorylation, the H⁺ protons flow back into the inner matrix compartment and finally get to mix with the electrons exiting the last complex via the electron transport chain and react with oxygen, producing water and heat. This removal of H⁺ protons in the inner compartment is critical to keep the proton motive force necessary to drive the nano rotary device in the assembly of the ATPs.\(^{[15]}\)

\[ 4H^+ + 4e^- + O_2 \rightarrow 2H_2O + \text{Heat}. \]

The heat generated in the reaction maintains the body’s core temperature.

This is the science in the TCM process of Qi transformations from ingested food to the final basic Qi form of Zhen Qi. Little wonder that the complex processes of Qi transformations are relegated to the three chambers of the ubiquitous Triple Burner. Zhen Qi represents ATP energy in metabolism (See Figure 3), giving us Proposition 1.

**Proposition 1:** ATP energy is represented by Zhen Qi (真气), the basic Qi form in transformations.

**Figure 3.** Zhen Qi, the final state of Qi formation, is ATP energy.

The number of mitochondrial factories in each human cell varies from hundreds to thousands. In heart muscle cells where the demand for ATPs is extremely high, the mitochondria take up about 40% of the cell’s cytoplasmic space. In the liver, there are 1000 to 2000 mitochondrial factories per cell. The mitochondrial factories keep us going.

### 3.3 Blood-Qi and Red Blood Cells (Hemoglobin)

In the conflation of blood and Qi as Blood-Qi, Qi assumes the life-force vitality of blood as a carrier of oxygen and nutrients, which are also viewed as forms of Qi. The Zhen Qi deployed in the Nutritive Qi category refers primarily to the subunits of the macronutrients carried in the blood. Here we review the transport of oxygen in the vitality of Air-Qi where the science is most transparent.

In the path of Air-Qi (oxygen) to the tissues, air taken into the lungs by respiration ends up in the alveolar sacs which are meshed in capillary beds where a first gas exchange occurs. As the capillary blood flows through, oxygen in higher concentration in the alveolar air crosses the membrane to the venous blood, oxygenating it, while carbon dioxide in the blood crosses into the sacs to be discharged in the out-breath. The oxygenated blood is pumped by the heart journeying to the brain, muscles, organs, and all the body tissues. Upon arrival at the capillary beds serving the destination tissue, another gas exchange takes place, where oxygen from the blood is released and
carbon dioxide from the tissue is absorbed by the blood to be discharged.

Life’s physiological activities ride on the safe transport of oxygen in the blood circulation system. The precious cargo of oxygen is carried in the red blood cells (RBCs). The oxygen molecules travel in a first-class hemoglobin cabin of four heme seatings, where each molecule is individually buckled down by an iron atom, and is released only upon arrival at its destination. There are about 270 million molecules of hemoglobin in each RBC to handle the volume of oxygen to be transported. An adult human body has about 20 to 30 trillion RBCs to transport 99% of oxygen in the blood circulation. TCM views the Qi-color of a healthy skin complexion as the robustness of Air-Qi (oxygen) in the blood.

To maintain the population of RBCs that have a lifespan of 100 to 120 days, about 2.4 million new RBCs are produced every second in the red marrow of large bones. In its lifetime, a mature RBC would have journeyed about 150,000 cycles in blood circulation, on an average of a minute per cycle.

The RBC’s devotion to its task exacts an unparalleled cell-sacrifice. As the RBC matures in 7 days, it sheds its nucleus, mitochondria and other organelles in order to pack in even more hemoglobin. The developed RBC, thus stripped, serves as a sack of hemoglobin, totally dedicated to the transport of oxygen, toiling 24/7. In its devotion, the RBC cell is dealt a fate of being nucleus-less, with neither identity nor soul. The RBCs form a class in servitude that represent an 80% majority of the cells in an adult human body.

TCM’s embrace of blood as a denser form of Qi accords it a nature of duality—the Yin character of the material liquid form, complementing the Yang character of the non-matter “gaseous” form. However, the transformation between the Qi forms is not simple like that of water and steam, requiring only heat, but is driven by a complex of chemical and electrical bioenergy in addition to heat, which is more akin to but more complicated than that of energy change in thermodynamics.

Qi has no theory of RBCs or of any of the other blood cells but in the same brush stroke of Qi, without the science of hematopoiesis, we find Blood-Qi deployable to subsume Wei Qi that represents the immune functions of lymphocytes and leucocytes (White Blood Cells), circulating in the blood that protect the body and fend off pathogens. In this regard, the TCM Spleen has a more relatable ing in the blood that protect the body and fend off pathogens (White Blood Cells), circulating in the blood that protect the body and fend off pathogens. In this regard, the TCM Spleen has a more relatable

4. Yuan Qi and Mitochondria

Of the three main components in the production of Zhen Qi, we can relate to Gu-Qi as sourced from food, and to Air-Qi as oxygen, but the premise of Yuan Qi is mystifying. Yuan Qi is strictly hereditary, bestowed in a fixed amount and is of a different essence from Postnatal Qi, which is produced by the body. The inherited prenatal Yuan Qi cannot be regenerated but can only be conserved and its depletion spells the expiration of life. The Daoist aspiration of longevity is an alchemy that refines Postnatal Qi to its purest essence to serve functionally as Yuan Qi, thereby conserving the prenatal Qi.

Although we can see the complex biochemical reactions of cellular respiration in the final phase of Qi transformation (from Zong Qi to Zhen Qi), the premise of Yuan Qi defies any attempts to relate it to a bioenergy concept. Bioenergy is electrochemical, stored in bonds, and bears no traits of inheritance.

As it turns out, what is inherited is not the Qi energy per se, but a genomic blueprint of the mitochondrial ATP factory from the mother. This small mitochondrial DNA (mtDNA) is a ringed structure of 37 genes and is distinct and separate from the cell’s DNA in the nucleus derived from the union of parental DNAs. The enzymes involved in the oxidative phosphorylation, the subunits of the ATP synthase, the NADH dehydrogenase and the cytochrome c oxidase in the function of the electron transport chain, are made from the encodings of the mtDNA genes.

After fertilization, the mitochondria in the mother’s egg cell continue to supply the energy needs while the mitochondria of the relatively tiny sperm, which are all in its tail, make no contribution, i.e., zero “child support.” It is this mtDNA from the egg cell that is passed down from mother to offspring in its entirety with no input from the sperm. This inheritance of the mtDNA is strictly a maternal lineage transmission. Genealogical researchers use mtDNA to trace our maternal lineage back in time, pushing the boundary in the quest of an Eve mitochondria. Nature’s trust in the maternal responsibility to pass down the genomic blueprint of the mitochondrial ATP factory from generation to generation has kept the flame of the human race burning from the beginning of time to the present.

As to Prenatal Qi and the Daoist alchemy of longevity, the fight against aging cannot succeed if the mitochondri-
al factories suffer a dysfunction and fail to keep up with life’s demand for ATPs. There is a rich compilation of herbal and physical therapies in TCM and Daoist alchemy that combat aging, which studied in mitochondrial biology, “may reveal previously unrecognized mitochondrial pathways and new therapeutics to manipulate them.”[18]

5. Yin-Yang Balance

Yin-Yang Balance is at the core of Qi theory and is pivotal in the functional harmony of the Zang-Fu organs and other entities. Homeostasis is a representation of this balance. Quite remarkably, the metaphysics of Yin-Yang Balance is captured fully with all its dynamics and multi-dimensionality in the ubiquitous Taijitu (太极图) or Taiji Diagram (Figure 4). The Qi harmony of the physiological entities in function is represented in the Taijitu. Wellness in TCM is a harmony of the body’s functional entities, just as musical harmony is of the orchestral entities in performance.

In metabolism, we see the Yin-Yang Balance of hydrogen protons and oxygen in the inner chambers of the mitochondrion that is forestalling combustion from getting out of control into a micro-Hindenburg disaster. The ATP in storage, localized at the tissues, maintains a Yin-Yang Balance between its incessant and varying consumption and its replenishment by production-on-demand on site.

However, the challenge remains to translate the application of the metaphysical Yin-Yang constructs in terms of bioenergy in physiological processes.

5.1 The Dao of Harmony

Physiological harmony is not just the balance of an entity but of a matrix of entities that are interrelated in function. The analysis of the multi-dimensional factors is complex but to integrate them in harmony in the dynamics of their interrelationships would seem nigh impossible as resolving imbalance in one would affect that of the others, requiring a recalibration of the matrix each time.

The problem may be cast in mathematical equations and solved by computers but the issue is that of relating the solutions to the physiological processes in real time. The body cannot relate to it. This is what renders the task of balancing the functional entities in harmony intractable. The ancient thinkers resorted to philosophy and the adaptability of the soft logic of Yin and Yang to come up with a practical solution in the art of Qi nurturing. The art develops the cognition of the functional effects of balancing as Qi is nurtured. The Qi thus nurtured holds the key to harmony.

Now while we may appreciate harmony, disharmony is what we cognize more readily. The states of disharmony are prevalent while harmony is a highly ordered state. The art is to cognize disharmony, discern the imbalance of the Qi of the matrix of entities, and then to resolve it towards harmony. Resolving imbalance involves a combination of Yin and Yang. Too Yin may be resolved by reducing Yin and/or increasing Yang, while too Yang, by reducing Yang and/or increasing Yin. However, in the art of Qi nurturing, imbalance is cognized as Qi being in excess or in deficiency, and is resolved by restraining what is in excess and invigorating what is in deficiency, thereby reducing the errors.

5.2 Perceptive Differentiation

To cognize Qi as being in excess or in deficiency requires the sensation to be able to differentiate between the error of being over or under. And the discipline to restrain excessive Qi or to invigorate deficient Qi requires the sensation to be able to differentiate between the degrees of error as well, so that the margin of errors can be reduced towards balance. The art of Qi nurturing develops both the perceptive differentiation in the sensation. The Qi nurtured thus comes equipped to resolve the Qi imbalance of the matrix and to keep reducing the margin of errors towards harmony.

