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RESEARCH ARTICLE

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Establishment of an anti-inflammation-based bioassay for the quality control of the 13-component TCM formula (Lianhua Qingwen)

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ABSTRACT

Context: Owing to the complexity of chemical ingredients in traditional Chinese medicine (TCM), it is difficult to maintain quality and efficacy by relying only on chemical markers.

Objective: Lianhua Qingwen capsule (LHQW) was selected as an example to discuss the feasibility of a bioassay for quality control.

Materials and methods: Network pharmacology was used to screen potential targets in LHQW with respect to its anti-inflammatory effects. An *in vitro* cell model was used to validate the prediction. An anti-inflammatory bioassay was established for the quality evaluation of LHQW in 40 batches of marketed products and three batches of destructed samples.

Results: The tumor necrosis factor/interleukin-6 (TNF/IL-6) pathway via macrophage was selected as the potential target of LHQW. The IC_{50} value of LHQW on RAW 264.7 was 799.8 μg/mL. LHQW had significant inhibitory effects on the expression of IL-6 in a dose-dependent manner ($p < 0.05$). The anti-inflammatory biopotency of LHQW was calculated based on the inhibitory bioactivity on IL-6. The biopotency of 40 marketed samples ranged from 404 U/μg to 2171 U/μg, with a coefficient of variation (CV) of 37.91%. By contrast, the contents of forsythin indicated lower CV (28.05%) than the value of biopotency. Moreover, the biopotencies of destructed samples declined approximate 50%, while the contents of forsythin did not change. This newly established bioassay revealed a better ability to discriminate the quality variations of LHQW as compared to the routine chemical determination.

Conclusions: A well-established bioassay may have promising ability to reveal the variance in quality of TCM.

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Biopotency; macrophage; network pharmacology; quality variation; interleukin-6

Introduction

Quality control is a key in ensuring the consistency of traditional Chinese medicine (TCM) preparations (Xiong et al. 2014). Due to the complexity and variance of chemical compositions of TCM (Lin et al. 2018), quality control is obviously a great challenge. The current quality control system in TCM mainly relies on chemical determination of marker component(s). However, most of the chemical components that are selected as quality control markers showed little association with the efficacy of TCM (Jiang et al. 2010; Lin et al. 2018). The evaluation method for qualitative and quantitative detection of these marker components cannot effectively guarantee the quality of TCM. For TCM formula with several herbal ingredients (usually more than 10 herbal medicines), it is more difficult to characterise quality (Wang et al. 2014; Zhu et al. 2019). The efficacy of TCM is often due to the overall effects of multiple components (Jiang et al. 2010; Lin et al. 2018), but none of the individual marker components can represent the overall efficacy.

In recent years, complex TCM preparations reveal advantages in the treatment of emerging communicable viral infections. For example, Maxingshigan-Yinqiaosan demonstrated good efficacy in the treatment of HINI virus infection at a multi-center, randomised, placebo-controlled trial (Wang et al. 2011). Lianhua Qingwen capsule (LHQW) is another good example, illustrating potent effectiveness in viral respiratory infections, including influenza and the coronavirus disease 2019 (COVID-19). In clinical observational studies and RCT trials for influenza, LHQW manifested beneficial effects both in alleviating patients' upper respiratory tract infection symptoms (fever, exertion, and cough) and in suppressing inflammatory cytokines (Duan et al. 2011; Hu et al. 2020). This efficacy may be explained by its ability to inhibit virus replication, as well as to inhibit the expression of

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pro-inflammatory cytokines (i.e., IL-6) (Ding et al. 2017; Li et al. 2020).

LHQW comprises of 13 herbal medicines, which poses a great challenge in quality profiling and control. Currently, only one marker chemical—forsythin, is determined in the quality control standard of LHQW in the Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission 2020). However, simply detecting one chemical marker does not link quality with the efficacy of LHQW because of the complexity and diversity of its chemical components (Jia et al. 2015; Wang et al. 2016) and the limited information on pharmacologically active components, as well as their dose-effect relationships. Therefore, it is imperative to establish a new quality evaluation method in improving the efficacy of LHQW.

Bioassay is a type of quality control method commonly used in biological preparations, including in complex components like TCM. Some scholars have proposed to utilise bioassays in quality control of TCM (Xiao et al. 2010). Theoretically, bioassays can profile the overall efficacy of chemicals in TCM preparations if a reasonable assay model has been chosen (Xiao et al. 2018). Most recently, network pharmacology has been used to screen and enrich potential targets and pathways by computing all the known components with the biological target network (Hopkins 2007, 2008; Li and Zhang 2013). This seems promising and enables network pharmacology and bioassays to be utilised in establishing a new quality control method for TCM preparations. In this study, we considered LHQW as an example to explore a bioassay method for the quality control of TCM.

