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A *Houttuynia Cordata*-based Chinese herbal formula improved symptoms of allergic rhinitis during the COVID-19 pandemic

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ABSTRACT

Background: The symptoms of coronavirus disease 2019 (COVID-19) such as hyposmia, rhinorrhea, nasal obstruction, and cough are similar to those of chronic allergic rhinitis (AR). Such symptoms can easily lead AR patients to unnecessary anxiety, misdiagnosis, and invasive diagnostic tests in the COVID-19 pandemic. Interleukin-6 (IL-6) is an important mediator for chronic AR and plays a crucial role in the inflammation of COVID-19. *Houttuynia cordata* (HC) has been shown to reduce nasal congestion and swelling by suppressing the activation of IL-6 and is used to fight COVID-19. A novel HC-based Chinese herbal formula, Zheng-Yi-Fang (ZYF), was developed to test effects on nasal symptoms of patients with AR in the COVID-19 pandemic.

Methods: Participants aged between 20 to 60 years with at least a 2-year history of moderate to severe perennial AR were enrolled. Eligible participants were randomly allocated to either the intervention group (taking ZYF) or the control group (using regular western medicine) for 4 weeks. The Chinese version of the Rhinosinusitis Outcome Measures was used to evaluate impacts on quality of life and nasal symptoms of participants with AR. In addition, the effect of ZYF on lipopolysaccharide (LPS)-induced IL-6 was investigated.

Results: Participants with AR taking ZYF improved their symptoms of nasal obstruction, nasal secretion, hyposmia, and postnasal drip in comparison with those of the control group. Meanwhile, ZYF exhibited inhibition of IL-6 secretion in the LPS-induced inflammatory model.

Conclusion: ZYF has potential effects to relieve nasal symptoms for AR during the COVID-19 pandemic.

Keywords: Allergic rhinitis; COVID-19 pandemic; Herbal formula; *Houttuynia cordata*

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INTRODUCTION

Allergic rhinitis (AR) is a common chronic inflammatory disease, which affects the health of nearly 30% of the world population.[1] It is characterized by inflammation of nasal mucosa with hypersensitivity resulting from all kinds of allergens.[2] Typical symptoms of AR, including sneezing, rhinorrhea, nasal congestion, and obstruction, seriously affect patients' quality of life and work.[3] The therapeutic strategies of AR mainly include avoidance of exposure to allergens, treatment with corticosteroids and/or antihistamines, and immunotherapy. However, these measures do not completely control AR symptoms.[4] Many AR patients seek complementary and alternative therapy, such as traditional Chinese medicine (TCM), due to their fear of the possible adverse effects of synthetic drugs they were taking.[5] In the recent decade, more and more studies of TCM for AR have indicated that the treatment of allergic disorders with herbal medicines is of safety and efficacy.[6,7]

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the cause of an outbreak of severe acute respiratory syndrome (SARS) associated with atypical pneumonia [coronavirus disease 2019 (COVID-19)] since December 2019.[8,9] COVID-19 causes a global pandemic and brings the cumulative numbers of confirmed cases and deaths globally to nearly 228 million and over 4.6 million respectively in September 2021.[10] The symptoms of COVID-19 such as hyposmia, rhinorrhea, nasal obstruction, and cough are similar to those of AR.[11] Such symptoms can easily lead AR patients to unnecessary anxiety, misdiagnosis, and invasive diagnostic tests in the COVID-19 pandemic.[12] Therefore, developing a suitable herbal formula to relieve the nasal symptoms of AR and COVID-19 will have physical and psychological benefits to patients with AR in the COVID-19 pandemic.