The body cannot relate to the hard solution by analysis and computation of the multi-dimensional factors of individual entities. So, pithily, the principle of perceptive differentiation in Qi offers a way to Yin-Yang balancing towards harmony that the body can relate to. The Way “seeks harmony by not seeking it,” but to practice the art of Qi nurturing—the Way of weiwuwei (无为) in the Dao of Harmony. The development of the art of Qi nurturing is inspired by the Dao of Harmony.

5.3 The Operative Principle of Qi in Balance

Qigong and Taijiquan practices are drawn from the ancient Qi-nurturing arts of dao yin tunique (导引吐纳) (“guiding-pulling and breathing”) that enhance yangsheng (养生) or health wellbeing by integrating body, mind and breath in the training. The art of Qi nurturing is grounded in the functional effects of yangsheng. Thus, inherent in the nature of Qi nurturing is its operational effect of Yin-Yang balancing that regulates the functions of the physiological entities.

The functional entities are interconnected by a network of meridians (Jing-luo) through which Qi courses. The operating principle of Yin-Yang balancing regulates the Qi dynamics, which restrains Qi that is excessive and invigorates Qi that is deficient. In other words, Qi nurturing
operationally serves as a homeostatic control in physiology that enhances Qi harmony, thus Wellness.

Qi nurturing is not just about the bioenergy per se, but is coupled with its invigorating or restraining function towards balance. That is, the operative principle of Yin-Yang balancing is built into the *daoyin tuna* practice of nurturing Qi. This leads to:

**Proposition 2:** Qi nurturing embodies the operative principle of Yin-Yang balancing towards harmony.

The Proposition implies that Qi nurturing operationally enhances *yangsheng* health wellbeing.

The operative principle of balancing in Qigong and Qi therapies can thus be applied to resolve Qi disharmony that represents an illness. Therefore, the proposition also forms the rationale of Chinese Medicine’s therapy of “ingesting Qi to cure illness” (*服气疗病*).

A TCM physician is trained to have a cognitive perception of Qi and to decipher the “patterns of disharmony of Qi” in clinical diagnosis. And Qigong practice cultivates the cognitive perception of a matrix of bioenergy transduced as signals of Qi sensation.

### 5.4 The Cognitive Perception of Qi

Qi experience is often described by Qigong or Taiji-quan practitioners as a warm and tingling sensation as well as a sudden surge of heat. These Qi sensations can be attributed to an increase in blood perfusion and a higher level of muscle activations, which extend the senses of vision, sound, touch, smell, and taste by external stimuli.

The bioenergy dynamics of the body’s functional entities can engender an internally driven Qi sensation. Dysfunction in one or more entities causes imbalance resulting in a physiological disharmony, which can give rise to a sensation of disorder or of being unwell. The psychophysics of TCM is the perception of the sensation of being unwell as Qi disharmony of the functional entities. This establishes a qualitative relationship between Qi as a sensation of Wellness and the stimulus bioenergy of the functional entities. In short, Qi represents the cognitive perception of a basket of sensations of Yin-Yang imbalances.

The cognitive perception of Qi cultivated by Qi nurturing in the practice of Qigong or Taijiquan incorporates the operative principle of balancing which can be harnessed to discipline and manipulate Qi. The ingenuity of the art of Qi nurturing is that the practice carves out a solution-path of invigorating Qi that is deficient and restraining Qi that is excessive that reduces via feedback the error margins of imbalance towards harmony. This is art working in science.

Additionally, the cognitive perception offers an insight of Qi as embodied in the Three Phases of *Essence, Energy, and Spirit* (*Jing Qi Shen*). Essence (*Jing*) represents the distilled essence of Qi material. Thus *Jing* and *Qi* are viewed as the material basis of the body part, and *Spirit* (*Shen*) the spirit of life, the mind part. More specifically, the cognitive perception of Qi represents the *Spirit Phase* of Qi.

**Proposition 3:** The cognitive perception of Qi represents the *Spirit* (*Shen*) of Qi in the Three Phases of *Jing Qi Shen*.

The practice of Qi nurturing has two components, the body part of exercise and breath, and the spirit part of meditation. The meditative or mind part is induced by the attentiveness and awareness of the practice. Both the body and meditative disciplines reinforce each other in the process of cultivating the cognitive perception of Qi, the achievement of which leads to the mastery of the art and the realization of the full flavor of the *Jing Qi Shen* of Qi. This is the prize of the *yangsheng* culture of nurturing longevity (*changshou*). It also provides a base for other spiritual experiences.

One does not have to achieve mastery to gain the *yangsheng* health benefits of Qi-nurturing practices. Qi may be evasive scientifically, but the body picks it up and relates to it readily. Beginning practitioners commonly experience Qi as a tingling or warm sensation in the hands in the first few days of practice. Initially, the sensation is intermittent, appearing and disappearing but over time gains clarity. Along with the developing perception of Qi, the body enjoys the clear benefits of health wellbeing which accrue and keep the practice going.

### 5.5 Some Other Qi Phenomena

The hard Qigong practice of Chinese kungfu relies on Qi training to strengthen the body’s constitution, building an “iron-shirt” for protection and to withstand hard strikes without injury. For example, by hitting sandbags, the Qi nurtured can strengthen the hand’s bone in breaking bricks. Qi can strengthen shin bones to break a baseball bat and can thicken skull bones. The science of bone strengthening in hard Qigong is explained in Wolf’s Law: Healthy bones adapt to become stronger if subjected to increasing loads placed on them. The load-force in hard Qigong practice stresses the bones, generating piezoelectricity, which transduces to electrochemical signals to the cells, stimulating bone growth. The Yin-Yang balance of Qi between load and growth is still maintained but at a higher level, the goalpost of Yin-Yang balance moved, so to speak.

The Qi-biomarker energies are also found in yoga and meditation practices. Most notably, the Tibetan monks’ practice of g-Tummo (Inner Energy) meditation can generate body heat to dry wet sheets draped on them, exposed...
in the Himalayan snowy winter clime. The monks in a deep meditative state can raise the temperatures of their fingers and toes by as much as 17 degrees Fahrenheit [19]. The g-Tummo has a somatic component that incorporates “vase breathing,” which is akin to abdominal breathing common in yoga and Qi-energetics but more demanding. A scientific study of the g-Tummo wet-sheet drying phenomenon is able to pinpoint the “forceful” breathing component as a crucial physical factor in generating body heat. Note that the g-Tummo meditation does not produce a sauna bath with the wet sheets, as the heat comes from the body. The body’s core temperature remains in the normal homeostatic range [20].

6. The Qi Crossroads of East and West

Western Medicine is pathogen-centric; it traces diseases to specific pathogens and finds medicine to treat them. However, the causes of illnesses cannot always be identified as in the chronic ailments of syndromes, and even if known, the cause-and-effect mechanisms are often not deterministic. The same initial conditions do not always produce the same final states of outcome as in the physical sciences with mathematical equations. We may trace the pathway from state A to state B, but the micro states of physiology are irreversible, as in thermodynamics. Cancerous cells can remain benign or become malignant and metastasize. Covid-19 infections can be asymptomatic or can develop into mild or severe cases.

TCM’s theory of health wellbeing is based on Qi theory and the Taiji Philosophy of Yin-Yang Balance [21]. TCM views disharmony within the functional entities and a weak Qi flow as ill health, and harmony and a robust Qi flow as good health.

If a functional entity were impaired, it could disrupt Qi flow and affect the body’s physiological function. The impairment can be due to: 1) pathogens or injuries; 2) irregularities of bioenergy dynamics, without specific pathogenic agents or injuries. Regardless, the body’s impaired physiological functions that impact Wellness are cognized as disharmony of Qi flow.

Without the science of pathogenesis, etiology, and pharmacology, Chinese Medicine relies on the diagnosis of “patterns of disharmony” and the operative principle of Qi therapy to resolve the impairments of physiological entities, whether or not caused by pathogens. TCM’s therapeutic strategy relies primarily on regulating Qi towards balance and harmony along a combination of pathways and less so on pathogens (Figure 4). Advantageously, the non-pathogen-specific and soft approach covers syndromes caused by stress and emotion as well, such as of vexation, anger, anxiety, fright, and grief.

Figure 4. Pathways to Yin-Yang Balance

Also, since regulating Qi dynamics to restore harmony involves all the related organs, TCM’s drug therapies are necessarily multi-herbal formulas, taking multiple pathways, and directed at multiple targets. The new science of network pharmacology is more conducive to TCM’s Qi therapies that target the network nature of biological processes [22].

Western Medicine may gain some insight by applying the operative principle of Qi to regulate the bioenergy dynamics. This may lead to the discovery of new indices of Qi Wellness in the bioenergy of homeostasis and the incorporation of Qi in Medical Science.

Chinese Medicine can develop the Qi cognition of the bioenergy dynamics of disease diagnostics and therapeutics of Western Medicine. TCM can incorporate the test data of glucose level, blood counts, and the profiles of metabolic, lipid or other health panels to complement its Qi-diagnostics.

If a TCM physician can be trained to develop the cognitive perception of Qi to decipher and manipulate Qi dynamics, Artificial Intelligence (AI) cannot be far behind. This brings to the forefront the inevitable role of AI in Chinese Medicine, which is being pursued [23].

7. Qi in the Sun-Earth System

Here we review briefly a scientific aspect of the cosmic nature of Qi in the framework of the Sun and Earth. The
key source of energy of the Sun-Earth system is the nuclear fusion energy of hydrogen isotopes taking place at the core of the sun, generating solar energy that the earth receives as radiant energy in the form of sunlight and heat.