Materials and methods

Drug and reagents

LHQW was provided by Shijiazhuang Yiling Pharmaceutical Co., Ltd (Shijiazhuang, China); the lot number and liquid chromatography profile is shown in Table 1 and Supplementary Figure 1. Among the samples, S40[a], S40[b] and S40[c] were self-made by treating S40 under a high temperature (60 °C), high humidity (95%) and light intensity (4500 lx) for 20 days (Chinese Pharmacopoeia Commission 2020). Lipopolysaccharide (LPS) was purchased from Sigma-Aldrich (Lot. 113M4068V, St. Louis, MO). Interferon-gamma (IFN-γ) was purchased from PeproTech (Lot. 061398, Rocky Hill). Dexamethasone (DXMS) was purchased from National Institutes for Food and Drug Control (Lot. 10029-201506, Beijing, China). The IL-6 assay kit was purchased from Beijing DAKEWE Biotechnology Co., LTD (Lot. 12-2060-096, Beijing, China). Dulbecco's modified Eagle medium (DMEM) was purchased from HyClone Company (Lot. SH3002201, Logan, UT). Foetal bovine serum, trypsin and penicillin-streptomycin solution were purchased from Gibco (Lot. 10082139, Lot. 25200056, Lot. 15070063, Grand Island, NY). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Laboratories (Lot. GD619, Kumamoto, Japan). Phosphate-buffered saline (PBS) was purchased from Beijing Solarbio Science & Technology Co., Ltd. (Lot. P1020-500, Beijing, China). Dimethyl sulfoxide (DMSO) was purchased from AMRESCO (Lot. 3304C252, Lardner, PA).

Network pharmacology analysis

Database construction

The molecule structure of the chemical components of LHQW was obtained from the TCM Database@Taiwan (TDT; http://tcm.cmu.edu.tw/). The targets of these components were predicted by using PharmMapper (http://59.78.96.61/pharmmapper), and scores greater than 4.0 were selected. Inflammation-related genes and protein targets were screened by using Online Mendelian Inheritance in Man (OMIM; http://www.omim.org/). The protein–protein interactions were obtained from the Database of Interacting Proteins (DIP; http://dip.doe-mbi.ucla.edu). The above targets were converted to UniProt ID format.

Table 1. Detailed information about 43 batches of LHQW samples.

No.	Batch no.	Lot no.	No.	Batch no.	Lot no.	No.	Batch no.	Lot no.
1	S01	1508114	16	S16	1510032	31	S31	1601061
2	S02	1508117	17	S17	1510035	32	S32	1601062
3	S03	1508118	18	S18	1510038	33	S33	1601063
4	S04	1508123	19	S19	1510048	34	S34	1601064
5	S05	1508129	20	S20	1510051	35	S35	1601065
6	S06	1508132	21	S21	1511008	36	S36	1601066
7	S07	1509081	22	S22	1511012	37	S37	1601067
8	S08	1509084	23	S23	1511050	38	S38	1601079
9	S09	1510002	24	S24	1511161	39	S39	1602001
10	S10	1510004	25	S25	1511181	40	S40	1602007
11	S11	1510017	26	S26	1601012	41	S40[a]	A1
12	S12	1510021	27	S27	1601016	42	S40[b]	A2
13	S13	1510024	28	S28	1601058	43	S40[c]	A3
14	S14	1510028	29	S29	1601059			
15	S15	1510029	30	S30	1601060			

The three destructive batches (A1, A2 and A3) were self-made in the laboratory. S40[a], S40[b] and S40[c] represent the destructed samples with high temperature (60°C), high humidity (95%) and light intensity (4500 lx).

Network construction and pathway enrichment analysis

The chemical components, targets and interactional proteins were used to construct a component-target-disease network using protein–protein interaction network (PPI; http://www.genome.jp/kegg/). The network visualisation was then analysed with Cytoscape software (version 3.2.1; http://cytoscape.org/). Candidate anti-inflammatory targets of LHQW were screened using the degree value (≥two-fold of the median value), betweenness centrality value and closeness centrality value (≥one-fold of the median value). Further, the putative targets were imported into Database for Annotation, Visualisation and Integrated Discovery (DAVID; https://david.ncifcrf.gov/) for Kyoto Encyclopaedia of Genes and Genomes (KEGG; https://www.kegg.jp/) pathway enrichment analysis. OMIM was used to screen the most likely inflammatory cell model of LHQW.

Bioassay setup

Cell culture

Murine macrophage RAW 264.7 cells were purchased from Peking Union Medical College cell bank. The cells were cultured in DMEM (containing 10% heat-inactivated FBS and 1% penicillin-streptomycin solution) at 37 °C under 5% CO_2. After 48 h, the cells were digested with trypsin and passaged at a ratio of 1:3. The cells in the logarithmic growth phase were used in subsequent experiments.