The mechanism of allergic rhinitis is a series of complex inflammatory reactions. When allergens go into the mucus layer, they are taken up by the antigen-presenting cells, and the helper T lymphocyte is activated to produce Th2 cytokines to interact with B lymphocyte.[13]

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The B lymphocytes then induce the synthesis of allergen-specific immunoglobulin E (IgE), and the allergen-specific IgE binds to the high-affinity IgE receptor (FcεRI) on the surface of mast cells,[14] leading to the release and synthesize of mediators such as histamine, leukotrienes, and prostaglandins. Histamine then stimulates the mucous glands, causing the secretion of mucous, and the mucus stuck in the nasopharynx further results in coughing, abnormal or rough (coarse) breathing sounds, and postnasal drip.[15] Past studies showed that interleukin-6 (IL-6) was a dominant proinflammatory mediator found in nasal discharge. Some other research using RT-PCR and immunohistochemistry techniques exhibited that IL-6 expression was increased in rhinosinusitis.[16-22] COVID-19 caused cytokine storm by upregulating the nuclear factor kappa B (NF-κB) pathway that induces the IL-6 amplifier.[23] IL-6 is a potential therapeutic target for the inflammation induced by COVID-19 and chronic AR.

Houttuynia cordata (HC) is a medicinal and edible Chinese medicine and many studies have shown that HC can reduce nasal mucosal congestion, swelling, and sinus ostium obstruction[24] and control focal infection of pathogenic bacteria. Moreover, HC is reported to be able to effectively repair pathological changes of nasal mucosa by suppressing FcεRI-mediated signaling activation in mast cells[25] to decrease the activation of IL-6, further reducing the production of inflammatory cytokines.[26]

Therefore, a novel Chinese herbal formula (called Zheng-Yi-Fang (ZYF) herbal tea bag) with HC as the major component was developed during the COVID-19 pandemic in Taiwan. A randomized control trial was conducted to test the efficacy of this herbal formula on patients with AR. Meanwhile, the IL-6 inhibition assay of this herbal formula *in vitro* was also investigated in this study.

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2. METHODS

2.1.Ethical statement

This study was approved by the Institutional Review Boards of Taipei Veterans General Hospital (TPEVGH IRB no. 2020-10-002B), Taipei, Taiwan. Written informed consent was obtained from all participants before the study.

2.2.Participants

This study enrolled individuals aged from 20 to 60 years with at least a 2-year history of moderate to severe perennial allergic rhinitis from Taipei, Taiwan, from January 2020 to December 2021. The history of moderate to severe perennial allergic rhinitis was defined as having a score of 2 or more on a 0-to-3-point scale (0, no symptoms and 3, severe symptoms), in 4 of 8 symptom categories (sneezing, itchy nose, running nose, stuffy nose, watery eyes, red eyes, itchy eyes, or itchy throat) based on patient recall of the previous 6 months. Exclusion criteria included having (1) a history of severe idiopathic anaphylactic reaction, (2) immunotherapy within 2 years for allergic rhinitis, (3) systemic corticosteroids within 3 months, (4) current or recent serious systemic disease, and (5) pregnancy or lactation.

2.3.*Study design and procedure*

The study design was a two-arm, randomized, parallel controlled trial with allocation concealment and assessor blinding. Randomization was performed by using a computer-generated random allocation sequence. Eligible participants were randomly allocated to either the intervention group (ZYF group) or the control group (using their regular western medicine) at a 1:1 ratio. The outcome assessor was blinded to the group assignment. A flowchart of the study design is presented in Fig. 1.

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2.4.Intervention

The HC-based Chinese herbal formula, called Zheng-Yi-Fang (ZYF), is composed of 11 herbal drugs. The components of and their proportions in ZYF are shown in Table 1, including 20% of *Houttuynia cordata* (yu xing cao), 16.5% of *Platycodon grandiflorum* (jie geng), 14% of *Scutellaria baicalnsis* (huang qin), 7% of *Pogostemon cabin* (huo xiang), 7% of *Eupatorium fortune* (pei lan), 7% of *Coix lacryma-jobi* (yi ren), 7% of *Prunus armeniaca* (xing ren), 7% of *Magnolia officinalis* (hou po), 7% of *Schizonepeta tenuifolia* (jing jie), 4.5% of *Pinellia ternate* (ban xia), and 3% of *Glycyrrhiza uralensis* (gan cao). Each ZYF (4 grams per pack) is steeped in 250 c.c. of water as a tea bag, once a day for 28 days.