The food/carbohydrates we ingest are produced in plants by the process of photosynthesis that uses sunlight energy and carbon dioxide in the atmosphere, with oxygen as a by-product. The ATP energy we use to run biological life comes from the burning of the food-fuel with oxygen, also from the atmosphere, with carbon dioxide as a by-product. Thus we have the cycling and recycling of oxygen and carbon dioxide in the Earth’s atmosphere and a production pathway transforming sunlight energy to ATP in our body. The Earth is also stores other forms of energy, notably, oil, coal, natural gas, and other fossil fuels. These are used directly or converted to electricity to run the economic and production engines of modern civilization, including food production.

Using the Qi paradigm, we can describe the solar energy and the nuclear fusion energy in the Sun as Sun-Qi, and all the converted forms of energy that sustain life on Earth, as Earth-Qi. This provides a Qi-representation, in modern terminology, of the philosophical model of Heaven-Man-Earth. The key source of Earth-Qi is the Sun-Qi. When the nuclear fuels in the sun run low, Sun-Qi depletes. Life on Earth ends when Sun-Qi runs out.

8. Conclusions

Qi was born as an energy concept under the auspices of the Grand Taiji Theory of Yin-Yang, of an earlier and non-scientific era, but not of an alternate universe. The challenge has been to explicate Qi without being entangled in the esoterica of its ancient trappings. The measurements of energy emissions from the body associated with Qi nurturing in the form of heat, light photons, and electrical charges, provide a concrete link of Qi to science. The life-force nature of Qi, a concept of the vitality of energy in life, which seems more philosophical than scientific, finds a basis in ATP energy, as the only energy currency negotiable in the life activities of physiology at the cellular level. The discussion of these and other phenomena of Qi serves to give science more familiarity of the old concepts.

What is to be reiterated is the Principle of Yin-Yang Balance at the heart of the concept of Qi. The perception of Qi embodies the operative principle of Yin-Yang balancing. So the very practice of the art of Qi nurturing forges a solution-path that leads to the harmony of the body’s functional entities, thus Wellness. In short, Qi nurturing serves to regulate the dynamics of bioenergy in homeostasis. This Yin-Yang art of Qi is inspired by the Dao of weiwei—Qi in the service of science.

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[6] Dr. Shin Lin is Professor of Cell Biology, Biomedical Engineering, and Integrative Medicine at University of California, Irvine. He is also a practitioner and teacher of Chinese Martial Arts, Qigong, and Taiji-quan with four decades of experience. https://mindbodyodylab.bio.uci.edu/.


ARTICLE
Detection of Puerarin from Pueraria Mirifica Tuber and Its Formulated Cosmetic Products

Lee Suan Chua1,2* Nurhirza Shaiful Yamin1,3

1. Institute of Bioproduct Development, Universiti Teknologi Malaysia, UTM Skudai, Johor Bahru, Johor, 81310, Malaysia
2. Department of Bioprocess and Polymer Engineering, School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, UTM Skudai, Johor Bahru, Johor, 81310, Malaysia
3. National Pharmaceutical Regulatory Agency, Lot 36, Jalan Universiti, Petaling Jaya, Selangor, 46200, Malaysia

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ABSTRACT
Pueraria mirifica (PM) has traditionally been used to relieve postmenopausal symptoms. Recently, its extract has been developed into various cosmetic products to promote skin rejuvenation and youthfulness. This study investigated the phytochemicals of PM tuber and compared between the tuber flesh and its outer peel. Puerarin which is one of the major isoflavones and being considered as the marker compound was used to determine the presence of PM extract in local cosmetic products. Puerarin could be ionized by a mass spectrometer at both negative and positive modes. The peak ionized at the negative mode showed to have a narrower peak width (0.2 min) and higher signal-to-noise ratio (30) for puerarin (1 mg/L). The results also found PM extract contained many C- and O-glycosylated isoflavones, especially from its peel extract. This explains the peel extract showed to have four times higher antiradical activity than those of flesh extract. Puerarin from the cosmetic products was recovered via successive methanolic sonication and followed by liquid-liquid extraction using ethyl acetate. Puerarin was successfully partitioned from the highly complex chemical mixture of cosmetic products with the recovery ranged from 89.1 % to 115 %. Hence, isoflavones was found to be higher at the outer peels than its tuber flesh. A simple and reliable method has been developed to analyse the presence of PM extract in cosmetic products based on the detection of puerarin after successive extraction via methanolic sonication and ethyl acetate partition.

1. Introduction

Pueraria mirifica (PM) or P. candollei var. mirifica is a plant can be found in northern Thailand and belongs to the Leguminosae family. The tubers of PM have been consumed by native Thai for generations to relieve postmenopausal symptoms including skin wrinkle, hair and memory losses1,2. Many scientific studies have proven the pharmacological activities such as estrogenic3,4,

*Corresponding Author:
Lee Suan Chua,
Institute of Bioproduct Development & Department of Bioprocess and Polymer Engineering, School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, UTM Skudai, Johor Bahru, Johor, 81310, Malaysia;
Email: chualeesuan@utm.my
antioxidant, antioestoporosis, anticancer, antimutagenic, neuroprotective, antidiabetic, of PM extracts. Few clinical studies proved that the administration of PM would promote the reduction of low-density lipoprotein.

Researchers revealed that the predominant phytochemicals in PM are isoflavones or commonly known as phytoestrogens. This is because isoflavones have structural similarities to estrogens with the hydroxyl groups at the C7 and C4 positions as estradiol molecule which could bind to estrogen receptors. Puerarin (8-C-glucoside of daidzein) accounts for about half of the total isoflavone content together with other small amount of isoflavones such as genistin (7-O-glucoside of genistein) and daidzin (7-O-glucoside of daidzein). Puerarin is a marker compound of the *Pueraria* genus. It is a heat sensitive compound and temperature may change the composition of isoflavone conjugates. Possibly, isoflavones may be altered after an extended period of storage even at room temperature.

The administration of PM extract at 20-100 mg/day for 6 months, or 100-200 mg/day for 12 months was found to relieve menopause symptoms without significant changes on hepatic, hematologic, and renal functions. A recommended safe dose of PM supplement was 1-2 mg/kg body weight/day or about 50-100 mg/day. However, the long-term effect of administration has not yet been clarified in detail. PM has yet to obtain regulatory approval as a dietary ingredient in Australia, Singapore, United States and European countries due to the lack of safety assessment.

In the present study, the phytochemicals of PM tuber were analyzed and compared between its flesh and peel extracts. Maceration was performed to extract phytochemicals from the PM tuber to avoid thermal degradation of isoflavones. A successive extraction by methanol and ethyl acetate was introduced to recover puerarin from the highly complex mixture of cosmetic products. The presence of puerarin was confirmed using liquid chromatography tandem mass spectrometry.

### 2. Methodology

#### 2.1 Chemical and Products

The tuber of PM was purchased from the province of Phatthalung, Thailand. The standard chemical, puerarin (≥ 98 %) was purchased from Sigma-Aldrich (St. Louis, USA). Three cosmetic creams containing *P. mirifica* extract were purchased from different regions of local markets in Malaysia. Product A has low viscosity and yellowish cream, whereas Product B and C were white and smooth cream.

#### 2.2 Maceration of *P. mirifica*

The tuber of *P. mirifica* was cleaned and peeled before sliced into thin and small pieces. Both flesh slices and peel were put on trays for oven drying at 65°C for 3 days until completely dryness. The dried samples were ground into powder. Ten grams of samples was macerated in 200 mL methanol (95%) in two different flasks for 5 days. The flasks were swirled on a daily basis and loosened the caps for gas release. The supernatant was decanted, filtered and evaporated to dryness using a rotary evaporator (Heidolph, Laborota 4003, Germany). The dried extracts were kept in a refrigerator for subsequent antiradical and LC-MS/MS analyses.

#### 2.3 Puerarin Extraction from Commercial Products

One gram of product creams was weighted and mixed with 5 mL methanol in individual tubes (10 mL). The tubes were vortexed for 1 min and sonicated for 10 min using an ultrasonic bath (Daihan Scientific, South Korea, 50 kHz, 400W) at 30°C. The mixture was then centrifuged at 6000 rpm for 60 min. The supernatant was decanted and filtered into ceramic evaporating dishes for drying in an oven at 65°C.

The dried extracts were re-constituted in 5% methanol (20 mL) and poured into a separating funnel (100 mL). Ethyl acetate (30 mL) was added into the funnel and the mixture was vigorously shaken for 5 min. Generated gas during extraction was released from the funnel from time to time. The mixture was left to separate into 2 significant
layers. Ethyl acetate which was located at the top layer was withdrawn. Another fresh ethyl acetate (20 mL) was added into the remaining aqueous solution for extraction again. The process was repeated for thrice and the collected ethyl acetate was combined for drying. The dried product extract was kept in a fridge at 4 °C for subsequent analysis.

2.4 Determination of Puerarin Recovery

The performance of puerarin extraction from commercial products was determined by spiking a known concentration of standard puerarin into products. One gram of product creams was taken and put into individual tubes. A 1 mL of puerarin (10 mg/L) was spiked into the individual tubes. The tubes were then topped up with methanol to 5 mL. The tubes were then well mixed for 1 min, sonicated for 10 min and centrifuged to harvest the supernatant as explained in the procedures of puerarin extraction from commercial products. The harvested supernatant was oven dried. The experiments without the procedure of puerarin spiking were performed as control (unspiked). The dried weights of product extracts were recorded for unspiked and spiked experiments.

Subsequently, the dried product extracts were re-constituted in 5% methanol (20 mL) and subjected to liquid-liquid chromatography using ethyl acetate as solvent for puerarin recovery. The combined ethyl acetate (60 mL) was dried and then re-constituted in methanol for LC-MS/MS analysis. The recovery of puerarin was determined using Equation (1).

\[
\text{Recovery (\%)} = \frac{[P_1]-[P_0]}{[P_{\text{spiked}}]} \times 100
\]

Where 
- \([P_0]\): Puerarin concentration in unsupplied product extract (mg/L)
- \([P_1]\): Puerarin concentration in spiked product extract (mg/L)
- \([P_{\text{spiked}}]\): Spiked concentration (2 mg/L)

2.5 LC-MS/MS for Puerarin

A hyphenated system consisted of ultra-high performance liquid chromatography (Waters Acquity UPLC; Milford, MA) and a triple quadrupole and linear-ion trap mass spectrometer (AB SCIEX 4000 QTRAP; Foster City, CA) was used to determine the concentration of puerarin. The strong wash and weak wash solution of the system were 10 and 50% of acetonitrile, respectively. The gradient of mobile phase consisted of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile) was: 0-5 min, 10% B; 5-15 min, 10-80% B; 15-20 min, 80% B; 20-25 min, 80-10% B; 25-30 min, 10% B for final washing.