Construction of an inflammatory model of RAW 264.7 cells

The induction time and inflammation model drug are important for a stable inflammatory cell model (Wongchana and Palaga 2012; Li et al. 2014, 2019). We explored the best induction time and inflammation model drug in RAW 264.7 cells (10^4 cells/well). Using the LPS (1 μg/mL) stimulation, we first used three different time points, 6, 12 and 24 h. Further, we used three

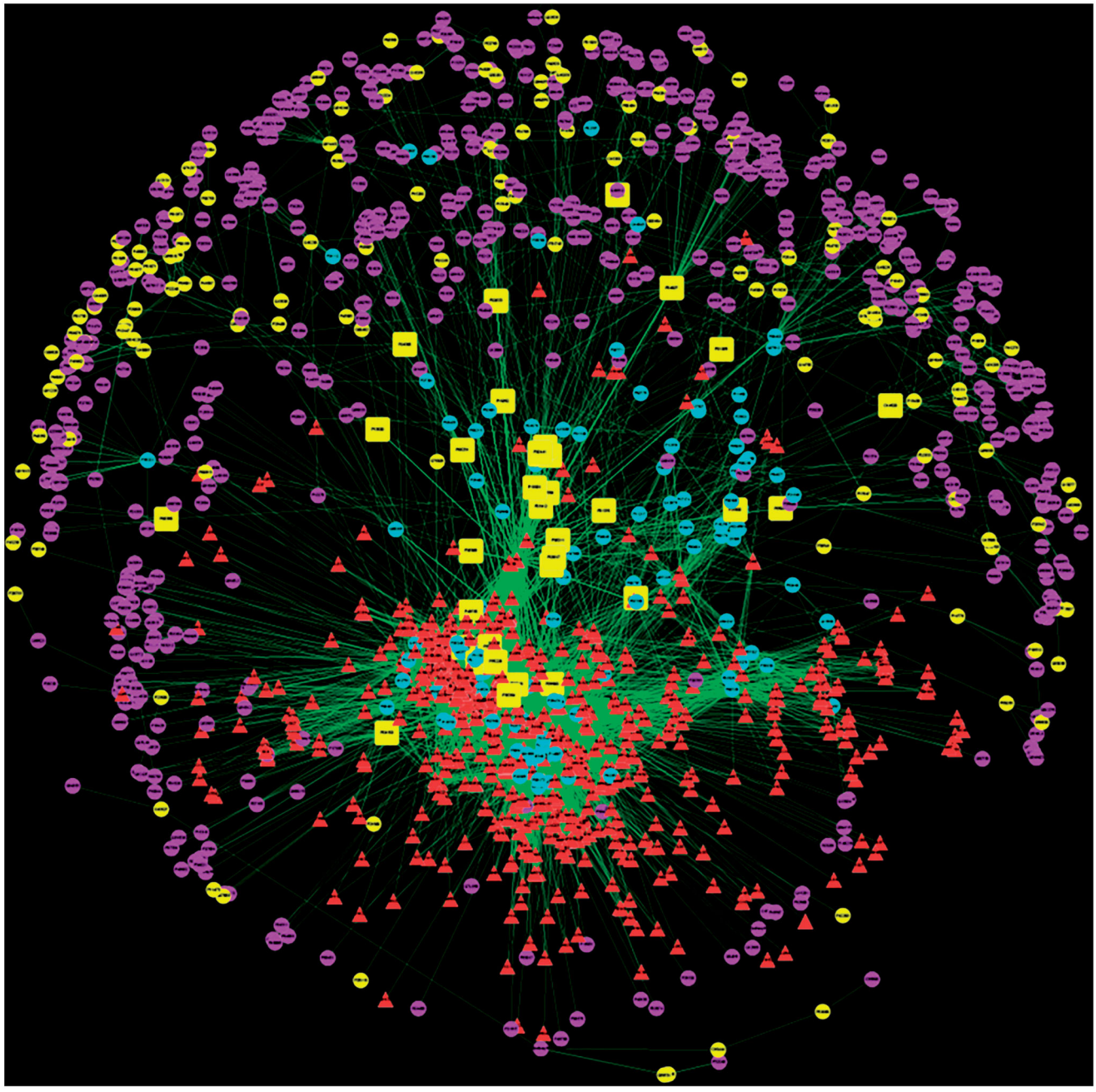

Figure 1. Component-target-disease interaction network of LHQW against inflammation.

inflammatory model drugs, LPS (0.25, 0.5, 1.0 and 2.0 μg/mL), IFN-γ (0.05, 0.1, 0.2 and 0.4 μg/mL), and a combined stimulation.

Inhibitory effect of LHQW on IL-6 expression

LHQW and DXMS were dissolved in DMSO and then diluted in DMEM (v/v, contain 0.4% DMSO). The RAW 264.7 cells (10^4 cells/well) were incubated with varying concentrations of LHQW (100–1000 μg/mL), with 24 h to calculate the cell viability using 10% CCK8. Based on the IC_{50} value of LHQW, we studied the effects of different concentrations of LHQW (containing the marketed and destructed sample; 50, 100, 200 and 400 μg/mL) on the expression of IL-6 in LPS/IFN-γ-induced RAW264.7 cells by enzyme-linked immunosorbent assay (ELISA), and selected DXMS (8 ng/mL) as the positive control. Specific groups were identified as follows: control group, LHQW-control group, model group, LHQW-treated group and DXMS group. Besides, we further formulated the inhibition rate formula of IL-6 (1).

