Author’s response to reviews

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

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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.

1. At present, we do not feel that there is sufficient evidence presented in your Background section to justify the testing of Ganluyin in an animal model. We would therefore ask you to expand this section to include as much referenced evidence as possible to explain why you would expect this treatment to have an effect in this model. This evidence should come from previous in vitro or animal work. Please note that we are unable to accept traditional medical use as sufficient justification for animal studies.

The authors’ Answer:
The each medicine flavor in Ganluyin has a significant effect on general inflammation of laboratory animals and human throat diseases (ulcers, sore throat, cough, etc.) [1, 2]; it has a broad-spectrum bacteriostatic effect, and it has obvious inhibitory effects on food bacteria and the main pathogens of oral throat; It can inhibit a variety of viruses, and also has an inhibitory effect on virus infection and proliferation. A previous study showed Suppression of the TNF-alpha Level Is Mediated by Gan-Lu-Yin (Traditional Chinese Medicine) in Human Oral Cancer Cells Through the NF-kappa B, AKT, and ERK-dependent Pathways [3]. The formula has been widely used to recuperate sore throat clinically in China, which has high cure rates for treatment of CP in clinical [4, 5]. In addition, some medicines in Ganluyin such as Dendrobium officinale and Ophiopogon japonicus all have the effect of improving immunity and analgesic.

The throat-clearing and throat-removing effect of Dendrobium officinale is mainly the anti-inflammatory effect of preventing and treating inflammation of the oral throat, and the bacteriostatic effect on pathogens of the oral throat. Animal experiments show that Dendrobium officinale has anti-inflammatory effects. Jian Liang [6] found through animal experiments and cell experiments that Dendrobium officinale polysaccharide (DOP) can reduce the secretion of NLRP3 inflammatory bodies by blocking the β-arrestin1 signaling pathway and achieve the therapeutic effect on ulcerative colitis (UC), And can regulate the immune response. Hou Shaozhen [7] found that freshly squeezed juice from Dendrobium officinale can reduce the number of writhing caused by acetic acid in mice, can significantly increase the pain threshold of mice in hot plate method, and can reduce the degree of xylene-induced ear swelling in mice and inhibit acetic acid Increased capillary permeability and inhibited the growth of cotton ball granuloma. In addition to its anti-inflammatory effect, DOP has a significant inhibitory effect on E. coli, Staphylococcus aureus, pneumococcus, and Bacillus subtilis, of which the most inhibitory effect on E. coli [8, 9]. In recent years, the Chinese market has released Dendrobium officinale tea and lozenges for clearing the throat and protecting the throat [10, 11].

Astragaloside in the water extract can reduce the degree of xylene-induced ear swelling in mice, and intraperitoneal administration is better than oral administration [12]. The mechanism may be to inhibit the biology of leukotriene B4 and leukotriene C4 in leukocytes. Synthesis, inhibits the increase of Ca2+ in leukocytes stimulated by fMLP, promotes the increase of intracellular cAMP levels, and thus achieves anti-inflammatory effects [13]. Network pharmacology suggested that baicalin mainly inhibits the production of inflammatory factors, inhibits the binding of inflammatory factors to the corresponding receptors, and blocks the initiation of inflammatory response through MAPK14, EGFR, TNFRSF1A, SELE and other targets [14].

Ophiopogon japonicus extract can reduce xylene-induced ear swelling and carrageenan or histamine-induced foot plantar swelling in mice [15], and can also inhibit the secretion of NO in RAW264.7 macrophages induced by LPS With the expression of TNF-α, it exerts an anti-inflam
inflammatory effect, and the extraction site of Ophiopogon japonicus and n-butanol has the strongest inhibitory effect on inflammatory factors [16]. Preparations based on Ophiopogon japonicus also have anti-inflammatory effects. For example, Ophiopogon japonicus syrup improves radiation pneumonitis in C57 mice by reducing IL-6, TNF-α, and transforming growth factor-β1 (TGF-β1), etc. [17]. Zhao Bo [18] found through animal experiments that compound Maidong pills may increase the activity of superoxide dismutase and reduce the content of malondialdehyde, nitric oxide, nitric oxide synthase and prostaglandin E2 in inflammatory tissue to achieve anti-inflammatory effect. Radix Scutellariae with Ophiopogon japonicus can treat recurrent oral ulcer by regulating the balance of T lymphocyte subsets [19].

Above all are suggested that the classic famous prescription Ganluyin may have the potential to develop new drugs for throat.

11. He ZH. Dendrobium officinale -based throat-clearing and throat-relieving tea. CN104904952A, 2015-09-16.
2. Please state in the methods section whether the specimen was released or euthanized. If euthanized, please clarify your euthanasia methods, including whether animals were anaesthetised and/or unconscious, injection dosages if applicable, methods used and rationale etc. Please try to be as detailed as possible.

