Author’s response to reviews

Title: Anti-inflammatory effect of Ganluyin, a Chinese classic prescription, in chronic pharyngitis rat model

Authors:
Ye-Hui Chen (chenyehui37@126.com)
Rong Luo (13736600301@163.com)
Shan-Shan Lei (leishan199006@126.com)
Bing Li (1174346047@qq.com)
Fu-Chen Zhou (1749676822@qq.com)
Hui-Ying Wang (3166656878@qq.com)
Xue Chen (1104370264@qq.com)
Xinglishang He (837479921@qq.com)
Yu-Zhi Wang (513463371@qq.com)
Liang-Hui Zhan (1164118257@qq.com)
Ting-Ting Lu (1732028049@qq.com)
Jie Su (sujie198169@126.com)
Qiao-Xian Yu (sstpfd@126.com)
Bo Li (boli19861023@163.com)
Gui-Yuan Lv (zjtcmly@163.com)
Su-Hong Chen (chensuhong@zjut.edu.cn)

Version: 4 Date: 05 Aug 2020

Author’s response to reviews:

Dear Editor:
Thank you for your work on our manuscript titled “Anti-inflammatory effect of Ganluyin, a Chinese classic Prescription, in chronic pharyngitis rat model” (BCAM-D-20-00842). We would
like to thank the reviewers for their hard work and their kindly suggestions. Based on the comment and request, we revised the manuscript. Here below is our description on revision according to the reviewers’ comments.

Reviewer 1:
1. The authors did not explain the bioavailability of GLY in any part of the manuscript since they told that it is clinically being used already. They should add it to introduction or even in discussion part.

The authors’ Answer:
Indeed, The GLY has been widely used in clinic already. In ancient Chinese medicine literature, GLY could be used for patients with nasopharyngeal discomforts among the people, such as sore throat or oral ulcers. Previous studies have demonstrated that GLY could inhibit cell migration [1, 2], block angiogenesis [3], and suppress secretion of some inflammatory cytokines. In human oral cancer cells, GLY extract could suppress tumor necrosis factor alpha (TNF-α) expression through the NF-κB, AKT, and ERK-dependent pathways [4]. One study combined the advantages of using a large-scale clinical database and a laboratory approach to investigate the potential benefits of using adjunctive GLY treatment in patients with CP [5]. They included all patients diagnosed with NPC during 1997-2009 and followed until 2011 in Taiwan. And used 1:1 frequency matching by age, sex, comorbidity, conventional treatment, and index year to compare the GLY users and non-GLY users (n = 2542 each). Finally, we could find patients treated with GLY had a lower risk of mortality and a better survival probability. The clinical efficacy of GLY is clear after treatment, and the sore throat gradually reduces or even disappears after treatment. The clinical bioavailability is more than 90% [6]. Current evidence indicates that the clinical total efficiency of GLY was better than conventional western medicine therapy or other treatments [7]. More than surprisingly, the use of GLY treatment also has not any adverse reactions and other clinical side effects [8]. It can be seen from the above that the Traditional Chinese Medicine (TCM) as a new complementary medicine and alternative therapies, has important guiding significance in clinical treatment [9]. Thus, the formula has been widely used to recuperate sore throat clinically in China, which has high cure rates for treatment of CP in clinical [10, 11].

Above all, I have added it to discussion part. (Discussion section, line 336-351, page 12).

The authors should explain if this plant combination is a standardized combination from sera plants. Otherwise the ingredients will change each time and they will not be able to arrange the dosage. And if they cannot arrange the dosage in standardized version of the product it cannot be used for treatment since it will have different ingredients each time with different efficacy. To be able to say that it can be used pharmacologically they should guarantee that each time they will have the same effect in long term use of the product which are prepared from different source of plants collected at different times.

The authors’ Answer:
The formula is a standardized formula, which is a Chinese classic famous Prescription. GLY comes from the “Tai Ping Hui Min He Ji Ju Fang” (hereinafter referred to as the “Ju Fang”) written by the medical scientist Chen Shiwen and others as early as the Song Dynasty. It has been more than 800 years ago history and then it has been included in many ancient books. In ancient Chinese medicine literature, GLY could be used for patients with throat diseases, such as ulcers, sore throat, cough, etc. Nowadays, the formula also has been widely used to recuperate sore throat clinically in China, which has high cure rates for treatment of CP in clinical. According to ancient records, Ganluyin (GLY), a classic Chinese medicine formula consisted of ten TCMs, including the root of Scutellariae Radix, Rehmanniae Radix, Rehmanniae Radix Praeparata, Asparagi Radix, Ophiopogonis Radix and Praeparata Cum Melle, the stem of Dendrobii Caulis, the aboveground of Artemisiae Scopariae Herba, the leaf of Eriobotryae Folium, the fruit of Aurantii Fructus. And the dosage is 7.4g for each flavor, 74g for one prescription totally. The extraction process is water extraction, specific methods can be seen in the manuscript. Also, these plants were purchased from Zhejiang Inte Pharmaceutical Co., Ltd. and grown in sera conditions in a standardized way. They can provide the guarantee for the growth, identification, authenticity, and quality standardize of these Chinese herbal medicines. Therefore, the plants, the dosages, and the extraction process, are all maintain fix and consistence. To be able to guarantee that each time they will have the same effect in long term use of the GLY for the patients.