(1) Inhibition rate (%) = [(Amodel − Asample)/Amodel] × 100%. (A, absorbance).

Method validation

Method validation was executed according to the Biological Assay for TCM Quality Control (Xiao et al. 2018). The intraday precision, inter-day precision, and repeatability were determined by measuring the inhibition rate of IL-6 according to formula (1).

Biopotency assay

The initial potency (1000 U/μg) of S40 was standardised using DXMS and then considered as the reference sample, while other

Table 2. Targets and related topological parameters of LHQW against inflammation.

UniProt ID	Protein names	Degree	Betweeness centrality	Closeness centrality
P35354	Cyclooxygenase-2	374	0.2541	0.5489
P35228	Inducible nitric oxide synthase	275	0.1121	0.4729
P23219	Cyclooxygenase-1	252	0.1011	0.4566
P37231	Peroxisome proliferator-activated receptor gamma	216	0.0646	0.4361
Q16539	Mitogen-activated protein kinase 14	160	0.0264	0.3978
P04637	Cellular tumour antigen p53	73	0.1455	0.369
P04150	Glucocorticoid receptor	66	0.0268	0.3378
P48736	Phosphatidylinositide 3-kinases	61	0.0057	0.3553
P05412	Transcription factor AP-1	45	0.0373	0.4002
P35968	Vascular endothelial growth factor receptor 2	43	0.0162	0.3418
P09960	Leukotriene A-4 hydrolase	36	0.0016	0.3204
P01375	Tumour necrosis factor-α	33	0.0184	0.3492
P00533	Proto-oncogene c-ErbB-1	28	0.0433	0.3404
P00441	human Cu/Zn superoxide dismutase	27	0.0277	0.3419
P15692	Vascular endothelial growth factor A	23	0.0191	0.343
P09211	Glutathione S-transferase P	22	0.001	0.3446
P09601	Heme oxygenase 1	22	0.0016	0.3457
P09917	Arachidonate 5-lipoxygenase	22	0.0041	0.3428
P16581	E-Selectin	19	0.0123	0.3347
P35869	Aryl hydrocarbon receptor	19	0.0044	0.3412
P05231	Interleukin-6	17	0.0092	0.3357
O14920	Inhibitor of nuclear factor kappa-B kinase subunit beta	17	0.0233	0.3379
P05067	Amyloid beta A4 protein	15	0.027	0.3721
P60568	Interleukin-2	13	0.0072	0.3327
P08253	Matrix metallopeptidase 2	12	0.0039	0.3323
P13500	Monocyte Chemotactic Protein 1	12	0.0121	0.333
P13726	Tissue factor	12	0.001	0.3371
P01584	Interleukin-1 beta	11	0.0062	0.332
P24385	*S*-Specific cyclin-D1	11	0.0164	0.3764
Q8NER1	Transient receptor potential vanilloid type-1	9	0.0034	0.2877
Q13936	Voltage-dependent L-type calcium channel subunit α-1C	9	0.0211	0.2759

Table 3. Top 10 components related to putative direct targets.

Compound name	Degree	Betweeness centrality	Closeness centrality
Quercetin	80	0.1057	0.3637
Kaempferol	49	0.0220	0.3344
beta-Sitosterol	45	0.0072	0.3249
7-Methoxy-2-methyl isoflavone	41	0.0025	0.3213
Stigmasterol	41	0.0038	0.3158
Medicarpin	37	0.0033	0.3194
Luteolin	36	0.0389	0.3398
Machiline	33	0.00161	0.3184
Quercetin	80	0.0021	0.3193
Formononetin	31	0.0031	0.3205

samples served as the test sample. The biopotency and confidence limits were calculated using Qualitative Response Potency software (version 2.0).

Statistics analysis

All data were presented as mean ± SD and were analysed with SPSS software program (version 25.0; SPSS Inc., Chicago, IL). A plot was generated in GraphPad Prism software (version 8.4.2; Inc., San Diego, CA). The significance level for all statistical tests was set at 0.05.

Results

Construction of a component target disease interactive network

Based on the interactive network, 661 chemical components, 107 drug-related targets, 169 inflammation-related targets and 561 interactional proteins were screened from LHQW. As shown in Figure 1, the yellow squares represent the shared targets of drugs and diseases; the yellow dots represent inflammation-related targets; the red triangles represent the chemical components; the blue dots represent the drug-related targets and the purple dots represent the interactional proteins. These findings indicate the complicated action against inflammation that is characteristic of LHQW.

Putative direct target of LHQW based on the interaction network

In the network, we designated 31 important targets with 'degree' >8, 'betweenness centrality' >0 and 'closeness centrality' >0.26 (Table 2). Furthermore, we extracted components related to potential direct targets (Table 3, Top 10), indicating that these targets and components may be interrelated and interact, and play an anti-inflammatory effect through multi-channel and multi-faceted coordination.

Target-pathway analysis

KEGG pathways of the above 31 eligible targets were enriched using DAVID. Finally, 22 inflammation-related pathways were screened, including the TNF/IL-6 signalling pathway, PI3K-Akt signalling pathway, influenza A, MAPK signalling pathway and Toll-like receptor signalling pathway (Figure 2). Among them, TNF/IL-6 signalling pathway was ranked most important, indicating that it may be the most important signalling pathways for LHQW against inflammation.