2.5.Measures

2.5.1. Evaluation of nasal symptoms and quality of life in AR

The Chinese version of the Rhinosinusitis Outcome Measures (CRSOM-31) is a validated instrument translated from the widely used Rhinosinusitis Outcome Measures 31 (RSOM-31), which contains seven domains including nasal symptoms, eye symptoms, sleep, ear symptoms, general symptoms, practical problems, and emotional consequences.[27] It is divided into 7 major areas with 6 questions about nasal cavity symptoms, 2 questions about eye symptoms, 4 questions about sleep quality, 5 questions about ear symptoms, 7 questions about overall symptoms, 4 questions about life influence, and 3 questions about emotional influence. Patients were asked to score each of the 31 items for their severity and importance to them, over the past two weeks. The total CRSOM-31 score was calculated as the sum of the severity multiplied by the importance scores. Higher scores indicate a greater impact of rhinosinusitis on quality of life.[28] The participants also filled out the adverse report during the period of taking the ZYF herbal formula.

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2.5.2. IL-6 inhibition assay

Preparation of the water extract ZYF

ZYF is composed of 11 herbal drugs and each herbal was purchased from Kaiser Pharmaceutical Co., Ltd. Aqueous Extract of those herbs with a total 100g raw herb (20g of *Houttuynia cordata,* 14g of *Scutellaria baicalnsis*, 16.5g of *Platycodon grandiflorum*, 7g of *Pogostemon cabin*, 7g of *Eupatorium fortunei*, 7g of *Coix lacryma-jobi*, 7g of *Prunus armeniaca*, 7g of *Magnolia officinalis*, 7g of *Schizonepeta tenuifolia*, 3.5g of *Pinellia ternata*, 4g of *Glycyrrhiza uralensis*) was submerged in 500g water for 30 minutes and boiled for 60 minutes, which was repeated twice. Then the decoction was filtered, collected, concentrated, and lyophilized.

Cell lines

Raw264.7, murine macrophages, was purchased from the Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan) and maintained in Dulbecco's modified Eagle's medium (DMEM, GIBCO-Life Technologies) supplemented with 10% heat-inactivated fetal bovine serum (FBS, HyClone, Marlborough, MA), 100 units/mL of penicillin and streptomycin (Biological Industries, Cromwell, CT), and 3.7 g/L of NaHCO3. Adherent Raw264.7 cells were detached by incubation with trypsin-EDTA (Invitrogen, Co., Carlsbad, CA). The cells were cultured in a 5% CO2 atmosphere at 37 °C.

Cell viability assay

Raw264.7 cells (5 × 104 cells) were seeded into 12-well plates and incubated overnight. Cells were stimulated with LPS (1 μg/mL) in the presence or absence of different concentrations of ZYF (0–800μg/mL) for 24 h. After incubation, each well was rinsed with phosphate-buffered saline (PBS) to remove unattached cells. The attached cells on the bottom of the well were fixed and stained with 1% crystal violet solution, as described previously.[29] The cells stained with crystal violet were dissolved by 30% acetic acid. Cell viability was determined by detecting the absorbance at 570–670 nm.

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Enzyme-linked immunosorbent assay (ELISA)

Raw264.7 cells (2 × 104 cells in 96-well plates) were treated with various concentrations of ZYF (0, 12.5, 25, 50, 100, 200, 400, and 800 μg/mL) and vehicle (0.1% DMSO) for 30 min, followed by LPS (100 ng/mL; E. coli O55:B5; Sigma Chemical L 2630, St. Louis, MO, USA) for 24 h. The levels of TNF-α and IL-6 in the cultured medium of Raw264.7 macrophages were measured using an ELISA kit (BioLegend, San Diego, CA, USA) according to the manufacturer's instructions. Standard curves for the assay system were obtained from a series of dilutions of the TNF-α and IL-6 (ranging from 0 to 800 pg/mL). A450 nm and A550 nm (reference absorbance) were determined on a TECAN Sunrise™ ELISA Reader (Tecan Group Ltd., Mannedorf, Switzerland). The levels of TNF-α and IL-6 by LPS individual stimulation were designated as 100% for each experiment.[30]

2.6. Statistical Analysis

All measured values were expressed as mean ± standard error of the mean. The differences between the intervention and control groups were analyzed using the one-way analysis of variance (ANOVA) with Tukey's post-hoc test. Differences were considered statistically significant at $p < 0.05$. Statistical analysis was performed by SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. RESULTS

3.1. Baseline characters of the intervention and the control groups

45 participants with AR were enrolled in this study. Three participants were excluded and 42 participants with AR (21 in each group) completed the study. No significant intergroup differences were observed in age, sex, and CRSOM-31 scores between the groups (Table 2).

