Effectiveness of LianhuaQingwen (LHQW) capsules for post-exposure prophylaxis of seasonal influenza virus infection in household setting: A multicenter, randomized, double-blind, placebo-controlled study

連花清瘟膠囊在聚居環境中對季節性流感密切接觸者的預防作用：一項多中心、隨機、雙盲、安慰劑對照研究

Study protocol version:2.0 (December 17, 2019)

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Positive effects of Lianhuaqingwen granules in COVID-19 patients: A retrospective study of 248 cases

Journal of Ethnopharmacology

- **Materials and methods:** We retrospectively collected 248 patients who met the moderate type COVID-19 diag_x0002_nostic criteria, and received treatment in Tongji Hospital. Patients were divided into control (158 cases, standard treatment) and LHQW treatment (90 cases, standard treatment combined with LHQW) groups according to the different treatments administered. All laboratory data were obtained after 5–7 days′ treatment.
- **Results:** In this study, the average patient age was 58.95 years and 131 patients were male. The two groups were comparable in demographic characteristics, symptoms, and treatment. Compared with in the control group, D dimer and erythrocyte sedimentation rate were significantly lower in the LHQW treatment group (2.47 ± 4.67 vs. 1.68 ± 3.61; 44.47 ± 30.24 vs. 35.39 ± 27.43; both $P < 0.05$). Lymphocyte counts, albumin and hemoglobin levels were higher in the LHQW treatment group than those in the control group (1.00 ± 0.46 vs. 1.13 ± 0.5; 34.39 ± 5.2 vs. 35.71 ± 4.76; 127.03 ± 16.58 vs. 131.11 ± 14.66; both $P < 0.05$).
- **Conclusion:** The study showed that LHQW significantly improved laboratory results of patients with COVID-19 and could be effectively applied alongside standard treatment of patients with moderate type COVID-19, providing preliminary clinical research evidence for the use of TCM in treatment of this disease.

Efficacy and safety of herbal medicine (Lianhuaqingwen) for treating COVID-19: A systematic review and meta-analysis

Integrative Medicine Research

- **Methods:** We conducted the literature search using six electronic databases from December 1, 2019, to June 2, 2020. Cochrane Risk of Bias tool was used to assess the quality of randomized controlled trials. Newcastle-Ottawa Scale was used to assess the quality of case control studies. Agency for Healthcare Research and Quality checklist was used to assess the quality of case series. All analyses were conducted by RevMan 5.3. For outcomes that could not be meta-analyzed were performed a descriptive analysis.
- **Results:** Eight studies with 924 patients were included. Three studies were RCTs, three were case control studies, and two were case series. The quality of the included studies was poor. Compared with patients treated by conventional treatment, patients treated by LH combined with conventional treatment have a higher overall effective rate (RR = 1.16, 95%CIs: 1.04~1.30, P = 0.01) and CT recovery rate (RR=1.21, 95%CIs: 1.02~1.43, P = 0.03). Patients of LH groups have a lower incidence of diarrhea (5.6% vs.13.4%), and have statistically significant (P = 0.026). But the rate of abnormal liver function in the combined medication group is higher than that in the single LH group.
- **Conclusion:** LH combined with conventional treatment seems to be more effective for patients with mild or ordinary COVID-19.

Efficacy and safety of Lianhuaqingwen for mild or moderate coronavirus disease 2019A meta-analysis of randomized controlled trials

Medicine

- **Methods and analysis** : We systematically searched the Medline (OVID), Embase, the Cochrane Library, and 4 Chinese databases from inception to July 2020 to include the RCTs that evaluated the efficacy and safety of LH in combination with usual treatment vs usual treatment for mild or moderate COVID-19. A meta-analysis was performed to calculate the risk ratio (RR) and 95% confidence interval (CI) for binary outcomes and mean difference (MD) for continuous outcomes.
- **Results :** A total of 5 RCTs with 824 individuals with mild or moderate COVID 19 were included. Compared with the usual treatment alone, LH in combination with usual treatment significantly improved the overall clinical efficacy (RR=2.39, 95% CI 1.61–3.55),increased the rate of recovery of chest computed tomographic manifestations (RR=1.80, 95% CI 1.08–3.01), reduced the rate of conversion to severe cases (RR=0.47, 95% CI 0.29–0.74), shorten the duration of fever (MD=_x0001_ 1.00, 95% CI - 1.17 to - 0.84).Moreover, LH in combination with usual treatment did not increase the occurrence of the adverse event compared to usual treatment alone.
- **Conclusion :** Our meta-analysis of RCTs indicated that LH in combination with usual treatment may improve the clinical efficacy in patients with mild or moderate COVID-19 without increasing adverse events. However, given the limitations and poor quality of included trials in this study, further large-sample RCTs or high-quality real-world studies are needed to confirm our conclusions.