The separation was carried out in a C18 reversed phase Acquity column (4.6 x 100 mm, 1.7 μm). The flow rate was 0.25 mL/min and the injection volume was 5 μL. The presence of puerarin was detected using the scan mode of enhanced product ion. The fragmentation pattern was matched to the product ions generated by standard puerarin. Untargeted mass screening was performed by the scan mode of enhanced mass spectra (EMS) linked to information dependent acquisition (IDA) with two parallel modes of enhanced product ion (EPI) at different collision energies ranged from 10 to 40 V. The capillary of ion source was maintained at 400 °C. The voltage was set at 5.0 kV and 4.5 kV for positive and negative ion modes, respectively. Nitrogen was used as ion source gas for nebulization, 40 psi; for drying solvent, 40 psi; curtain gas, 10 psi; collision gas, high; declustering potential, 40 V, and collision exit energy, 10 V. The scan rate was 1000 amu/s. A serial dilution of puerarin standard solution ranged from 0.5 to 10 ppm was prepared for the construction of calibration curve. Sample was filtered with nylon membrane filter (0.22 μm) before injection.

2.6 Antiradical Assay

The radical scavenging activity of samples was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. A 3.5 mL methanolic DPPH (0.1 mM) was added into 0.5 mL sample with different concentrations. The solution was incubated for 30 min and the absorbance of the solution was measured using a UV-Vis spectrophotometer (Shimadzu UV-1800, Tokyo, Japan) at 517 nm. The DPPH reagent added with methanol only was used as blank. The inhibition of samples was calculated from Equation (2).

\[
\text{Inhibition (\%)} = \frac{A_b - A_s}{A_b} \times 100
\]

Where 
- \(A_b\) = absorbance of blank,
- \(A_s\) = absorbance of sample or standard

3. Results and Discussion

3.1. *Pueraria Mirifica* Tuber Extract and Its Antioxidant Capacity

Maceration was conducted for the flesh and peel of PM tuber. The results found to produce yellowish and brown extracts from the flesh and peel samples, respectively. Higher yield of extract (9.4 %) was obtained from the peel samples which also exhibited higher radical scavenging activity (Table 1). As a comparison, the EC50 of peel extract...
tract was about four times higher than the flesh extract. This was because the higher amount of flesh extract was required to achieve EC50 (7.4303 mg/mL) than that of the peel extract (1.8636 mg/mL). In another word, the antioxidant capacity was about 57.7 % per milligram of peel extract as observed from the concentration dependent inhibitory curve. This value was about four times higher than that of flesh extract (15.6 % per milligram of flesh extract). The result was in good agreement with the findings of Zhang et al. [18] who reported higher free radical scavenging activity exhibited by the outer bark of Pueraria lobata roots. The total inhibition of DPPH radicals can be seen at 8 mg/mL of peel extract, whereas only 60% of inhibition was exhibited by flesh extract at the similar concentration (Figure 2). The antioxidant capacity of PM tuber was found to be lower than that of Pueraria thunbergiana Benth (16.4 μg/mL) as reported by Son et al. [19]. The researchers reported that higher antioxidant capacity could be due to the high total phenolic and isoflavone content. Many bioactivities reported for the genus Pueraria were attributed to isoflavone glycosides, mainly puerarin [15]. Table 1 shows the peel extract possessed higher concentration of puerarin (4.6553 mg/g extract) which was about four times higher than the flesh extract (1.0731 mg/g extract).

Table 1. Yield and antioxidant capacity of Pueraria mirifica tuber flesh and peel extracts

<table>
<thead>
<tr>
<th>Sample</th>
<th>Extract colour</th>
<th>Extraction yield (%)</th>
<th>Puerarin (mg/g extract)</th>
<th>IC50 (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuber flesh</td>
<td>Yellowish</td>
<td>7.1 ± 0.9</td>
<td>1.073080863</td>
<td>7.4303</td>
</tr>
<tr>
<td>Tuber peel</td>
<td>brown</td>
<td>9.4 ± 1.8</td>
<td>4.655276066</td>
<td>1.8636</td>
</tr>
</tbody>
</table>

Figure 2. The inhibitory curves of (a) flesh and (b) peel extracts in a concentration dependent manner

3.2 LC-MS/MS Detection of Pueraria Mirifica Tuber Extract

In the subsequent experiments, both flesh and peel extracts were subjected to LC-MS/MS analysis. The fragment ions of puerarin in both negative and positive ion modes are presented in Figure 3. The characteristic ions of m/z 297 and m/z 267 as reported by Li et al. [20] were also detected in this study. The peak width of puerarin was found to be narrower at the negative ion mode (0.2 min) than that of positive ion mode (0.5 min) at 0.5 % peak height. A narrow peak width is always better for peak resolution, even though MS detector does not have difficulty in compound identification with overlapped peaks. Furthermore, the signal to noise ratio of puerarin was higher at the negative ion mode (30) than that of positive ion mode (10.7). Both calibration curves showed to have a good linearity in the range of 0.5 to 10 mg/L with the coefficient correlation of 0.9980 and 0.9563 at the negative and positive ion modes, respectively.

The untargeted mass screening also found to detect many flavonoids, especially isoflavones from PM tuber (Table 2). The detected compounds have mostly higher content in peel extract. In line with the findings of Cheng et al. [21], the outer bark of PM tuber also contained higher isoflavonoids. This also explains peel extract exhibited higher antioxidant capacity due to the presence of isoflavones.

3.3 Recovery of Puerarin from Cosmetic Products

Since the wide application of PM in cosmetic formulation, three commercial cosmetic creams with PM extract as one of the major ingredients was purchased from the local markets of Malaysia. They were extracted by methanol and then back extraction by ethyl acetate using the technique of liquid-liquid extraction. The yield of methanolic
Figure 3. Total ion chromatograms (a and b) and mass spectra of puerarin (c and d) detected at the negative (c) and positive (d) ion modes.
Table 2. Compounds detected at negative and positive modes in the flesh and peel extracts of *Pueraria mirifica* tuber

<table>
<thead>
<tr>
<th>Rt (min)</th>
<th>Negative ion (m/z)</th>
<th>Fragment ions</th>
<th>Compound</th>
<th>Flesh</th>
<th>Peel</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.33</td>
<td>133</td>
<td>133/115</td>
<td>malic acid</td>
<td>1.58E+09</td>
<td>1.19E+09</td>
<td>[22]</td>
</tr>
<tr>
<td>7.50</td>
<td>253</td>
<td>223/207/195/180/155/135/133</td>
<td>diadzein</td>
<td>1.23E+09</td>
<td>1.76E+09</td>
<td>[23]</td>
</tr>
<tr>
<td>8.64</td>
<td>269</td>
<td>269/224/180/159</td>
<td>apigenin</td>
<td>6.19E+08</td>
<td>9.18E+08</td>
<td>[24]</td>
</tr>
<tr>
<td>14.30</td>
<td>271</td>
<td>271/253/225</td>
<td>naringenin</td>
<td>3.54E+08</td>
<td>1.06E+08</td>
<td>[25]</td>
</tr>
<tr>
<td>11.71</td>
<td>339</td>
<td>339/324/281/253/241/183</td>
<td>glyceollidin</td>
<td>0</td>
<td>1.16E+09</td>
<td>[26]</td>
</tr>
<tr>
<td>4.26</td>
<td>415</td>
<td>415/307/297/267bp/253/209</td>
<td>puerarin</td>
<td>1.36E+09</td>
<td>1.48E+09</td>
<td>Standard</td>
</tr>
<tr>
<td>3.89</td>
<td>431</td>
<td>431/311/283/255/227/211</td>
<td>vitexin (apigenin-8-C-glucoside)</td>
<td>0</td>
<td>1.03E+09</td>
<td>[24]</td>
</tr>
<tr>
<td>4.67</td>
<td>445</td>
<td>445/325/282/254/225</td>
<td>glycitin</td>
<td>0</td>
<td>8.29E+08</td>
<td>[26]</td>
</tr>
<tr>
<td>4.99</td>
<td>563</td>
<td>563/323/311/283/239</td>
<td>apigenin-6,8-C-pentoside-hexoside</td>
<td>8.75E+07</td>
<td>1.42E+09</td>
<td>[27]</td>
</tr>
<tr>
<td>3.37</td>
<td>577</td>
<td>577/457/294/266/222</td>
<td>puerarin glycoside</td>
<td>0</td>
<td>5.60E+08</td>
<td>[28]</td>
</tr>
<tr>
<td>5.28</td>
<td>611</td>
<td>611/564/269bp</td>
<td>apigenin diglucoside</td>
<td>1.02E+09</td>
<td>1.42E+09</td>
<td>[29]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rt (min)</th>
<th>Positive ion (m/z)</th>
<th>Compound</th>
<th>Flesh</th>
<th>Peel</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.26</td>
<td>255</td>
<td>255/109/227/137</td>
<td>daidzein</td>
<td>7.83E+08</td>
<td>1.42E+09</td>
</tr>
<tr>
<td>5.12</td>
<td>255</td>
<td>255/237(-18)/227(-28)/199(-28-28)/137</td>
<td>dihydroxyflavone</td>
<td>2.39E+08</td>
<td>7.98E+08</td>
</tr>
<tr>
<td>8.60</td>
<td>271</td>
<td>271/197/153</td>
<td>apigenin</td>
<td>2.59E+08</td>
<td>0.00E+00</td>
</tr>
<tr>
<td>7.50</td>
<td>285</td>
<td>285/270/229/213/139</td>
<td>calycosin</td>
<td>0</td>
<td>5.49E+08</td>
</tr>
<tr>
<td>8.96</td>
<td>301</td>
<td>301/286/283/240/187/123</td>
<td>pratensein</td>
<td>0</td>
<td>5.45E+08</td>
</tr>
<tr>
<td>6.40</td>
<td>315</td>
<td>315/297/287/237/209/137</td>
<td>dihydroxy-dimethoxyisoflavone</td>
<td>0</td>
<td>3.07E+08</td>
</tr>
<tr>
<td>4.26</td>
<td>417</td>
<td>417/307/297/267bp/163</td>
<td>puerarin (daidzin 8-C-glucoside)</td>
<td>1.25E+09</td>
<td>2.60E+09</td>
</tr>
<tr>
<td>3.67</td>
<td>433</td>
<td>433/397/367/313/283/267/255/165</td>
<td>3′-hydroxy-puerarin</td>
<td>0</td>
<td>1.45E+09</td>
</tr>
<tr>
<td>5.15</td>
<td>433</td>
<td>433/397/367/313/283/267/255/165</td>
<td>3′-hydroxy-puerarin (isomer)</td>
<td>7.66E+08</td>
<td>1.64E+09</td>
</tr>
<tr>
<td>4.52</td>
<td>447</td>
<td>447/351/327/297/269/255/165/</td>
<td>apigenin C-glucuronide</td>
<td>0</td>
<td>1.64E+09</td>
</tr>
<tr>
<td>4.00</td>
<td>549</td>
<td>549/417/307/297/267</td>
<td>puerarin pentoside</td>
<td>4.25E+08</td>
<td>2.76E+09</td>
</tr>
<tr>
<td>3.32</td>
<td>579</td>
<td>579/417/307/297/267bp/239</td>
<td>puerarin glycoside</td>
<td>1.66E+08</td>
<td>1.23E+09</td>
</tr>
<tr>
<td>1.96</td>
<td>579</td>
<td>579/417/307/297/267bp/239</td>
<td>puerarin glucoside isomer</td>
<td>0</td>
<td>7.41E+08</td>
</tr>
<tr>
<td>4.10</td>
<td>579</td>
<td>579/447/429/393/381/327/297/269</td>
<td>swertisin pentoside</td>
<td>0</td>
<td>8.76E+08</td>
</tr>
</tbody>
</table>