In addition, according to your suggestions, we will consider the plan of developing a complete set of fingerprints of different batches prepared from different source of plants collected at different times in the future, in order to further perfect the standard of the dosage for the patients.

The compositions and full details of GLY were shown in Table S1 in the supplementary data. Please see the attached file for details.

Table S1. The composition and full details of GLY

Table S1. The composition and full details of GLY
<table>
<thead>
<tr>
<th>No.</th>
<th>Chinese name</th>
<th>Abbreviation</th>
<th>Botanical name</th>
<th>Plant family</th>
<th>Part used</th>
<th>Original proportions</th>
<th>Weight (g)</th>
<th>Batch numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Huang Qin</td>
<td>HQS</td>
<td>SCUTELLARIAE RADIX</td>
<td>Labiatae</td>
<td>Root</td>
<td>17.4180304</td>
<td>180</td>
<td>304</td>
</tr>
<tr>
<td>2</td>
<td>Shi Hu</td>
<td>SHD</td>
<td>DENDROBII CAULIS</td>
<td>Ranko</td>
<td>Stem</td>
<td>17.4181126</td>
<td>181</td>
<td>126</td>
</tr>
<tr>
<td>3</td>
<td>Di Huang</td>
<td>DH</td>
<td>DREHMANNIAE RADIX</td>
<td>Scrophulariaceae</td>
<td>Root</td>
<td>17.41812016</td>
<td>190</td>
<td>101</td>
</tr>
<tr>
<td>4</td>
<td>Shu Di Huang</td>
<td>SD</td>
<td>DREHMANNIAE RADIX PRAEPARATA</td>
<td>Scrophulariaceae</td>
<td>Root</td>
<td>17.41812016</td>
<td>181</td>
<td>2016</td>
</tr>
<tr>
<td>5</td>
<td>Tian Dong</td>
<td>TD</td>
<td>DASPARAGI RADIX</td>
<td>Liliaceae</td>
<td>Root</td>
<td>17.41901016</td>
<td>190</td>
<td>1019</td>
</tr>
<tr>
<td>6</td>
<td>Mai Dong</td>
<td>MD</td>
<td>DOPHIOPOGONIS RADIX</td>
<td>Liliaceae</td>
<td>Root</td>
<td>17.41812059</td>
<td>181</td>
<td>2059</td>
</tr>
<tr>
<td>7</td>
<td>Yin Chen</td>
<td>YC</td>
<td>CARTEMISIAE SCOPARIAE HERBA</td>
<td>Compositae</td>
<td>Aboveground</td>
<td>17.41808024</td>
<td>180</td>
<td>8024</td>
</tr>
<tr>
<td>8</td>
<td>Pi Pa</td>
<td>PP</td>
<td>YERIOBOTRYAE FOLIUM</td>
<td>Rosaceae</td>
<td>Leaf</td>
<td>17.4180428</td>
<td>180</td>
<td>428</td>
</tr>
<tr>
<td>9</td>
<td>Zhi Qiao</td>
<td>ZQ</td>
<td>ZGGLYCYRRHIZAE RADIX ET RHIZOMA PRAEPARATA CUM MELLE-Leguminosae</td>
<td></td>
<td>Fruit</td>
<td>17.41810052</td>
<td>181</td>
<td>10052</td>
</tr>
<tr>
<td>10</td>
<td>Zhi Gan Cao</td>
<td>ZGC</td>
<td>GLYCYRRHIZAE RADIX ET RHIZOMA PRAEPARATA CUM MELLE-Leguminosae</td>
<td></td>
<td>Root</td>
<td>17.41902013</td>
<td>190</td>
<td>2013</td>
</tr>
</tbody>
</table>
Fig. 1. GLY water extract. 1 = naringin, 2 = neohesperidin, 3 = baicalin, 4 = wogonoside. (A) Comparative with mixed reference standards solution. a = naringin standard, b = hesperidin standard, c = neohesperidin standard, d = baicalin standard, e = wogonoside standard. (B)

Fig. 2. Comparative with single reference standards solution. a = naringin standard, (C) c = neohesperidin standard, (D) d = baicalin standard, (E) e = wogonoside standard. (F)

The data that show the comparative with reference standards in the supplementary data. Please see the attached file for details.