3.2. CRSOM-31 changes in the intervention and the control groups

Compared with the control group, changes in nasal symptoms scores (-23.1 ± 1.9 in the intervention group *vs* -4.2 ± 0.8 in the control group, $p < 0.01$) and overall influence scores

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(-15.0 ± 1.3 in the intervention group *vs* -3.0 ± 0.7 in the control group, $p < 0.01$) of CRSOM-31 in the intervention group significantly decreased after 4 weeks of taking ZYF (Fig.2). In further analysis of nasal symptoms, AR participants taking ZYF showed that their scores of nasal obstruction (-5.1 ± 0.8 in the intervention group *vs* -2.0 ± 0.3 in the control group, $p < 0.01$), nasal secretion (-4.0 ± 0.9 in the intervention group *vs* -1.1 ± 0.6 in the control group, $p < 0.05$), hyposmia (-3.5 ± 0.5 in the intervention group *vs* 0.3 ± 0.1 in the control group, $p < 0.01$), and postnasal drip (-5.2 ± 1.0 in the intervention group *vs* -0.3 ± 0.1 in the control group, $p < 0.05$) decreased significantly when compared with the control group (Fig. 3).

No side effects were reported after taking 4 weeks of ZYF herbal tea bag.

3.3. Effects of ZYF on LPS-induced inflammation of Raw264.7 cells

Inhibiting inflammation is a clinical therapeutic strategy to reduce acute inflammation-related swelling, so examining whether ZYF abrogates acute inflammation is a pivotal issue. Therefore, targeting pro-inflammatory cytokine production may be a potential strategy for ameliorating inflammation-related diseases. We then analyzed the secretion of pro-inflammatory cytokines in lipopolysaccharide (LPS)-stimulated Raw264.7 cells after the ZYF treatment. As shown in Fig.4, ZYF could downregulate the secretion of LPS-induced IL-6 in a concentration-dependent manner, while it did not synergistically enhance LPS-induced TNF-α expression.

4. Discussion

This study demonstrates that HC-based Chinese herbal formula (ZYF) improves the nasal symptoms and overall feelings of patients with AR without adverse events. ZYF also exhibited the ability to suppress IL-6 expression in LPS-induced inflammatory cell assays. ZYF is composed of herbs traditionally used to treat patients suffering from epidemics in TCM theories, with their capacities to fight against various viruses and allergic disorders.[31-34] A recent study demonstrated that Th2 cytokines modulate expressions of angiotensin-

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converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) in airway epithelial cells from AR, implying some interactions of AR in the setting of COVID-19 infection.[35] ZYF has the dual properties of regulating allergy and being antiviral, so it may be suitable for patients with AR during the epidemic period of COVID-19.

The inflammation caused by AR has been reported to be a Th1/Th2 imbalance combined with a Th2 lymphocyte dominant immune response that triggers the accumulation of Th2 cells at the inflammatory sites of individuals.[36] Previous studies showed that Chinese herbal formulae for AR possess multiple effects through anti-allergic, anti-inflammatory, or immunomodulatory activities. Such functions include the inhibition of the release of mediators from mast cells (such as histamine), inhibition of allergic inflammation reaction, and down-regulation of serum IgE levels or the activity of lymphocyte and/or macrophage.[37]

The major component of ZYF, HC, can effectively repair pathological changes of nasal mucosa by suppressing FcεRI-mediated signaling in mast cells, such as Syk, Lyn, and NF-κB activation,[25] decreasing the production of inflammatory cytokines, such as IL-6.[26] Platycodon grandiflorum, the second major component of ZYF, has been shown to inhibit IL-6 in allergic reactions.[38] Scutellaria baicalnsis, the third major component of ZYF, significantly suppressed the secretion of IL-6 in murine macrophages. Baicalin, a main component of Scutellaria baicalnsis, has been reported to inhibit pulmonary inflammatory cytokines including IL-6, IFN- γ, and TNF-α and decrease the ratios of Th1/Th2 and Th17/Treg.[39] In addition, Magnolia officinalis has been demonstrated antioxidant activity in allergic responses.[40] These data revealed that the coordination of these herbs contributed to the anti-allergic activities of ZYF.