Table 3. Puerarin content in cosmetic products and their radical scavenging activity

<table>
<thead>
<tr>
<th>Product</th>
<th>Yield of methanolic extract (%)</th>
<th>Yield of ethyl acetate extract (%)</th>
<th>Puerarin (µg/mL)</th>
<th>DPPH (%)</th>
<th>Puerarin recovery from spiked experiments (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.8 ± 0.7</td>
<td>20.6 ± 7.7</td>
<td>0.3095 ± 0.0254</td>
<td>32.1 ± 2.4</td>
<td>115.8</td>
</tr>
<tr>
<td>B</td>
<td>3.8 ± 0.6</td>
<td>32.4 ± 9.8</td>
<td>0.4353 ± 0.0341</td>
<td>3.1 ± 0.01</td>
<td>96.0</td>
</tr>
<tr>
<td>C</td>
<td>5.6 ± 0.6</td>
<td>38.1 ± 8.2</td>
<td>0.5255 ± 0.0309</td>
<td>4.4 ± 0.4</td>
<td>89.1</td>
</tr>
</tbody>
</table>
and ethyl acetate extract, as well as the recovered puerarin concentration are listed in Table 3. Lipophilic puerarin could be partitioned and recovered from the complex aqueous mixture into the organic phase of ethyl acetate. It was found that the concentration of puerarin was ranged between 0.3095 to 0.5255 µg/mL (Table 3). The measured radical scavenging activity was varied from 3.1 % to 32.1 % independent on the detected concentration of puerarin in the products. This was because the cosmetic products contained a mixture of plant extracts which might be extracted out together with puerarin, and thus contributing to the synergistic antioxidant capacity.

A spiked experiment was also conducted to reveal the performance of puerarin extraction from commercial cosmetic products. Puerarin could be recovered from the highly complex product mixture effectively (Table 3). A good recovery ranged from 89.1 % to 115.8%, was obtained for puerarin. The results revealed that the recovery of puerarin was strongly affected by the texture of sample matrices. Products with thick or liquidified texture should be well homogenized prior to analyses.

4. Conclusions

The concentration of puerarin was found to be about 4 times higher in the peel extract of PM tuber than its flesh extract. The higher concentration puerarin in the peel extract was also showed to have higher radical scavenging activity. The detection which was performed by a triple quadrupole mass analyser exhibited better identification power at the negative ion mode. Successive extraction by methanolic sonication and ethyl acetate partition was found to be effective in recovering puerarin from the highly complex cosmetic products.

References


ARTICLE
Acupuncture as an Alternative Therapy in the Management of Burning Mouth Syndrome (BMS)

Letícia Dussarrat Brito Oliveira*  Maria da Luz Rosário de Sousa*  Maria Lúcia Bressiani Gil
Cássia Maria Grillo  Vera Lúcia Rasera Zotelli  Edson Shizuo Tanaka

Department of Health Sciences and Children’s Dentistry, School of Dentistry of Piracicaba, State University of Campinas, São Paulo, Brazil

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ABSTRACT

In the routine of dental care, complaints of burning sensation, burning tongue and dry mouth are frequent. Due to the complexity of etiology and diagnosis, treating patients with these sensations is a challenge for clinicians. This study aimed to assess acupuncture as an alternative therapy for patients with burning mouth syndrome (BMS). Clinical data were collected from visits of patients to the School of Dentistry of Piracicaba, State University of Campinas, with the application of a protocol of acupuncture points: LI-4 (Hegu), HT-7 (Shenmen), SP-6 (Sanyinjiao), ST-36 (Zusanli), REN-23 (Lianquan), REN-6 (Qihai), LI-11 (Quchi), ExHn3 (Yintang), LV-3 (Taichong), ExHn12 (Jinjin), and ExHn13 (Yuye) to treat the symptoms of BMS, from August 2017 to March 2020. The sample consisted of 6 volunteers – 5 female and 1 male patients – aged 40 to 79 years. The intensity of BMS was assessed before and after each session with self-reported verbal numerical rating scale (VNRS) during the treatment. On average, each patient had 8.16 sessions using 9.92 acupuncture points per session. Variation of VNRS during the treatment showed a decline in burning mouth in most patients, and 83.34% of the sample showed partial or total improvement of symptoms. This study showed that acupuncture was effective in relieving burning mouth, reducing it by 43%, representing an alternative therapy in the management of symptoms of burning mouth syndrome.

*Corresponding Author:
Letícia Dussarrat Brito Oliveira,
Department of Health Sciences and Children’s Dentistry, School of Dentistry of Piracicaba, State University of Campinas, São Paulo, Brazil;
Email: leticiadussarrat@yahoo.com.br
Maria da Luz Rosário de Sousa,
Department of Health Sciences and Children’s Dentistry, School of Dentistry of Piracicaba, State University of Campinas, São Paulo, Brazil;
Email: luzsousa@fop.unicamp.br
1. Introduction

The International Association for the Study of Pain (IASP) defines burning mouth syndrome (BMS) as “a burning pain in the tongue or other mucous membranes associated with normal signs and laboratory findings lasting at least 4 to 6 months” [1].

Although no consistent data are available about the prevalence of BMS in Brazil, estimates indicate that around 4.5% of the population has BMS [2].

The prevalent mean age of patients with BMS is higher among individuals from 55 to 65 years, but it can also affect adults between 30 and 40 years, rarely affecting younger people [2,3,4,5], and more women are affected when compared to men.

BMS has complex multi-factor etiopathogenesis, involving systemic, local, psychogenic factors, and possibly an interaction among them. Therefore, its diagnosis and specific therapy for each case represent challenges for dental surgeons [6].

Different types of treatments are found in the literature, such as topical treatments based on clonazepam; systemic treatments with alpha-lipoic acid, selective serotonin reuptake inhibitor and amisulpride inhibitor; and cognitive behavioral therapy. Other possibilities based on the opinion of specialists and clinical practice, but not yet assessed, include topical treatments based on capsaicin, doxepin and lidocaine, in addition to systemic treatments [7]. Low-level laser therapy has been an alternative treatment for BMS, since it helps reduce the symptoms of BMS [8].

Few studies have analyzed acupuncture as a therapy for BMS, with evidence of its effective impact on relieving and treating burning sensation by stimulating microcirculation of the oral cavity [9]. It is a non-invasive method that influences the control of peripheral sympathetic nervous system activity, and stimulation promoted by acupuncture associated with minimal local and systemic adverse effects makes it an alternative treatment for BMS [10].

From a pilot study patients reported a reduction of pain and better able cope with their oral symptoms [11]. Then, this study aimed to evaluate acupuncture as an alternative therapy for the symptoms of burning mouth syndrome (BMS).

2. Method

2.1 Participants

After analyzing the medical records of patients treated with acupuncture at the Acupuncture Clinic of the School of Dentistry of Piracicaba from August 2017 to March 2020, whose main complaint was burning mouth, a sample was obtained of 6 patients: 5 female and 1 male individuals, aged between 40 and 79 years, mean age 62 years. The inclusion criterion was data collection from at least 8 points (75%) of the protocol of acupuncture points for BMS during the treatment sessions; medical records were excluded from this analysis which used acupuncture points other than those of the protocol.

The treatments used traditional acupuncture performed by experienced dentists-acupuncturists.