Efficacy of Lianhua Qingwen Compared with Conventional Drugs in the Treatment of Common Pneumonia and COVID-19 Pneumonia: A Meta-Analysis

Evidence-Based Complementary and Alternative Medicine

- **Methods:** Seven English and Chinese databases were used to search for qualifiedexperimental studies as of July 27, 2020. All data were extracted directly from the included studies, and no special conversion formula was used. *e weighted mean difference (WMD), 95% confidence interval (CI), and odds ratio (OR) were used for evaluation.
- **Results.** Forty-two studies involving 3793 subjects met the qualification criteria. For common pneumonia, a short duration of flu-like symptoms (WMD= −1.81, 95% CI= −2.12 to −1.50, P < 0.001), sputum (WMD= −1.10, 95% CI= −1.50 to−0.70, P < 0.001), pulmonary rale (WMD = −2.03, 95% CI = −2.74 to −1.31, P < 0.001), pulmonary imaging improvement (WMD= −1.88, 95% CI = −2.28 to −1.47, P < 0.001), curative effect (OR =3.65, 95% CI = 2.81 to 4.76, P < 0.001), and healing period (WMD = −1.68, 95% CI =−2.62 to −0.74, P < 0.001) were associated with the Lianhua Qingwen group; subgroup analysis based on flu-like symptoms showed statistically significant improvements in fever and cough. For COVID-19 pneumonia, improvements in flu-like symptoms (OR = 3.18, 95% CI= 2.36 to 4.29, P < 0.001), shortness of breath (OR =10.62, 95% CI =3.71 to 30.40, P < 0.001), curative effect (OR = 2.49, 95% C= 1.76 to 3.53, P < 0.001), healing period (WMD = −2.06, 95% CI =−3.36 to −0.75, P = 0.002), and conversion of severe cases (OR = 0.46, 95% CI = 0.27 to 0.77, P = 0.003) were associated with the Lianhua Qingwen group; subgroup analysis indicated statistically significant improvements of fever, cough, fatigue, and muscle pain in the Lianhua Qingwen group compared to the conventional drug group. Regarding adverse reactions, no significant difference was detected for common pneumonia (OR = 0.75, 95% CI = 0.54 to 1.05, P = 0.097)
- **Conclusions:** Lianhua Qingwen combined with conventional drugs may be a promising therapy for treating common pneumonia and COVID-19 pneumonia.

A meta-analysis for Lianhua Qingwen on the treatment of Coronavirus disease 2019 (COVID-19)