Previous studies showed that HC and Scutellaria baicalnsis potentially blocked spike protein/ACE2 interaction dose-dependently, while Scutellaria baicalnsis and Magnolia officinalis inhibited 3CL protease activity.[41] Besides, emerging evidence suggests that IL-

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6 plays a crucial role in the pathophysiology of cytokine-driven immune-inflammatory responses to COVID-19,[42] and therapeutics with the potential to attenuate IL-6 may slow disease progression and subsequent mortality.[43-46] Our research results showed that ZYF suppressed the secretion of IL-6 in murine macrophages, which implied that ZYF may have the potential to prevent COVID-19-induced inflammation. Therefore, ZYF may be beneficial for patients with AR in the COVID-19 pandemic due to its multiple actions.

However, there are some limitations of the study. First, the sample size of trials is small. Second, other Th2 cytokines expressions (such as IL-13) related to AR are not investigated in this study. Studies about the effects of ZYF on more inflammatory cytokines and on virus plaque assay of COVID-19 merit further research in the future.

In conclusion, ZYF is helpful for AR in the COVID-19 pandemic to relieve nasal symptoms and psychological impacts.

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Table 1 The components and proportion of Zheng-Yi-Fang (ZYF) prescription

ZYF(4g/pack) Ingredient composition	**percentage**
Houttuynia cordata	20%
Platycodon grandiflorum	16.5%
Scutellaria baicalnsis	14%
Pogostemon cabin	7%
Eupatorium fortunei	7%
Coix lacryma-jobi	7%
Prunus armeniaca	7%
Magnolia officinalis	7%
Schizonepeta tenuifolia	7%
Pinellia ternata	4%
Glycyrrhiza uralensis	3.5%

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Table 2 The demographic data of participants with allergic rhinitis

	control group with regular western medicine (N=21)	intervention group with ZYF (N=21)	Statistical Verification (P Value)
Average age	49.77±3.77	49.09±4.9	p=0.92
Gender (male :female)	8:13	8:13	p=1.00
RSOM-31	121.54±10.11	131.55±12.11	P=0.80