2.2 BMS Treatment Protocol

![Figure 1. Bleeding at acupuncture points](image)

Acupuncture points from the BMS treatment protocol are: LI4 (Hegu), HT7 (Shenmen), SP6 (Sanyinjiao), ST36 (Zusanli), REN23 (Lianquan), REN6 (Qihai), LI11(Qu-chi), ExHn3 (Yintang), LV3 (Taichong), ExHn12 (Jinjin), and ExHn13 (Yuye); with bleeding performed at the two last points.

Dong Bang® disposable stainless steel needles 0.25mm x 30mm were used. Perpendicular insertion was used in most acupuncture points, with Yintang acupuncture point needled more superficially and parallel to the skin, towards the base of the nose. The application was unilateral and the needle retention time was 20 minutes in each session.

For bleeding of extra points ExHn12 (Jinjin) and ExHn13 (Yuye), the acupuncturist wore disposable rubber gloves, stabilized the tongue with gauze, and bled the points with perforation using disposable stainless steel lancets (Sterilance Advantive®, Wuxi Xinda Medical Device Co, Ltd, Jiangsu Province, People’s Republic of China); ending with tamponing of perforations by gentle compression of gauze over the acupuncture points (Figure 1).

Medications taken by patients and the clinical characteristics of tongues were recorded.

2.3 Burning Mouth Assessment

Verbal numerical rating scale (VNRS) was used to measure the effects of acupuncture, with 0 (zero) meaning absence of burning mouth, and 10 (ten), high level of burning, as verbally reported by patients in the beginning and at the end of each session.
In order to analyze the evolution of symptoms during the treatment, each individual was evaluated separately, given the significant variation in the number of sessions, enabling to assess the progress of symptom remission, session by session, during the treatment.

2.4 Statistical Analysis

The statistical analysis was performed using Excel 2010 spreadsheet and data were analyzed by the reduction of Verbal Numerical Rating Scale (VNRS).

2.5 Ethical Aspects

All patients signed an informed consent approved by the Research Ethics Committee of the School of Dentistry of Piracicaba, State University of Campinas.

3. Results

Most participants reported taking medication to control chronic diseases, including arterial hypertension, anticoagulants, anxiolytics and antidepressants, drugs for cholesterol control, among others, as indicated in Table 1.

Table 1. Medications taken by patients comprising the study sample

<table>
<thead>
<tr>
<th>Patient</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diazepam, Antihypertensive agents, Lipitor, Noxipen, Omeprazole</td>
</tr>
<tr>
<td>2</td>
<td>Omeprazole, Benzodiazepine, Tranax, Neurontin, Gabapentin, Lamotrigine, Omeprazole</td>
</tr>
<tr>
<td>3</td>
<td>Sertraline, Lipitor, Noxipen, Omeprazole</td>
</tr>
<tr>
<td>4</td>
<td>Tranax, Lamotrigine, Omeprazole</td>
</tr>
<tr>
<td>5</td>
<td>Sertraline, Omeprazole, Neurontin, Aspirin, Motrin</td>
</tr>
<tr>
<td>6</td>
<td>Atarneol, Neurontin, Aspirin</td>
</tr>
</tbody>
</table>

Most patients (80%) reported burning sensation in the tongue, and at the time of diagnosis, some characteristics of the tongue were common to patients, such as white coating (100% of patients), teeth marks (83.33%), deviation downwards (83.33%), swelling (83.33%), moisture (83.33%) and weakness (83.33%) (Figure 2).

Table 2. Sites affected and duration

<table>
<thead>
<tr>
<th>Patient</th>
<th>Site Affected</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gingiva, Palate</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Buccal mucosa, Tongue</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>Tongue</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>Tongue</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>Palate, Tongue</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Buccal mucosa</td>
<td></td>
</tr>
<tr>
<td>Average duration</td>
<td>Tongue</td>
<td>36</td>
</tr>
</tbody>
</table>

In total, 49 acupuncture sessions were provided to the sample, using on average 8.16 sessions per patient and 9.92 acupuncture points per session.

In the total sample, 87% of the points selected for treatment were from the BMS treatment protocol and 13% were additional points, due to sporadic secondary changes presented by patients during the therapy. Among these additional points not from the BMS protocol, the most frequently used were PC6 (Neiguan) for anxiety and restlessness, LU7 (Lieque) to tonify defensive Qi and release...
emotional tension, HT5 (Tongli) to relieve stress and clear the heat of the heart, and REN12 (Zongwang) to tonify stomach Qi and disperse heat and moisture.

All treatments used at least 75% of the acupuncture points from the BMS protocol, as illustrated in Figure 3.

Figure 3. Use of acupuncture points (in %) from BMS treatment protocol per patient

The illustration below shows the individual results of initial and final VNRS from the first sessions of each patient (Figure 4).

Figure 4. Variation of VNRS in initial sessions of treatments according to the protocol

Variations in initial VNRS during the treatment show a decline in burning sensation in most patients, with 5 out of 6 patients (83.34% of the sample) showing partial or total improvement in burning mouth sensation. Patient 2 interrupted the treatment in the third session and did not complete it.

4. Discussion

BMS affects five out of 100,000 individuals, affecting around 1.3 million American adults; it is more frequent in post-menopausal and elderly or middle-aged women, regardless of ethnicity or socioeconomic condition [12]. Prevalence of BMS in the world population varies between 3.7% and 5.4% [13], which justifies further studies like this one to analyze potential therapies.

Discomfort starts suddenly, with low intensity [14], which may be accompanied by other symptoms, whose intensity may vary between individuals, which was also observed in the sample of this study, where initial VNRS varied from 2 to 10 among participants.

Studies have reported a relationship between burning mouth and emotional factors such as depression and anxiety, although it is not known whether these psychological conditions have an etiological contribution to the syndrome [15,16], in agreement with the findings of this study, in which 66.66% of all patients were taking an anti-anxiety or antidepressant drug.

It is a consensus in the literature that BMS proportionally affects more women than men, more frequently reported among elderly people, mean age 65 years, but it may also occur among younger people [2,4,17,18], which is consistent with the results of our study, whose sample consisted of 83.33% female patients, mean age 62 years.

In BMS, tongue is the site presenting most symptoms, bilaterally and symmetrically in most cases, more commonly found in the anterior two thirds (71% to 78%); symptoms may also affect the dorsum and lateral borders, hard palate, alveolar ridge and lips, and may affect more than one site of the same patient [19], consistent with data of our study, where tongue was frequently affected in the sample; however, the clinical findings of tongues presented surprising characteristics, since the reported burning symptoms would lead to a hypothesis of heat and dryness in the perspective of Traditional Chinese Medicine (TCM), contradicting the recurrent aspects of white coating and moisture in most individuals of the sample. Also, absence of clinical signs was observed in both perspectives of Western medicine and TCM, which makes the reporting of symptoms by the patient essential for the differential diagnosis of BMS.

The common tongue characteristics of the patients in this study show Qi running counterflow, stagnant Qi in the stomach, with deficiency of Qi and Yin. Then, acupuncture points were used to regulate the affected functions: LI-4 (Hegu) promotes analgesia for disorders of the head and face region; HT-7 (Shenmen) eliminates heat from the heart’s energy channel by calming the Shen; SP-6 (Sanyinjiao) nourishes blood and Yin and reassures the Shen; ST-36 (Zusanli) strengthens and tonifies Qi, nourishes blood and reassures the Shen; REN-23 (Lianquan) regulates and redirects the flow of inverted Qi, eliminates fire and perverse heat; REN-6 (Qihai) strengthens Qi and harmonizes blood, LI-11 (Quchi) filters heat and cools blood; EXHn3 (Yintang) eliminates wind and reassures the Shen; LV-3 (Taichong) nourishes blood, eliminates internal wind.

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and mobilizes Qi; ExHn12 (Jinjin) and EXHn13 (Yu ye) expel heat and wind.

Sardella et al. (2006) studied the spontaneous remission of BMS symptoms, analyzing 48 women and 5 men, mean age 67.7 years, who presented BMS for an average of 5.5 years, and concluded that due to the therapies adopted, 26 patients (49%) showed no improvement in symptoms, 15 (28.3%) showed a small improvement and 10 (18.9%) reported worsened symptoms, with only 2 patients (3.7%) presenting full spontaneous remission of symptoms. Then, studies analyzing therapeutic practices that can relieve symptoms, even partially, are important as an alternative to improve the quality of life of patients with BMS. The protocol adopted in this study showed reduced symptoms in 83.34% of the sample.

Changes in microcirculation caused by acupuncture happened not only at the skin level, but also in deeper areas [20].

Acupuncture is a non-invasive method that can influence the control of peripheral vascular sympathetic activity [21]. Stimulation promoted by acupuncture associated with minimal local and systemic adverse effects make it an alternative treatment for diseases caused by poor systemic circulation [22], despite the challenge of standardizing this methodology as it is a TCM technique, requiring many sessions, which can lead BMS patients to dependence on therapy due to the psychogenic components that involve the etiopathogenesis of BMS [10]. Our study also observed difficult standardization of this methodology, considering that several sessions required additional acupuncture points, not from the BMS treatment protocol, for tonification or due to secondary complaints that emerged during the treatment. These additional acupuncture points included PC-6 (Neiguan) for anxiety and restlessness, LU-7 (Lieuque) to tonify defensive Qi and release emotional tension, HT-5 (Tongli) to relieve stress and clear heart heat, and REN-12 (Zhongwan) to harmonize, tonify and strength spleen Qi and stomach Qi and promote stomach Qi and the middle Jiao.

Scardina (2010) conducted a study that analyzed a sample of 30 individuals with BMS, mean age 65.4 years, 10 male and 20 female individuals; and a control group of 30 healthy individuals, mean age 62.06 years, 10 male and 20 female individuals. All participants were treated with acupuncture using the following acupuncture points: SI1 (Shaoze), SJ1 (Guanchong), LI4 (Hegu), SJ21 (Ermen), ST5 (Daying), ST6 (Jiache), and REN24 (Chengjiang), with LI4 (Hegu) in common with the protocol adopted in our study. Scardina found a significant change in the oral microcirculation, promoting relief of burning mouth symptoms after a three-week treatment. Such relief lasted 18 months after acupuncture therapy, leading the author to conclude that acupuncture may be a valid option for the treatment of BMS. This finding is consistent with the results of our study, where reduced VNRS was observed throughout the sessions. In future studies we intend to add acupuncture points that help further tonify Qihua, remove obstruction, improve flow, descend Yang that has ascended, and harmonize the three San Jiaos such as ST40 (Fengleng), REN12 (Zhongwan), ST21 (Liangmen), and SP15 (Daheng).