Complementary Therapies in Medicine

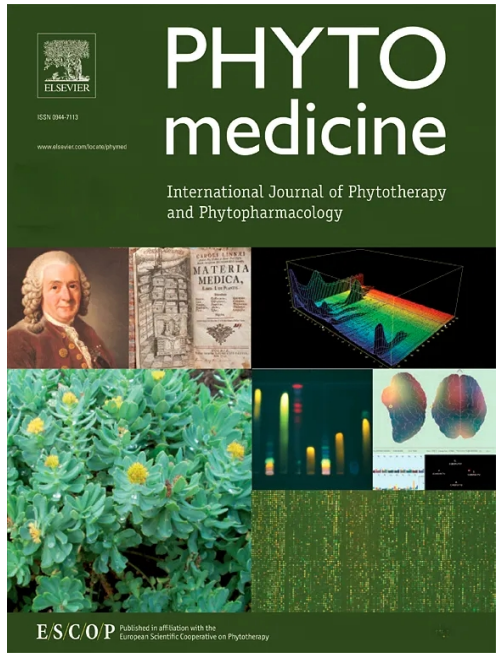
- **Methods**: Seven databases (PubMed, EMBASE, CENTRAL, CNKI, VIP, CBM and Wanfang) were searched to include all appropriate clinical trials that explore the efficacy of LQ on the treatment of COVID-19.
- **Result**: A total of 3 trials including 245 COVID-19 patients were eventually enrolled. Compared with the control group, the LQ group showed great significant difference on reducing the rate of clinical change to severe or critical condition[RR = 0.38, 95 %CI (0.17,0.85), P < 0.05]and the fever time (SMD =-0.57,95 %CI (-0.96,-0.17), P<0.05),as well as the significant improvement on the disappearance rate of the clinical symptoms: fever [RR = 1.36,95 %CI (1.14,1.61), P < 0.05],cough[RR = 1.99,95 %CI (1.39,2.86), P < 0.05],fatigue[RR = 1.52,95 %CI (1.15,2.01), P < 0.05] and anhelation [RR = 4.18,95 %CI (1.99,8.81), P < 0.05], but no significance on expectoration[RR = 2.46,95 %CI (0.81,7.51), P < 0.05].
- **Conclusion**: The clinical application of LQ on the treatment of COVID-19 has significant efficacy in improving clinical symptoms and reducing the rate of clinical change to severe or critical condition. Nevertheless, due to the limited quantity and quality of the included studies, more and higher quality trials with more observational indicators are expected to be published.

Arbidol combined with the Chinese medicine Lianhuaqingwen capsule versus arbidol alone in the treatment of COVID-19

Medicine

- **Methods:** 108 patients with COVID-19 were divided into 2 groups, including 40 patients in the arbidol group and 68 patients in the arbidol + LH group. Patients in the arbidol + LH group received 200mg of arbidol and 1400mg of LH per 8hour, and the arbidol group was given 200mg arbidol per 8hour. Blood routine examination, blood biochemistry detection, SARS-CoV-2 nucleic acid detection, and chest CT scans were performed to evaluate the clinical effects between the 2 groups.
- **Result:** No statistically significant differences were observed between the 2 groups in terms of preoperative characteristics including the baseline characteristics, laboratory indicators, and chest CT. On day 7 after admission, patients in the arbidol + LH group showed a higher level of Lymphocytes count, and a lower level of serum amyloid A and C-reactive protein levels (P<.05). Moreover, the median time from admission to the first negative result of the SARS-CoV-2 nucleic acid detection was shorter in the arbidol + LH group (P<.05). Analysis based on CT scan results showed a better extinction of lung inflammation in the arbidol + LH group. No apparent side effects were found in both groups. No patients were transferred to the intensive care unit (ICU) treatment.
- **Conclusion:** Arbidol combined with LH treatment may be more effective in improving the prognosis and accelerating the SARS-CoV-2 clearance in patients with COVID-19.

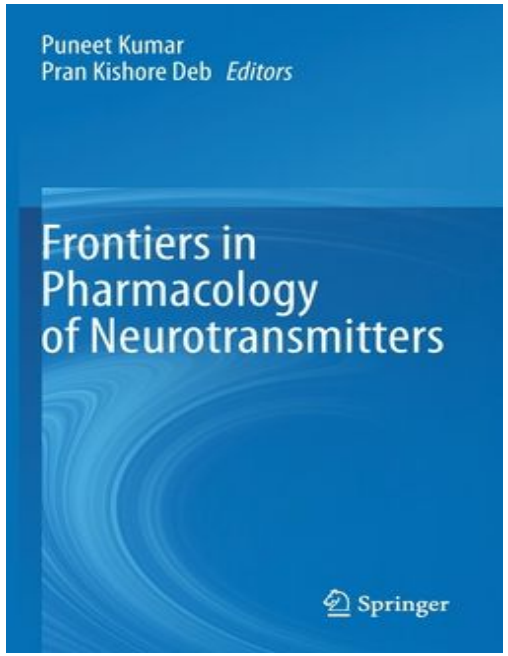
Efficacy and safety of Lianhuaqingwen capsules, a repurposed Chinese herb, in patients with coronavirus disease 2019: A multicenter, prospective, randomized controlled trial