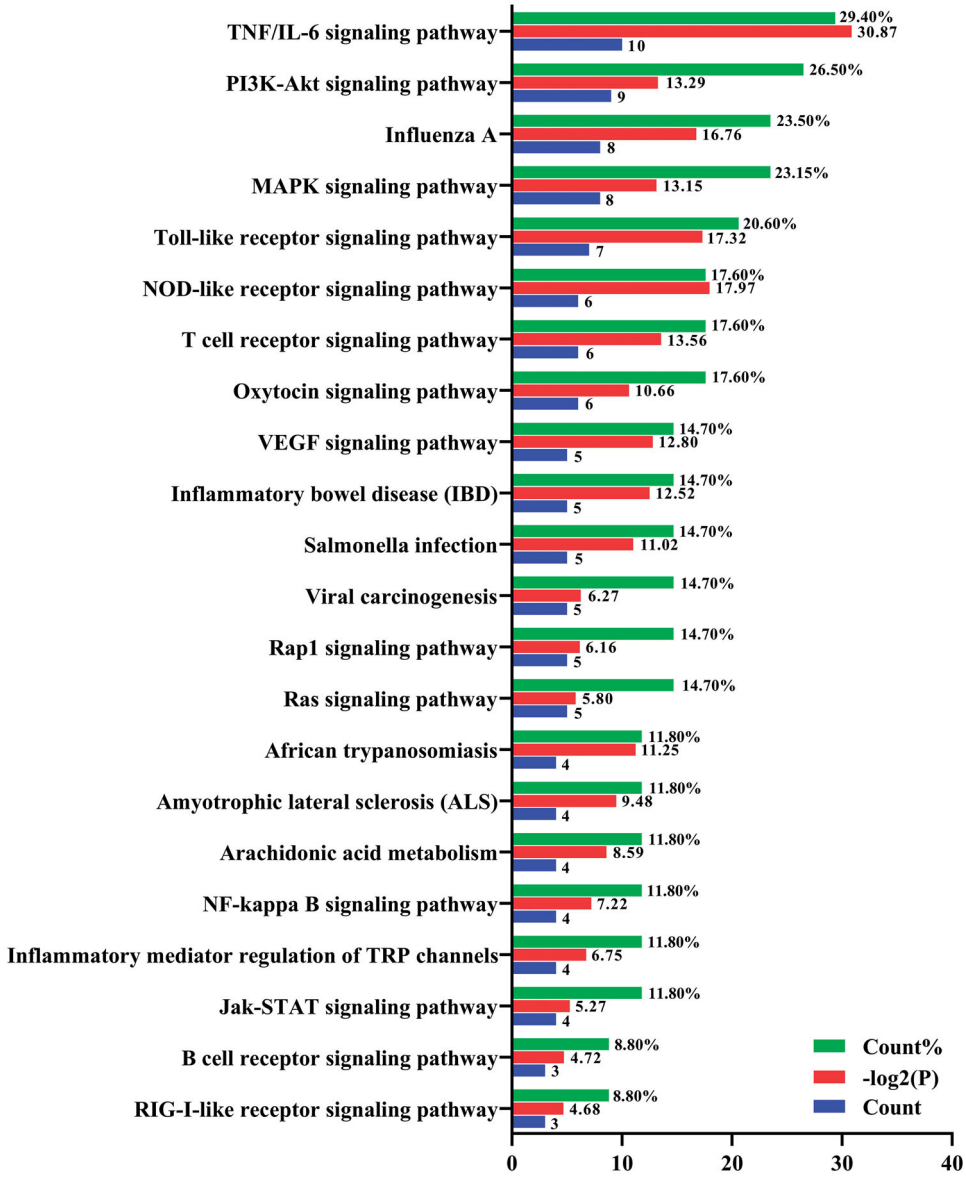

Figure 2. Putative direct targets of LHQW against inflammation.

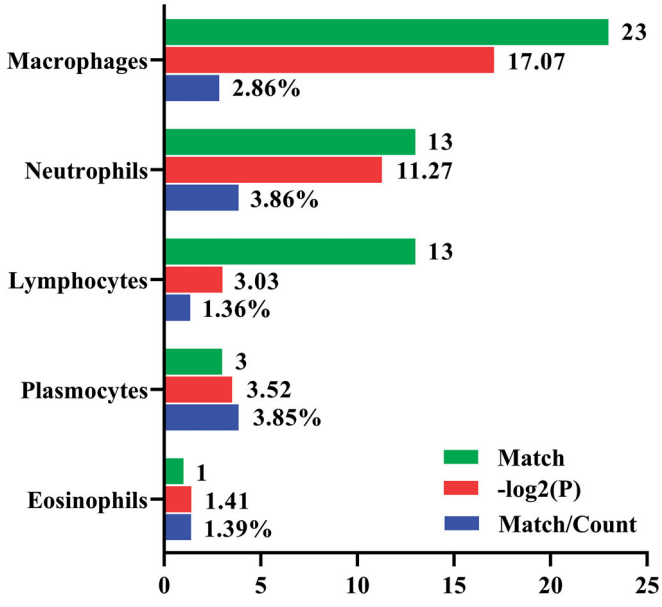

Figure 3. Five kinds of inflammation-related cells associated with LHQW.