5. Conclusions

This study demonstrated that acupuncture was effective in relieving burning mouth, reducing it by 43%, representing an alternative therapy in the management of symptoms of burning mouth syndrome.

Declaration of Competing Interest

The authors declare no conflict of interests in this study.

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CASE REPORT

Chinese Prescription Kangen-karyu against Metabolic Syndrome: Successful Treatment of Three Patients

Tsutomu Kitazawa¹  Kazuyuki Hiratani¹  Chan Hum Park²  Takako Yokozawa³*

1. Shinseikai Toyama Hospital, Toyama, 939-0243, Japan
2. Institute of New Frontier Research Team, Hallym Clinical and Translational Science Institute, Hallym University, Chuncheon, 24252, Republic of Korea
3. Graduate School of Science and Engineering for Research, University of Toyama, Toyama, 930-8555, Japan

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ABSTRACT

Metabolic syndrome is the cluster of diseases, which is manifested by central obesity, impaired glucose tolerance, lipodystrophy, and high blood pressure. These metabolic syndrome-related traits significantly increase the risk of type 2 diabetes, adverse cardiac events, stroke, and hepatic steatosis. In the past decade, several organizations have proposed different diagnostic criteria. The use of traditional Chinese medicine to treat metabolic syndrome has received increasing attention due to its wide availability. In this paper, we report a case of three patients with metabolic syndrome with improved administration of 7.5 g of Kangen-karyu extract per day for 6 months. We present and discuss evidence supporting the possibility of using Kangen-karyu for metabolic syndrome.

1. Introduction

Metabolic syndrome, a major public health problem worldwide today, is a cluster of clinical, metabolic, and biochemical abnormalities, such as central obesity, high blood pressure, impaired glucose tolerance, and dyslipidemia. These metabolic syndrome-related traits significantly elevate the risk of type 2 diabetes mellitus, adverse cardiovascular events, stroke, and hepatic steatosis. The pathogenesis of metabolic syndrome is multifactorial, with the interplay of environmental, nutritional, and genetic factors [1-3]. Thus, metabolic syndrome has become one of the major burdens of the health care system.

The concept of metabolic syndrome was first presented by Kylin in 1923, who described a syndrome involving hypertension, hyperglycemia, and gout [4]. However, this concept did not attract much attention until Dr. Reaven mentioned syndrome X, clustering of metabolic risk factors, in 1988, which is similar to metabolic syndrome [5]. The WHO (World Health Organization) and EGIR (European Group for the Study of Insulin Resistance) released their diagnostic criteria for metabolic syndrome [6,7]. The definition of metabolic syndrome in Japan, which is characterized by the accumulation of visceral fat with the co-occurrence of several risk factors, was established in 2005 [8].

Epidemiological studies demonstrated that a condition clustering several risk factors like metabolic syndrome...
will lead to a greater risk of cardiovascular diseases than a single risk factor [9-11]. The accumulation of visceral fat is essential for the diagnosis of metabolic syndrome and is considered to be located upstream of the cascade clustering several risk factors and subsequent cardiovascular diseases. Therefore, the main purpose of the treatment of metabolic syndrome is the effective reduction of multiple risk factors and the following cardiovascular diseases through the reduction of visceral fat. In the treatment of metabolic syndrome, oriental medicine is an outstanding example of alternative and complementary medicine with a long history, a unique theoretical system, and a variety of herbal remedies. Many traditional Chinese medicine (TCM) has been tested for the treatment of metabolic syndrome. We have conducted pre-clinical animal experiments to investigate the effectiveness of multi-target treatment of TCM for various human diseases. In our previous studies, Kangen-karyu (a crude drug consisting of Salviae Miltiorrhizae Radix, Cnidii Rhizoma, Carthami Flos, Paeoniae Radix, Aucklandiae Radix, and Cyperi Rhizoma, as shown in Table 1; Guan-Yuan-Ke-Li in Chinese), a TCM modified from Kan-shin No. 2 (Guan-xin No. 2 in Chinese) [12], may play a protective role against metabolic syndrome [13-15]. Kangen-karyu has been used clinically as a treatment for cardiovascular disease, including angina pectoris and cerebrovascular diseases [16,17]. The results of our previous study provide important evidence that this prescription ameliorates metabolic syndrome.

Based on these results, Kangen-karyu was administered to three patients with metabolic syndrome, and report its therapeutic usefulness.

Table 1. Composition of Kangen-karyu.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Botanical name</th>
<th>Family name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salviae Miltiorrhizae Radix</td>
<td><em>Salvia miltiorrhiza</em> Bunge</td>
<td>Labiatae</td>
</tr>
<tr>
<td>Cnidii Rhizoma</td>
<td><em>Cnidium officinale</em> Makino</td>
<td>Umbelliferae</td>
</tr>
<tr>
<td>Paeoniae Radix</td>
<td><em>Paeonia lactiflora</em> Pallas</td>
<td>Paeoniaceae</td>
</tr>
<tr>
<td>Carthami Flos</td>
<td><em>Carthamus tinctorius</em> L.</td>
<td>Compositae</td>
</tr>
<tr>
<td>Aucklandiae Radix</td>
<td><em>Aucklandia lappa</em> Dene.</td>
<td>Compositae</td>
</tr>
<tr>
<td>Cyperi Rhizoma</td>
<td><em>Cyperus rotundus</em> L.</td>
<td>Cyperaceae</td>
</tr>
</tbody>
</table>

2. Case Presentation

2.1 Case 1

A 68-year-old male with hypertension, hypercholesterolemia, borderline diabetes, and obesity reports an improvement in metabolic syndrome when administered Kangen-karyu extract. This patient subsequently changed his lifestyle and continued to receive conventional treatments: antihypertensive drugs (amlodin: 5 mg/day, azilva: 20 mg/day) and an antilipidemic agent (lipitor: 5 mg/day). However, he came to our hospital, seeking to recover his functional level with medicine including herbal medicine. Kangen-karyu prescription (7.5 g/day) was administered three times a day. Before Kangen-karyu prescription administration, his initial anthropometric measurements included a BMI of 30.3 kg/m²: body weight 82.6 kg (182.1 lb), height 165 cm (5.41 ft), and an abdominal circumference of 105.8 cm (3.47 ft), which classified him as obese. In the blood pressure test, his systolic blood pressure (SBP)/diastolic blood pressure (DBP) was 133/85 mmHg. Serum hemoglobin A1c (HbA1c) or glycated hemoglobin was 7.3%, displaying poorly regulated blood glucose. Serum lipids levels were as follows: total cholesterol: 230 mg/dL, low-density lipoprotein (LDL)-cholesterol: 142 mg/dL, LDL-cholesterol/high-density lipoprotein (HDL)-cholesterol: 2.3, and triglycerides: 353 mg/dL, indicating dyslipidemia. When Kangen-karyu extract was administered for 6 months, BMI and abdominal circumference were slightly reduced. The levels of triglycerides, total cholesterol, LDL-cholesterol, LDL-cholesterol/HDL-cholesterol, and HbA1c were reduced. Meanwhile, other parameters such as liver and kidney function parameters [aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen and creatinine (Cr)] were not affected by Kangen-karyu extract administration. However, physical and subjective symptoms such as headache, feeling of heaviness in the head, and mottled skin have improved. In addition, the tongue coating was slightly improved when Kangen-karyu was administered. Herein, we present evidence supporting the use of Kangen-karyu prescription for metabolic syndrome.

2.2 Case 2

Kangen-karyu extract was effective for a 64-year-old woman with hypertension and hypercholesterolemia. This patient also changed her lifestyle and continues to receive existing treatments: antihypertensive drugs (amlodin: 5 mg/day, micardis: 20 mg/day), but has not received hypolipidemia drugs. She presented to our hospital, seeking to recover her functional level with herbal medicine, and
was administered 7.5 g of Kangen-karyu extract per day. Before Kangen-karyu prescription administration, her initial anthropometric measurements included a BMI of 25.2 kg/m²: body weight 58.0 kg (127.8 lb), height 152 cm (4.99 ft), and an abdominal circumference of 90.5 cm (2.97 ft), which classified her as obese. In the blood pressure test, her SBP/DBP was 133/88 mmHg. Serum HbA1c was 5.7%, displaying regulated blood glucose. Serum lipids levels were as follows: total cholesterol: 246 mg/dL, LDL-cholesterol: 179 mg/dL, HDL-cholesterol: 54 mg/dL; LDL-cholesterol/HDL-cholesterol: 3.3, and triglycerides: 183 mg/dL, indicating dyslipidemia. After 6 months, administration of Kangen-karyu prescription showed a slight decrease in BMI and abdominal circumference. Her SBP/DBP was reduced from 133/80 to 115/77 mmHg. The level of total cholesterol was reduced from 246 to 235 mg/dL. The elevated level of LDL-cholesterol was slightly decreased, and HDL-cholesterol was slightly elevated on treatment with Kangen-karyu prescription during the follow-up period. At that time, oral administration of Kangen-karyu prescription significantly decreased the elevated serum triglyceride level. Hepatic functional parameters (AST and ALT) also decreased at the 6-month follow-up. However, there was no change in HbA1c for Kangen-karyu treatment. Regarding the score using the questionnaire, it showed amelioration to 75% of the level on non-administration. The physical and subjective symptoms involving headache, stiff shoulders, feeling heavy in the head, and mottled skin had improved. According to the tongue diagnosis, there was a notable improvement in vessels below the tongue following the treatment of Kangen-karyu prescription for 6 months, although the tongue color, fur color, and thickness ameliorated only slightly. The results reported herein identified the therapeutic usefulness of Kangen-karyu to treat blood stasis.