Phytomedicine

- **Methods:** We did a prospective multicenter open-label randomized controlled trial on LH capsule in confirmed cases with Covid-19. Patients were randomized to receive usual treatment alone or in combination with LH capsules (4 capsules, thrice daily) for 14 days. The primary endpoint was the rate of symptom (fever, fatigue,coughing) recovery.
- **Results:** We included 284 patients (142 each in treatment and control group) in the full-analysis set. The recovery rate was significantly higher in treatment group as compared with control group (91.5% vs. 82.4%, p =0.022). The median time to symptom recovery was markedly shorter in treatment group (median: 7 vs. 10 days, p< 0.001). Time to recovery of fever (2 vs. 3 days), fatigue (3 vs. 6 days) and coughing (7 vs. 10 days) was also significantly shorter in treatment group (all p < 0.001). The rate of improvement in chest computed tomographic manifestations (83.8% vs. 64.1%, p < 0.001) and clinical cure (78.9% vs. 66.2%, p = 0.017) was also higher in treatment group. However, both groups did not differ in the rate of conversion to severe cases or viral assay findings (both p > 0.05). No serious adverse events were reported.
- **Conclusion:** In summary, LH capsules confer therapeutic effects on Covid-19 by improving the recovery rate of symptoms, shortening the time to symptom recovery, and improving the recovery of chest radiologic abnormalities. In light of the efficacy and safety profiles, LH capsules could be considered for the treatment of Covid-19. Future double-blind, prospective, randomized controlled trials are needed to fully evaluate the efficacy of LH capsules in a larger patient population.

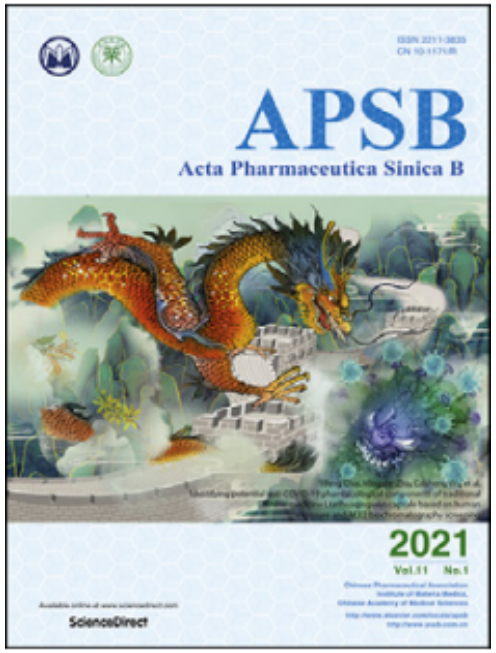
Broad Anti-Viral Capacities of Lian-Hua-Qing-Wen Capsule and Jin-Hua-Qing-Gan Granule and Rational use Against COVID-19 Based on Literature Mining



Frontiers in Pharmacology

- **Methods:** In order to collect sufficient data on anti-viral effects of LHQWC and JHQGG, we employed Pubmed (https://pubmed.ncbi.nlm.nih. gov), Ovid (https://ovidsp.ovid.com/), CNKI (https://www.cnki. net), WANFANG (http://www.wanfangdata.com.cn/index.html) and WEIPU (http://www.cqvip.com/) database by searching either the full name of formulae, such as “Lianhua Qingwen Capsules”, “Jinhua Qinggan Granules”, or names of individual medicinal herbs, or active ingredients, together with “virus” as keywords. In addition, bioactive components that were proposed to be antivirals were included via network pharmacology-based prediction and analysis. In order to describe broad-spectrum anti-viral activities of LHQWC and JHQGG, we grouped antiviral data collected as mentioned, and built a network in forms of “Formula-herb-virus Baltimore classification of viruses”. To further interpret the common and distinctive anti-viral activities of LHQWC and JHQGG in terms of holism theory of TCM, we classified the anti-viral actions reported for LHQWC and JHQGG into being either associated with viral life cycle or responsible to host immune responses and inflammation.
- **Result:** Based on literature mining, we found that both LHQWC and JHQGG were endowed with multiple antiviral activities by both targeting viral life cycle and regulating host immune responses and inflammation. In addition, from literature analyzed, JHQGG is more potent in modulating viral life cycle, whereas LHQWC exhibits better efficacies in regulating host anti-viral responses.
- **Conclusion:** When translating into clinical applications, oral administration of LHQWC could be more beneficial for patients with insufficient immune functions or for patients with alleviated symptoms after treatment with JHQGG