The authors’ Answer:
The animal specimens were euthanized after completing the experiment, anesthetized rats by intraperitoneal injection of 2% pentobarbital sodium, the injection dosages was 0.3ml/100g, which was proved to be applicable through literature review and previous experimental experience. The rationale of this methods is mainly related to blocking the upward activation system of the brainstem network structure, promoting the binding of the central GABA to the GABA receptor, producing a hyperpolarizing inhibitory synaptic effect, and thus having anticonvulsant effects. The mechanism of action is pentobarbital sodium generally acts on the post-synaptic membrane of the CNS. Barbiturates have the effect of pseudo-gamma-aminobutyric acid (GABA) and can bind to GABA receptors. Small doses of barbiturates make GABA reduce the dissociation of its receptors and maintain the permeability of chloride channels. Large doses of barbiturates can directly activate chloride channels in the absence of GABA, thereby reducing the excitability of post-synaptic neurons. As the dose increases, barbiturates cause animal sedation, hypnosis, analgesia, and anesthesia. Larger doses can inhibit the cortical motor center and have anticonvulsant effects.

3. Due to the animal experimentation undertaken in this study, we require that you have ethics approval from either an IRB (Institutional Review Board) or IACUC (Institutional Animal Care and Use Committee). Please refer to our guidelines for research involving animals at: https://www.biomedcentral.com/getpublished/editorial-policies#research+involving+animals to ensure that your study complies with these rules. Please note that we may request proof of ethics approval at any time. Please also state whether any guidelines for the standard care of animals were followed, and if so state which ones. This information must be inserted in the section 'Ethics Approval and Consent to Participate'.

The authors’ Answer:
Our samples have been certified by ethics approval, please see the attached file for details.

4. We note that the current submission contains some textual overlap with other previously published works.

The authors’ Answer:
In the original text, the highlighted yellow parts from line 24 to 25 is revised to:
The study aim is to evaluate the anti-inflammatory efficacy of GLY and its potential mechanisms in a CP rat model.
In an acute inflammation model, xylene was used to induce ear edema on the mice ear surface, and carrageenan was injected subcutaneously into the right hind paws of the animal to induce the paw edema of rats.

In the original text, the highlighted yellow parts from line 32 to 35 is revised to: levels of interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor (TNF-α), and prostaglandin E2 (PGE2) were measured by ELISA in serum, and protein expressions of cyclooxygenase-2 (COX-2) and nuclear factor kappa-B p65 (NF-κB p65) of throat were detected by Western blot.

In the original text, the highlighted yellow parts from line 72 to 77 is revised to: The current clinical treatment of CP was mainly through the use of glucocorticoids, antibiotics, and nonsteroidal anti-inflammatory drugs (NSAIDs) to improve the symptoms [11, 12]. However, the treatment of CP during the long-term use of medicine such as aspirin, dexamethasone, and other drugs, could cause gastric mucosal, liver and kidney damage and other obvious side effects, which are difficult to be tolerated.

In the original text, the highlighted yellow parts from line 147 to 149 is revised to: The 10 herbs of GLY were purchased from Zhejiang Inte Pharmaceutical Co., Ltd. All the herbs were soaked for 12 h in pure water, which is 10 times their weight and then decocted for 1 h.

In the original text, the highlighted yellow parts from line 166 to 175 is revised to: After the last dose, rats were taken orally once a day for one month and one hour. 0.1 mL of freshly prepared carrageenan (1%, w/v) in physiological saline (0.9%, w/v) was injected into the plantar tissues of the right hind paw of each rat except normal group to induce inflammation. And the 0.1 mL physiological saline was injected in the normal group. The uninjected left hind paws were used as controls. The thickness of both hind paws of each animal were measured using a vernier calipers at 1 h before the induction and 1 ~ 6 h after the induction. Calculating the rate of increase in the paw thickness (paw edema) of the right hind paw (foot swelling) in the following formula: the increase rate = (B-A) / A * 100%, where A and B represent the paw thicknesses before and at different time points before and after induction respectively.

In the original text, the highlighted yellow parts from line 180 to 187 is revised to: After the last dose, Mice were taken orally once a day for one month and one hour. To induce inflammation, each mouse except normal group was induced by applying 20 μL xylene on both sides of its right ear, and the left ear was used as a normal control. After a further 0.5 h, anesthetize mice using a respiratory anesthesia machine and photograph auricle microcirculation. Then, cut the back ear along the base line of the auricle, and then use the left and right ear punches to punch a small round piece of the same part as the left and right ears with an ear piercer and weigh it. Edema is defined as the difference in weight between the two ears.

In the original text, the highlighted yellow part in line 273 is revised to: GLY can markedly inhibit acute paw edema caused by carrageenan injection

In the original text, the highlighted yellow parts from line 275 to 276 is revised to: At the same time points, compared with the model group similarly
In the original text, the highlighted yellow part in line 348 is revised to:
Results indicated that the expression levels of

In the original text, the highlighted yellow parts from line 350 to 351 is revised to:
significantly reduced the expression levels of COX-2

All indicated above are revised in the manuscript and marked with yellow high light. Thank you and all the reviewers for the kind advice.
Sincerely
Rong Luo