2.3 Case 3

A 65-year-old man with high blood pressure, dyslipidemia, type 2 diabetes, chronic kidney disease, and hyperuricemia was previously diagnosed with metabolic syndrome. Subsequently, the patient continued to receive existing treatments: an antilipidemic agent (livaro: 1 mg/day) and antihypertensive agent (micardis: 20 mg/day). In addition, Kangen-karyu prescription (7.5 g/day) was treated three times a day for 6 months. Before Kangen-karyu prescription administration, his initial anthropometric measurements included a BMI of 27.4 kg/m²: body weight 81.6 kg (179.7 lb), height 173 cm (5.68 ft), and an abdominal circumference of 98.4 cm (3.23 ft), which classified him as obese. His SBP/DBP was 130/86 mmHg. Serum HbA1c was 6.1%, displaying poorly controlled blood glucose. Serum lipids levels were as follows: total cholesterol: 216 mg/dL, LDL-cholesterol: 118 mg/dL, HDL-cholesterol: 46 mg/dL, and triglycerides: 306 mg/dL, indicating hyperlipidemia. In addition, his serum Cr level of 1.69 mg/dL had moderately impaired renal function (eGFR 33.0 ml/min/1.73 m²) according to the Modification of Diet in Renal Disease equation [19]. The level of serum uric acid was 8.6 mg/dL, representing hyperuricemia derived from kidney disease. This patient was also an alcoholic, and alcohol consumption was assessed by questioning. He was categorized as a heavy consumer (≧30 g alcohol/day) according to the average daily alcohol consumption proposed by Agarwal [19]. Therefore, enzymes related to the hepatobiliary system and myocardial infarction were determined to assess their effects on the relationship between metabolic syndrome and alcohol consumption. ALT, gamma-glutamyl transpeptidase (γ-GTP), and creatine phosphokinase (CPK) were found to be poorly controlled. There were no significant changes in the activities of AST, alkaline phosphatase, or lactate dehydrogenase. During the administration of Kangen-karyu extract, BMI and the abdominal circumference showed no changes, but the systolic/diastolic blood pressure decreased from 130/86 to 126/70 mmHg. The level of total cholesterol had reduced from 216 to 197 mg/dL at the 6-month follow-up. The increased levels of LDL-cholesterol and LDL-cholesterol/HDL-cholesterol were slightly reduced on treatment with Kangen-karyu prescription. Oral treatment of Kangen-karyu prescription significantly decreased the elevated triglyceride level. Other parameters such as eGFR, Cr, uric acid, AST, ALT, γ-GTP, and CPK were improved by the Kangen-karyu treatment. At that time, the physical and subjective symptoms such as cold limbs, fatigue, and insomnia had partially disappeared. There was a slight improvement in the tongue coating. Kangen-karyu extract exhibits good efficacy in the administration of lifestyle-induced metabolic syndrome.

3. Discussion

Therapy for metabolic syndrome is multifaceted, as is the syndrome itself. Effective preventive approaches include lifestyle modification, mainly weight loss, exercise and diet, and the treatment comprises the appropriate use of pharmacological drugs to decrease the particular risk factors [20]. TCM has received much attention as potential sources of novel therapeutic drugs due to their multiple beneficial effects and absence of side effects toxic and/or toxicity [21]. Up to now, many TCM have been tested for the treatment of metabolic syndrome. We chose Kangen-karyu, TCM modified from herbal formular
of Kan-shin No. 2 \[12\]. It has been clinically used as an administration for cardiovascular disorders, involving cerebrovascular disorder and angina pectoris \[16,17\]. Kan-gen-karyu is being proposed as a new therapeutic material based on preclinical studies related to various human diseases \[13,14,22-27\]. To add to these findings, we reported evidence supporting its preventive and/or therapeutic potential against metabolic syndrome in a rat model \[14\]. Treatment of Kangen-karyu significantly improved high-fructose-induced metabolic syndrome such as hypertension, hyperlipidemia, and hyperglycemia, through the reductions of triglyceride and cholesterol contents with the modulation of sterol regulatory element-binding protein-1 (SREBP-1) and the nuclear factor-kappa B signaling pathway in the liver. We also reported the lipid-regulatory activity of Kangen-karyu on type 2 diabetes-induced dyslipidemia \[15\]. The results of our pre-clinical experiment provide scientific evidence that the use of this prescription for the treatment of metabolic syndrome is becoming increasingly popular due to its wide availability. We administered Kangen-karyu extract to metabolic syndrome patients, and evaluated its treatment-based usefulness.

In the present cases, there was an improvement in metabolic syndrome following the treatment of Kangen-karyu prescription for 6 months. Most notably, the levels of serum triglycerides, total cholesterol, and LDL-cholesterol decreased following the administration of Kangen-karyu prescription. The body weight, BMI, abdominal circumference, and blood pressure reduced compared with before treatment, as shown in Figure 1. At that time, the somatic and subjective symptoms had partially improved. Here, we present a therapeutic effect of Kangen-karyu based on metabolic parameters.

In TCM, tongue diagnosis plays an important role in the organ’s meridians and conditions. The characteristic of the tongue reflects the condition of organ and imbalance of Qi and blood. TCM practitioners observe the tongue appearance such as the body color and coating of tongue to determine the pathogenic factors. The color of tongue reflects the state of the Yin organs, blood, and nutritive Qi. Coating of tongue is observed from the tip to root and reflects the hot or cold aspects of the body \[28,29\]. The tongue provides a geographic map of organ systems; characteristics of the tongue in each of these areas provide information critical to the TCM diagnosis \[28\]. Therefore, tongue diagnosis was evaluated by TCM doctors who collected

![Kangen-karyu (Guan-Yuan-Ke-Li in Chinese)](image)

**Kangen-karyu administration (7.5 g per day in 3 divided doses for 6 months)**

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>- 68-year-old man</td>
<td>- 64-year-old woman</td>
<td>- 65-year-old man</td>
</tr>
<tr>
<td>- Existing treatments</td>
<td>- Existing treatments</td>
<td>- Existing treatments</td>
</tr>
<tr>
<td>- antihypertensive agents</td>
<td>- antihypertensive agents</td>
<td>- antihypertensive agent</td>
</tr>
<tr>
<td>- antilipidemic agent</td>
<td>- antilipidemic agent</td>
<td>- antilipidemic agent</td>
</tr>
</tbody>
</table>

**Metabolic syndrome-related improvement effects by Kangen-karyu administration**

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Clinicopathologic parameters</td>
<td>- Anthropometric indicators</td>
<td></td>
</tr>
<tr>
<td>- serum total cholesterol</td>
<td>- BMI</td>
<td></td>
</tr>
<tr>
<td>- serum LDL-cholesterol</td>
<td>- abdominal circumference</td>
<td></td>
</tr>
<tr>
<td>- serum triglycerides</td>
<td>- blood pressure</td>
<td></td>
</tr>
</tbody>
</table>

![Figure 1. Clinical effects of Kangen-karyu.](image)
the information with a digital camera. The researchers checked tongue characteristics, such as the coat color, coat weight, coat surface, tongue action, and vessels below the tongue. In case 2, there was a notable improvement in vessels below the tongue following the treatment of Kangen-karyu extract for 6 months, although the tongue color, fur color, and thickness ameliorated only slightly. Most notably, vessels below the tongue were recovered by Kangen-karyu administration to near-normal levels. These in cases 1 and 3 revealed a slight improvement in the tongue coating of the treatment group.

It has been proposed that metabolic syndrome develops as a result of the reciprocal action of several environmental factors. In particular, alcohol consumption is one of the most prevalent habits in the general population. The harmful effects of heavy alcohol consumption are due to an increase in plasma triacylglycerol and increased blood pressure. Each of these factors is a component of metabolic syndrome. In case 3, interesting findings were obtained with regard to enzymes related to the hepatobiliary system and myocardial infarction: the levels of AST, ALT, γ-GTP, and CPK decreased compared with non-administration. Although the association of metabolic syndrome with alcohol consumption is complex and controversial, as both protective and detrimental effects have been reported, we report evidence to support the use of Kangen-karyu as an adjunctive therapy for patients with lifestyle-induced metabolic syndrome. The administration for metabolic syndrome involves the management of a cluster of chronic diseases such as high blood pressure, dyslipidemia, diabetes mellitus, and obesity. However, TCM has received much attention as a source of multi-target strategies due to its multiple beneficial effects and absence of toxic and/or side effects. The present cases provide strong evidence to support the administration of Kangen-karyu as a therapeutic agent to prevent the progression of metabolic syndrome.

The definition of metabolic syndrome in Japan, which is characterized by the accumulation of visceral fat, accompanied by the concurrence of multiple risk factors, was established in 2005. The accumulation of visceral fat is essential for the diagnosis of metabolic syndrome and is considered to be located upstream of the cascade clustering multiple risk factors and subsequent cardiovascular diseases. Therefore, the primary aim of treatment for metabolic syndrome is the effective reduction of multiple risk factors and subsequent cardiovascular diseases by lower visceral fat. TCM is an excellent system in complementary and alternative medicine. It shows marked and unique potential in the management of metabolic syndrome. Its potential in the treatment of metabolic syndrome has not been reviewed, but is promising regarding the development of new, effective therapies. Therapy with Kangen-karyu is aimed to correct maladjustments and restore the self-regulatory ability of the body to ameliorate metabolic syndrome. The present study provides important evidence that the use of Kangen-karyu may become a new therapeutic strategy against metabolic syndrome.

4. Conclusions

We report scientific evidence supporting the use of Kangen-karyu as an adjunctive therapy for patients with metabolic syndrome. Kangen-karyu shows good efficacy in the treatment of patients with metabolic syndrome.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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