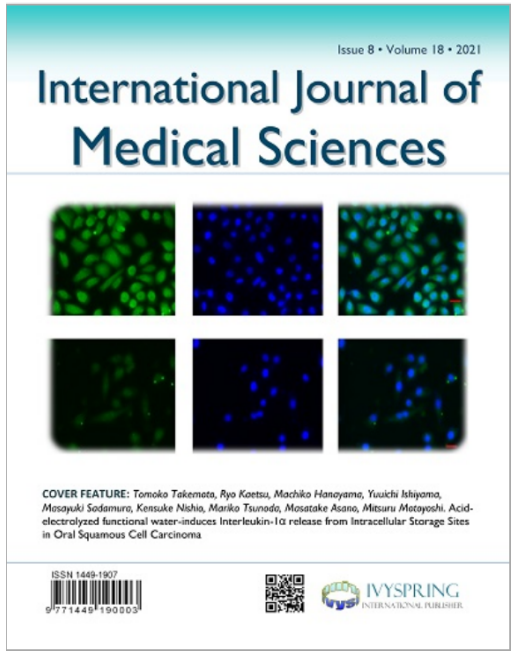
Identifying potential anti-COVID-19 pharmacological components of traditional Chinese medicine Lianhuaqingwen capsule based on human exposure and ACE2 biochromatography screening



Acta Pharmaceutica Sinica B

- **Methods:** Analysis of LHQW component profiles in human plasma and urine after repeated therapeutic dosing was conducted using a combination of HRMS and an untargeted data-mining approach, leading to detection of 132 LHQW prototype and metabolite components, which were absorbed via the gastrointestinal tract and formed via biotransformation in human, respectively. Together with data from screening by comprehensive 2D angiotensin-converting enzyme 2 (ACE2) biochromatography, 8 components in LHQW that were exposed to human and had potential ACE2 targeting ability were identified for further pharmacodynamic evaluation.
- **Results:** Results show that rhein forsythoside A, forsythoside I, neochlorogenic acid and its isomers exhibited high inhibitory effect on ACE2.
- **Conclusions:** For the first time, this study provides chemical and biochemical evidence for exploring molecular mechanisms of therapeutic effects of LHQW capsule for the treatment of COVID-19 patients based on the components exposed to human. It also demonstrates the utility of the human exposure-based approach to identify pharmaceutically active components in Chinese herb medicines.

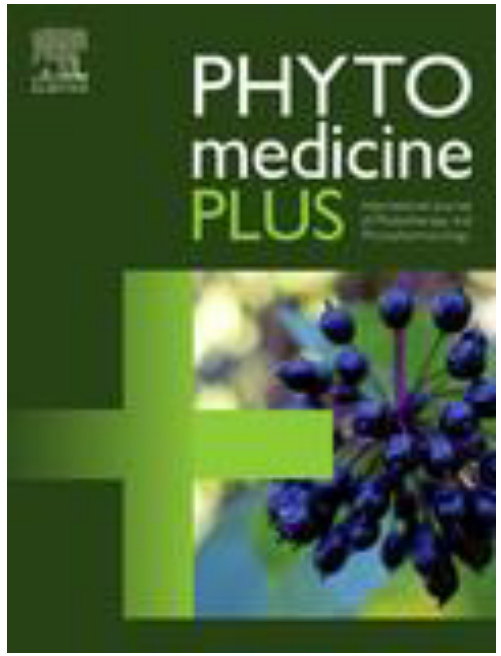
A network pharmacology based approach for predicting active ingredients and potential mechanism of Lianhuaqingwen capsule in treating COVID-19