Table 4. Biopotency conversion between LHQW (batch) S40 and DXMS.

	LHQW (batch) S40 (reference drug)		DXMS (positive drug)	
No.	Dose/μg·mL^{-1}	Inhibition rate/%	Dose/ng·mL^{-1}	Inhibition rate/%
1	50.00	6.94	1.00	7.68
2	100.00	33.87	2.00	21.59
3	200.00	41.95	4.00	35.96
4	400.00	75.25	8.00	59.86

Macrophages and their role in LHQW against inflammation

The enrichment analysis of putative targets was carried out, and the corresponding *p*-values were calculated by the hypergeometric distribution. The results show that the matching target number of macrophages was the largest (Figure 3), which indicates that macrophages may be the main functional cells for LHQW against inflammation.

Optimisation of the inflammatory model

As shown in Figure 4(A), the expression of IL-6 increased over time, and the peak was at 24 h. With LPS stimulation alone, the

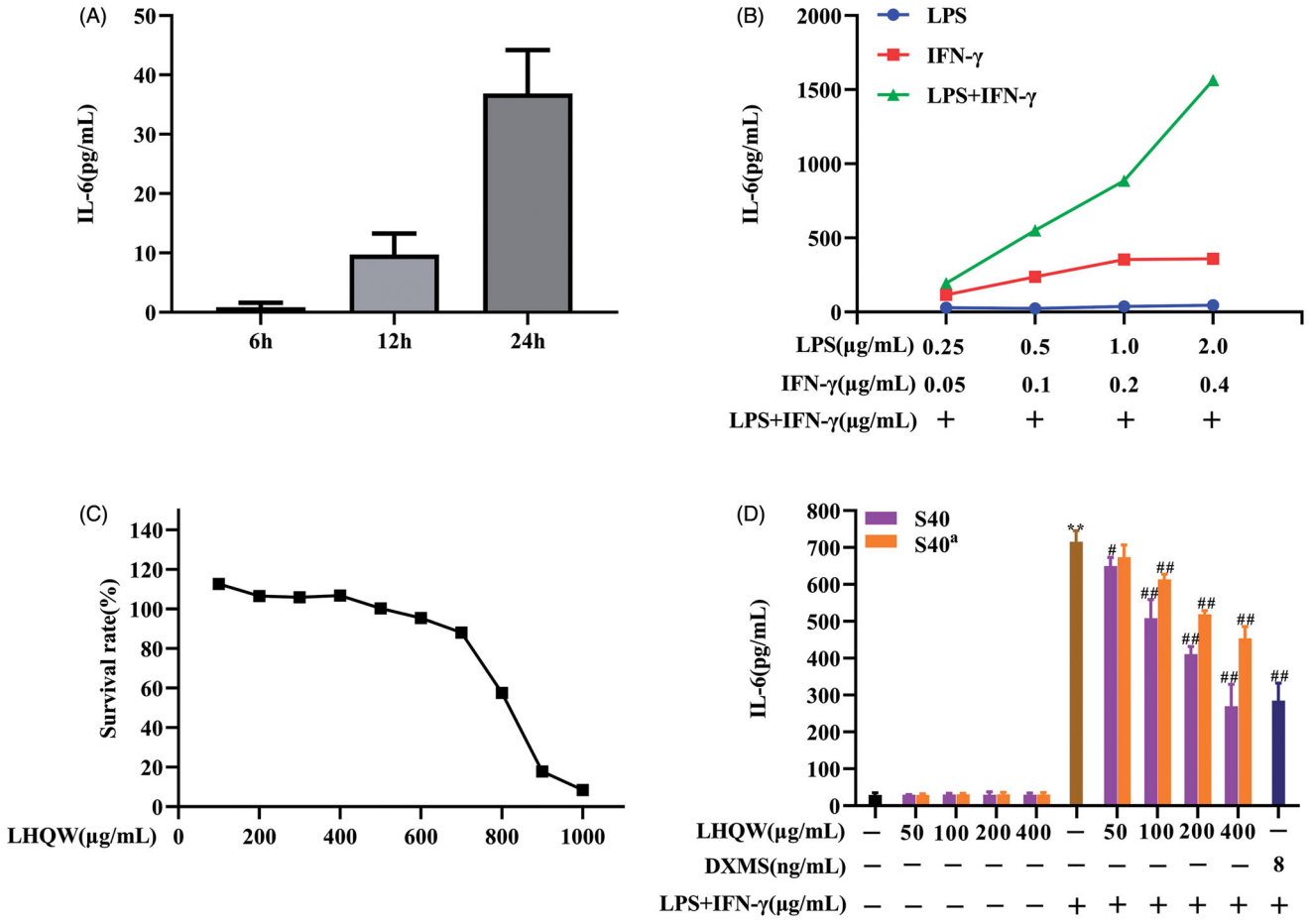

Figure 4. LHQW inhibited IL-6 expression in RAW 264.7 cells. (A) The cells were treated with LPS (1 μg/mL) for 6, 12 and 24 h. (B) The influence of LPS (0.25, 0.5, 1.0 and 2.0 μg/mL), IFN-γ (0.05, 0.1, 0.2 and 0.4 μg/mL), and the combined stimulation on the expression of IL-6. (C) Cell viability in different concentrations of LHQW (100–1000 μg/mL). (D) Effects of LHQW (containing marketed and destructed sample) on the expression of IL-6. S40 represent the marketed sample, and S40^a represent the destructed sample with high temperature. $^{**}p < 0.01$ *vs.* control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. model group.

expression of IL-6 was below 50 pg/mL. While IFN-γ stimulation, the expression of IL-6 increased compared to that of LPS, and the expression was stable (Figure 4(B)). The expression of IL-6 after combination stimulation was higher than that of LPS alone and LPS (1.0 μg/mL) combined with IFN-γ (0.2 μg/mL), while that of LPS (2.0 μg/mL) combined with IFN-γ (0.4 μg/mL) was beyond the linear range. Therefore, LPS (1.0 μg/mL) combined with IFN-γ (0.2 μg/mL) were combined for 24 h.