I quite agreed that the extract also contains with others components in the minor amount. Ganluyin (GLY), a classic Chinese medicine formula consisted of ten TCMs, including the root of Scutellariae Radix, Rehmanniae Radix, Rehmanniae Radix Praeparata, Asparagi Radix, Ophiopogonis Radix and Praeparata Cum Melle, the stem of Dendrobii Caulis, the aboveground of Artemisiae Scopariae Herba, the leaf of Eriobotryae Folium, the fruit of Aurantii Fructus. By searching databases such as Traditional Chinese Medicine System Pharmacology (TcmSP) and reviewing extensive literature, we could find some others compounds in GLY. The Scutellariae Radix contains about 75 active compounds, the most important of which are baicalin, wogonoside, scutellarein, chrysin, dihydrobaicalin, eriodyctiol, 5,7,4'-trihydroxy-6-methoxyflavanone, isoborneol, etc [1]. The Rehmanniae Radix and Rehmanniae Radix Praeparata contains about 76 active compounds, the most major of which are isoacteoside, rehmaglutin A, rehmaglutin D, 5-HMF, catalpol, leonurus glycoside, pilose glycoside, etc [2, 3]. The Asparagi Radix contains about 32 active compounds, the most major of which are quercetin, aspafilioside A, palmitic acid, methylprotodioscin, gracillin, etc [4]. The Ophiopogonis Radix contains about 64 active compounds, the most important of which are ophiopogonin P, ophiopogonin Q, glycoside C, nolinospiroside, ophiofurospiside, ophiopogonone B, β-sitosterol-β-d-glucoside, trans-p-coumaric acid, etc [5]. The Praeparata Cum Melle contains about 87 active compounds, the most main of which are asernestioside A, betaine, coumarin, crystal VI, rhamnocitrin-3-O-glucoside, 3-hydroxy-2-picoline, isoferulic acid, bifendate, isoflavanone, etc [6]. The Dendrobii Caulis contains about 190 active compounds, the most major of which are naringenin, dihydrogen resveratrol, dendroflorin, citrusin C, citronellol, isochastaftoside, dihydrosyringin, uridine, adenosine, syringic acid, vanillic acid, dendrocanidin A, 7-methoxycoumarin-6-O-β-D-pyranoglucose, essential oils, etc [7, 8]. The Artemisiae Scopariae Herba contains about 53 active compounds, the most important of which are scoparone, methylugenol, salicylic acid, rutin, vanillin, Artepillin C, eupatilin, 5-hydroxyferulate, quercetin, etc [9]. The Eriobotryae Folium contains about 142 active compounds, the most main of which are myrcene, myristic acid, pyrene, diphenacyprone, anisaldehyde, arjunolic acid, hesperidin, neohesperidin, methyl salicylate, oleanolic acid, ursolic acid, p-xylene, etc [10]. The Aurantii Fructus contains about 17 active compounds, the most chief of which are hesperidin, neohesperidin, naringin, naringenin, myrcene, nobiletin, N-methyltyramine, etc [11]. We also found some others components in the extract, like the hesperidin, but the content is too low to be detected. We will further increase the detection range and sensitivity in the future. Thank you very much for your kindly comments!


All indicated above are revised in the manuscript and described exactly what amendments have been made to the manuscript text and where these can be viewed in this response letter. Thank you and all the reviewers for the kind advice.

Sincerely
Rong Luo

Dear Editor:
Thank you for your work on our manuscript titled “Anti-inflammatory effect of Ganluyin, a Chinese classic Prescription, in chronic pharyngitis rat model” (BCAM-D-20-00842). We would like to thank the reviewers for their hard work and their kindly suggestions.

Based on the comment about the repetition rate of ithenticate, we have revised the manuscript and polished the native language according to the Turnitin originality report. The final duplicate check rate is 25%, of which 20% are cited references. And the references have been properly cited. Please see the attachment for the specific final Turnitin check report.

All indicated above are revised in the manuscript. If our manuscript has any question, please let me know immediately. I will deal with them as soon as I can. Thank you and all the reviewers for the kind advice.

Sincerely
Rong Luo

Dear Editor:
Thank you for your work on our manuscript titled “Anti-inflammatory effect of Ganluyin, a Chinese classic Prescription, in chronic pharyngitis rat model” (BCAM-D-20-00842). We would like to thank the reviewers for their hard work and their kindly suggestions.

I am sorry that the repetition rate of our manuscript "BCAM-D-20-00842R2" is too high (46%), mainly because the description of some experimental methods is similar to related literature, and the reference itself has a certain repetition rate. We have submitted a new manuscript BCAM-D-20-00842R3 named “Anti-inflammatory effect of Ganluyin, a Chinese classic Prescription, in chronic pharyngitis rat model”. However, your letter showed still BCAM-D-20-00842R2. I wonder if you still used "BCAM-D-20-00842R2" for duplicate check?

And the new manuscript “BCAM-D-20-00842R3” final duplicate check rate is 25%, of which 20% are cited references. And the references have been properly cited. Please see the attachment for the specific final Turnitin check report.

If our manuscript has any question, please let me know immediately. I will deal with them as soon as I can. Thank you and all the reviewers for the kind advice. Looking forward to receiving your reply soon.

Sincerely
Rong Luo