International Journal of Medical Sciences

- **Methods:** In this study, an integrating network pharmacology approach including pharmacokinetic screening, target prediction (targets of the host and targets from the SARS-CoV-2), network analysis, GO enrichment analysis, KEGG pathway enrichment analysis, and virtual docking were conducted.
- **Result:** Finally, 158 active ingredients in LHQW-C were screen out, and 49 targets were predicted. GO function analysis revealed that these targets were associated with inflammatory response, oxidative stress reaction, and other biological processes. KEGG enrichment analysis indicated that the targets of LHQW-C were highly enriched to several immune response-related and inflammation-related pathways, including the IL-17 signaling pathway, TNF signaling pathway, NF-kappa B signaling pathway, and Th17 cell differentiation. Moreover, four key components (quercetin, luteolin, wogonin, and kaempferol) showed a high binding affinity with SARS-CoV-2 3-chymotrypsin-like protease (3CL pro).
- **Conclusion:** The study indicates that some anti-inflammatory ingredients in LHQW-C probably modulate the inflammatory response in severely ill patients with COVID-19.

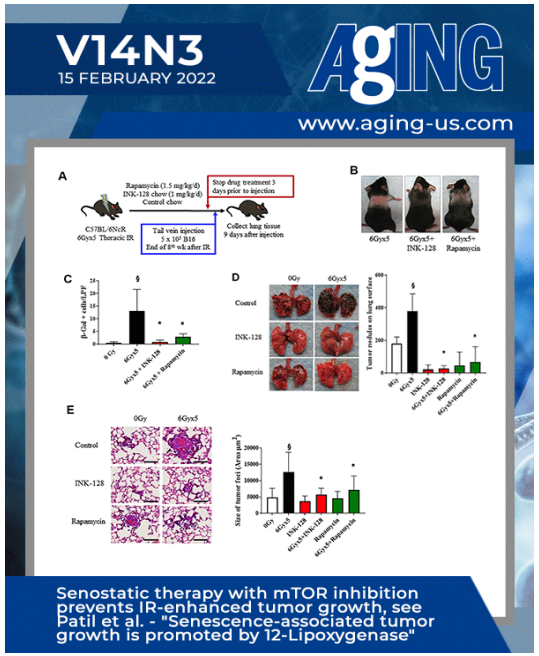
Insights into forsythia honeysuckle (Lianhuaqingwen) capsules: A Chinese herbal medicine repurposed for COVID-19 pandemic



Phytomedicine Plus

- **Methods:** Using the full names of LH capsules Lianhuaqingwen, Lianhua Qingwen and SARS-COV-2, COVID-19 as the keywords of the search terms, systemically search for existing related papers in various databases such as Web of Science and PubMed. And completed the collection of clinical data in ClinicalTrials.gov and Chinese Clinical Trial Registry. Last but not least, we have sorted out the anti-inflammatory and antiviral mechanisms of LH capsules through literature and Selleck.
- **Results:** This review systematically sorted out the active ingredients in LH capsules. Furthermore, the related pharmacological and clinical trials of LH capsule on SARS-CoV-2, IAV and IBV were discussed in detail. Moreover, the present review provides the first summary of the potential molecular mechanism of specific substances in LH capsules involved in resistance to SARS-COV-2 infection and the inhibition of cytokine storm syndrome (CSS) caused by IL-6.
- **Conclusion:** This review summarizes the available reports and evidence that support the use of LH capsules as potential drug candidates for the prevention and treatment of COVID-19. However, TCM exerts its effects through multiple targets and multiple pathways, and LH capsules are not an exception. Therefore, the relevant mechanisms need to be further improved and experimentally verified.

New tale on LianHuaQingWen: IL6R/IL6/IL6ST complex is a potential target for COVID-19 treatment