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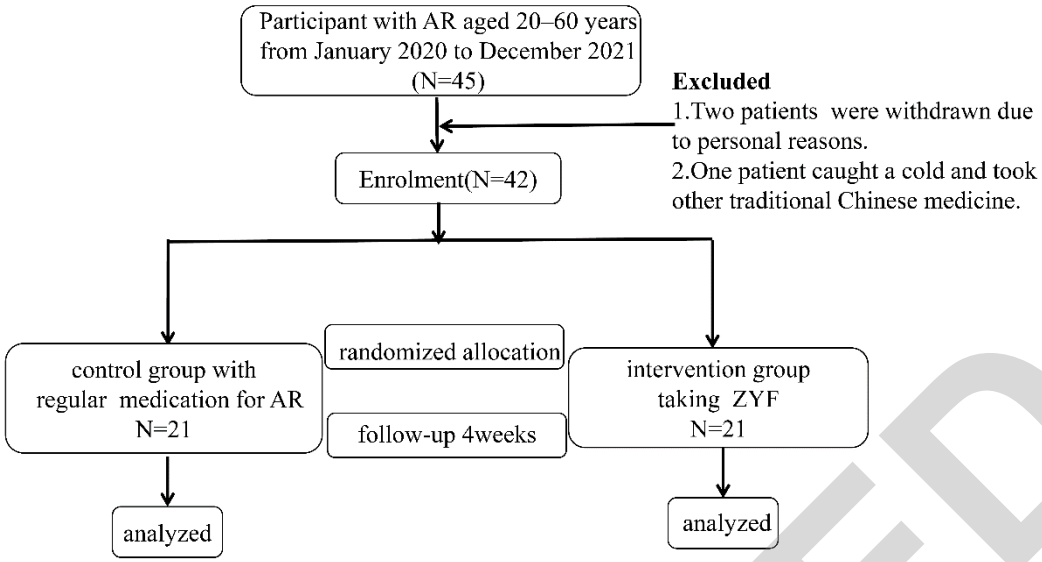
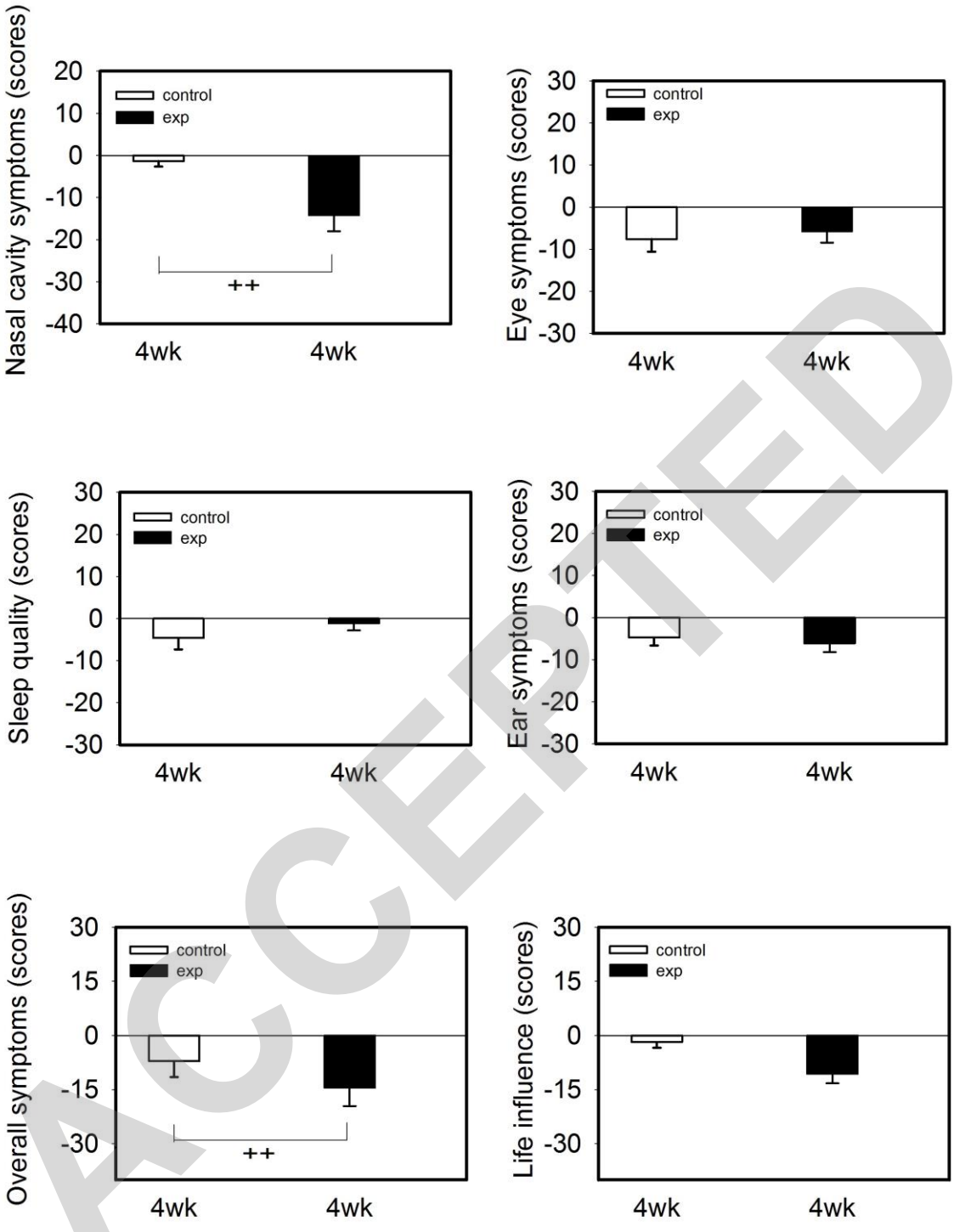