Experimental validation

The cell survival experiments showed that IC_{50} was 799.8 μg/mL, and the maximal non-toxic concentration was 500 μg/mL (Figure 4(C)). Therefore, LHQW with 50, 100, 200 and 400 μg/mL were selected for further study. As displayed in Figure 4(D), compared to the control group, the expression of IL-6 was no significant in the LHQW-control group (all $p > 0.05$). The expression of IL-6 in the model group ($p < 0.01$) was significantly higher than that in the control group, which indicates that the inflammation model met the experiment. The LHQW-treated group and DXMS groups had a significant dose-dependent inhibitory effect on the expression of IL-6 (all $p < 0.05$). These results suggest that LHQW has obvious anti-inflammatory activity.

Method validation of bioassay

There were good linear regressions for the expression of IL-6 at 15.6–1000 pg/mL. The calibration curve was $Y = 0.0015X - 0.0077$ ($r = 0.9999$), which indicates that the method had good linearity. The average inhibitory rates of intra-day precision, inter-day precision and repeatability were 61.29, 64.74 and 64.53% and the relative standard deviations (RSDs) were 4.41, 13.24 and 10.44%, respectively. The RSDs were all less than 15%. Therefore, it is validated that the precision and repeatability met bioassay requirements.

Biopotency analysis of LHQW

After coordinate transformation of the inhibition rate and dose, as shown in Table 4, the effects of 1 U S40 was equivalent to 0.0194 ng of DXMS. As shown in Figure 5(A,B), both deviations from the straight line and parallel line were greater than 0.05, indicating that the reference and test samples had good linear relationships. As shown in Figure 5(C), there are obvious differences in biopotency of marketed samples. The potency ranged from 404 to 2171 U/μg, while the average potency was 954 U/μg, and the coefficient of variation (C.V) was 37.91%. These findings show that the quality of marketed samples have obvious