AGING

- **Methods:** The main active ingredients, therapeutic targets of LHQW, and the pathogenic targets of COVID-19 were screened using the TCMSP, UniProt, STRING, and GeneCards databases.
- **Results:**According to the "Drug-Ingredients Targets-Disease" network, Interleukin 6 (IL6) was identified as the core target, and quercetin, luteolin, and wogonin as the active ingredients of LHQW associated with IL6. The response to lipopolysaccharide was the most significant biological process identified by gene ontology enrichment analysis, and AGE-RAGE signaling pathway activation was prominent based on the interaction between LHQW and COVID-19. Protein-protein docking analysis showed that IL6 receptor (IL6R)/IL6/IL6 receptor subunit beta (IL6ST) and Spike protein were mainly bound via conventional hydrogen bonds. Furthermore, protein-small molecule docking showed that all three active ingredients could bind stably in the binding model of IL6R/IL6 and IL6ST. Our findings suggest that LHQW may inhibit the lipopolysaccharide-mediated inflammatory response and regulate the AGE-RAGE signaling pathway through IL6. In addition, the N-terminal domain of the S protein of COVID-19 has a good binding activity to IL6ST, and quercetin and wogonin in LHQW may affect IL6ST-mediated IL6 signal transduction and a large number of signaling pathways downstream to other cytokines by directly affecting protein-protein interaction.
- **Conclusion:**These findings suggest the potential molecular mechanism by which LHQW inhibits COVID-19 through the regulation of IL6R/IL6/IL6ST.

Lianhuaqingwen exerts anti-viral and anti-inflammatory activity against novel coronavirus (SARS-CoV-2)

Pharmacological Research

- **Methods:** The antiviral activity of LH against SARS-CoV-2 was assessed in Vero E6 cells using CPE and plaque reduction assay. The effect of LH on virion morphology was visualized under transmission electron microscope. Pro-inflammatory cytokine expression levels upon SARS-CoV-2 infection in Huh-7 cells were measured by real time quantitative PCR assays.
- **Results:** LH significantly inhibited SARS-CoV-2 replication in Vero E6 cells and markedly reduced pro-in-flammatory cytokines (TNF-α, IL-6, CCL-2/MCP-1 and CXCL-10/IP-10) production at the mRNA levels. Furthermore, LH treatment resulted in abnormal particle morphology of virion in cells.
- **Conclusions:** LH significantly inhibits the SARS-COV-2 replication, affects virus morphology and exerts antiinflammatory activity in vitro. These findings indicate that LH protects against the virus attack, making its use a novel strategy for controlling the COVID-19 disease.

Theoretical Study of the anti-NCP Molecular Mechanism of Traditional Chinese Medicine Lianhua-Qingwen Formula (LHQW)

- **Computational methods:** 1.Molecule docking.
  2.Molecular dynamics (MD) simulation.
  3.Decomposition of binding free energy between ligand and residue.
  4.Construction of component-target-pathway network.
- **Results:** It is shown that the docking scores of three components in LHQW including Rutin,Forsythoside E, and Hyperoside to main protease of SARS-CoV-2 are very large as -9.1, -9.0 and -8.7 kcal/mol, respectively, which are even better than those of Lopinavir at -7.3 kcal/mol. Importantly, the binding modes between active compounds and protein were verified via molecular dynamics (MD) simulation and calculation all the binding free energies at MM-PBSA level. Note that these donor-acceptor systems were stabilized by non-polar interactions including hydrogen bonds and hydrophobic interactions. At last, from the constructed component-target-pathway network, it is shown that the components in LHQW are related important pathways to improve the human immunity such as T cell, B cell receptor signaling, natural killer cell mediated cytotoxicity, as well as anti inflammatory pathways including Fc epsilon RI, ErbB, MAPK signaling and so on.
- **Conclusion:** Therefore, LHQW has not only anti-viral effect but also anti-inflammatory and immunity mechanisms. This is also consistent with the character of TCM, i.e., comprehensive therapy complex disease with multi components, multi targets, and multi pathways.

Research Article

Efficacy and Safety of Lianhuaqingwen Capsules for the Prevention of Coronavirus Disease 2019: A Prospective Open-Label Controlled Trial

- To determine the safety and efficacy of LH capsules for the prevention of COVID-19, we conducted a prospective open-label controlled trial of LH capsules on subjects who had close contact with people infected with COVID-19.

- We included 1976 patients, 1101 in the treatment group and 875 in the control group. The rate of positive nucleic acid tests in the treatment group was significantly lower than that in the control group (0.27% vs. 1.14%, p=0.0174) during the quarantine medical observation period (14 days).

- Among secondary close contacts, the rate of positive nucleic acid tests in the treatment group was significantly lower than that in the control group (0.09% vs. 0.71%, respectively; p=0.0485).