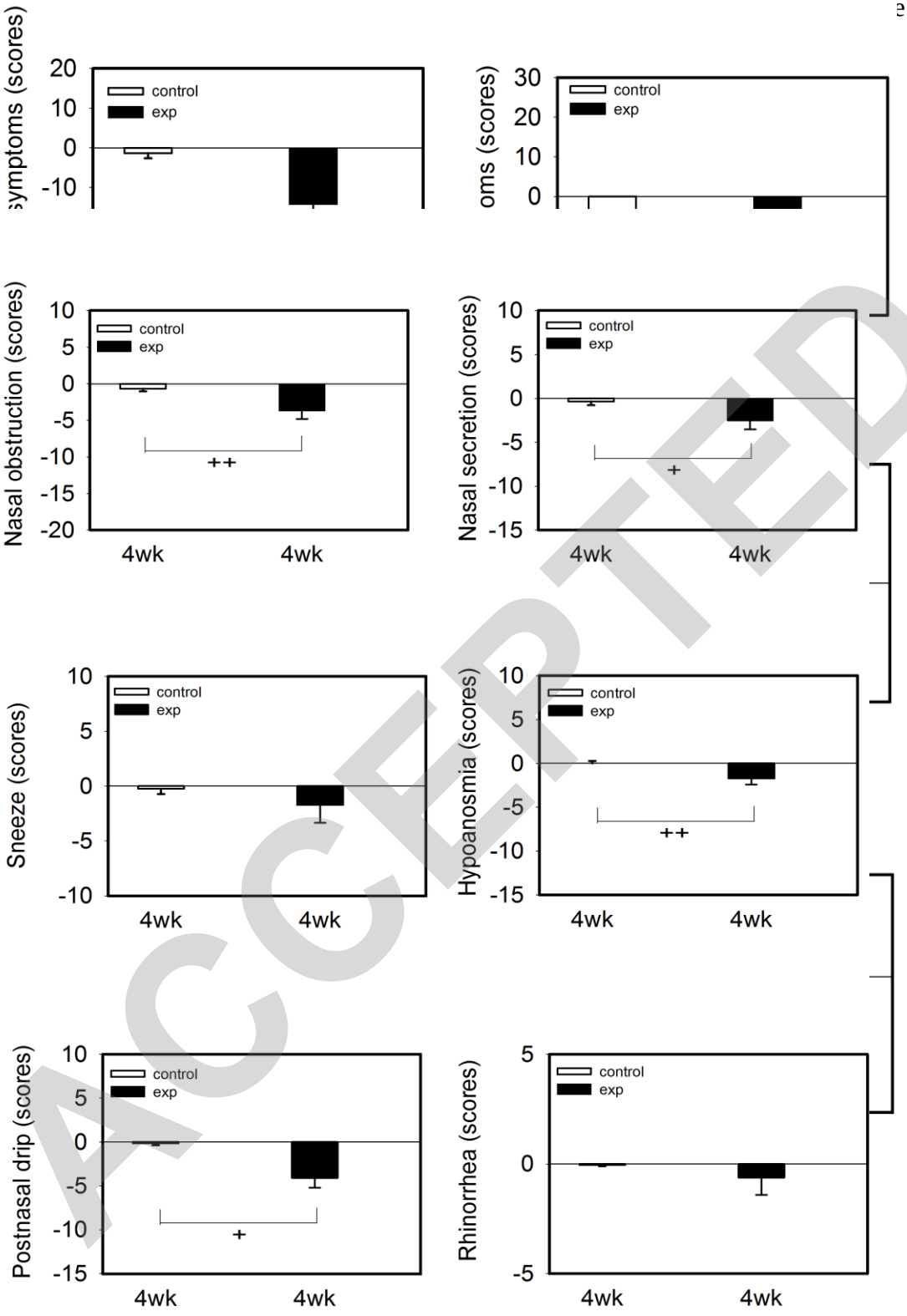
Fig.1 Flow chart of this trial

ZYF=Zheng-Yi-Fang herbal tea

ACCEPTED

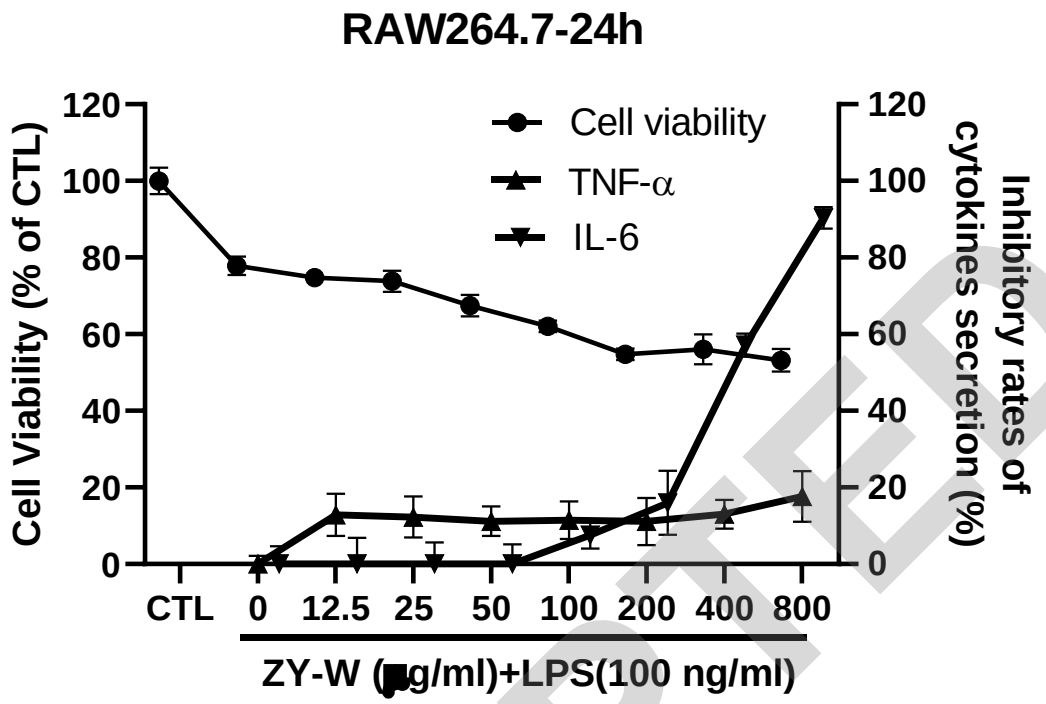

Nasal cavity symptoms (scores)
20
10
0
-10
-20
-30
-40
control
exp
++
4wk
4wk
Eye symptoms (scores)
30
20
10
0
-10
-20
-30
control
exp
4wk
4wk
Sleep quality (scores)
30
20
10
0
-10
-20
-30
control
exp
4wk
4wk
Ear symptoms (scores)
30
20
10
0
-10
-20
-30
control
exp
4wk
4wk
Overall symptoms (scores)
30
15
0
-15
-30
control
exp
++
4wk
4wk
Life influence (scores)
30
15
0
-15
-30
control
exp
4wk
4wk

ACCEPTED


ymptoms (scores)
20
10
0
-10
control
exp
oms (scores)
30
20
10
0
control
exp
Nasal obstruction (scores)
10
5
0
-5
-10
-15
-20
control
exp
++
4wk
4wk
Nasal secretion (scores)
10
5
0
-5
-10
-15
control
exp
+
4wk
4wk
Sneeze (scores)
10
5
0
-5
-10
control
exp
4wk
4wk
Hypoanosmia (scores)
10
5
0
-5
-10
-15
control
exp
++
4wk
4wk
Postnasal drip (scores)
10
5
0
-5
-10
-15
control
exp
+
4wk
4wk
Rhinorrhea (scores)
5
0
-5
control
exp
4wk
4wk

ACCEPTED

Fig. 4 The inhibitory effects of ZYF on cell viability and cytokine secretion in LPS-induced RAW264.7 cells.



CTL=control; ZY-W=Zheng-Yi-Fang water extract; LPS=lipopolysaccharide

ACCEPTED