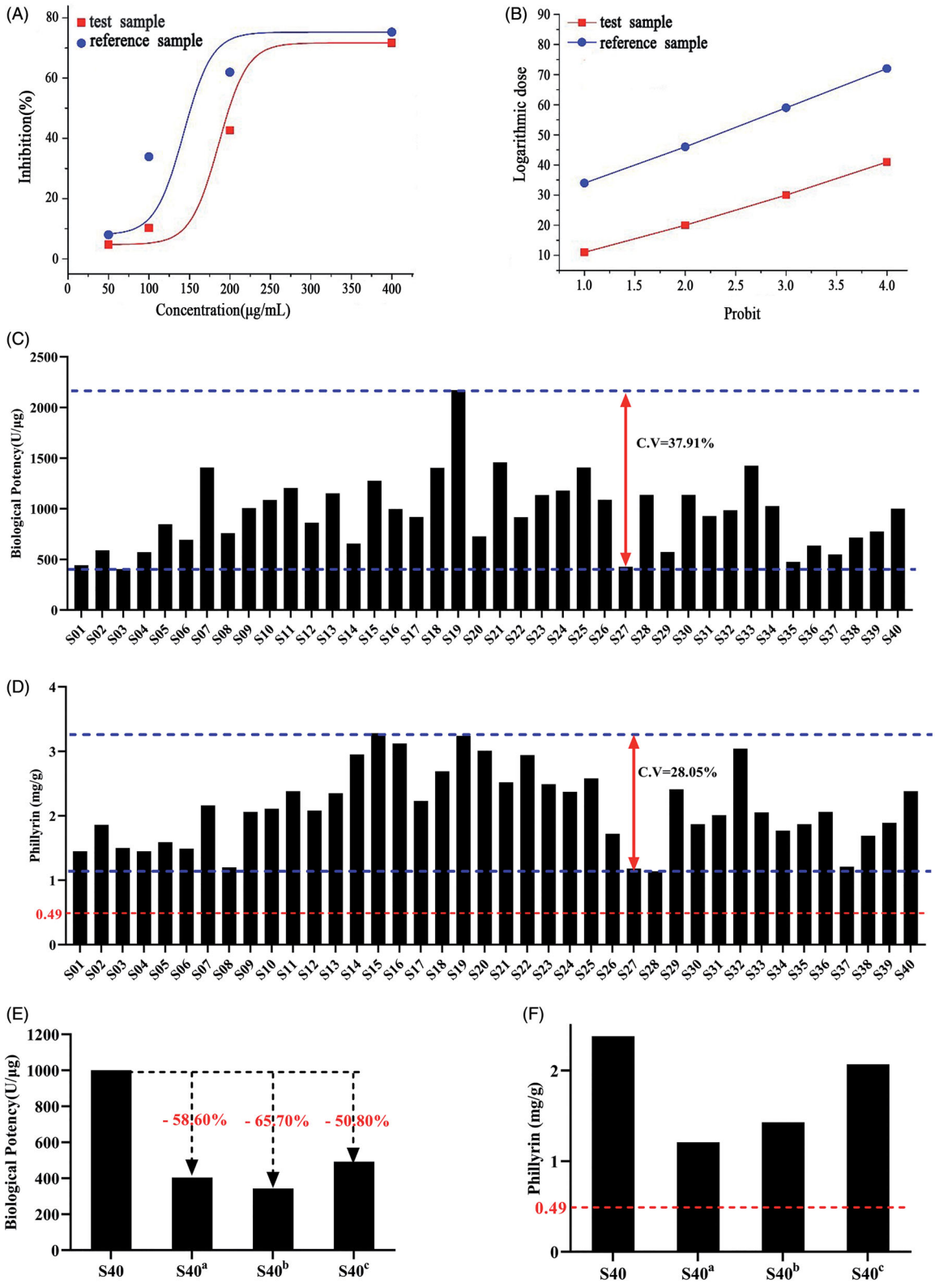

Figure 5. Validation of bioassay of IL-6 for LHQW. (A) The relationship between concentration and inhibition rate. (B) The relationship between logarithmic doses and probit. (C), (D) The biopotency and content of forsythin in 40 batches of marketed samples. (E), (F) The biopotency and content of forsythin in S40, $S40^a$, $S40^b$ and $S40^c$. S01-S40 represent the marketed samples, and $S40^a$, $S40^b$ and $S40^c$ represent the destructed samples with high temperature, high humidity and light intensity. The biopotency of other samples was calculated based on that of S40. CV: coefficient of variation.

variations. Our previous study (Gao et al. 2020) found that the C.V of chemical evaluation was 28.05% (Figure 5(D)), suggesting that bioassays can more sensitively characterise the quality variations of LHQW. Furthermore, compared with the marketed sample (S40), the forsythin content of $S40^a$, $S40^b$ and $S40^c$ was also up to standard (Figure 5(E)). However, the anti-inflammatory activity of $S40^a$, $S40^b$ and $S40^c$ decreased by 58.60, 65.70 and 50.80%, respectively (Figure 5(F)). These findings

demonstrate that it is difficult for chemical component to reflect quality damage, but bioassays can more effectively distinguish the quality of marketed samples and destructed samples.

Discussion

Regarding the selection of bioassay model for LHQW, we have some considerations. Firstly, the FDA's requirements for LHQW bioassay fell in anti-inflammation. When LHQW was approved for clinical trials by FDA in the United States, it was to improve influenza-related symptoms, rather than directly inhibit virus replications. Secondly, from the perspective of improving influenza-related symptoms, the core effects of LHQW are to improve the inflammation-mediated symptoms, which have been illustrated in the literature (Dong et al. 2014; Ding et al. 2017; Gao et al. 2020; Li et al. 2020). Therefore, we selected anti-inflammatory effects for evaluating the quality of LHQW.

Based on the network pharmacology approach, we found that TNF/IL-6 signalling pathway may be the most important one for LHQW against inflammation. IL-6 is a downstream effector directly involved in inflammatory reaction, which can directly lead to flu-related symptoms. IL-6 is an important medium that causes fever and acute-phase reaction (Wu et al. 2010; Lee et al. 2011; Nakajima et al. 2013), as well as the primary cause of symptom formation (Svitek et al. 2008; McGill et al. 2009). IL-6 is closely related to the pathology of respiratory inflammatory diseases (Ullah et al. 2015; Jevnikar et al. 2019). Furthermore, IL-6 has also been implicated in the severity, diagnosis, and treatment of COVID-19 (Herold et al. 2020; Liu et al. 2020; Zhang et al. 2020). Therefore, IL-6 can be used as an important candidate biomarker for the bioassay of LHQW. Then, this target has been validated in macrophage cell line (RAW 264.7) -based *in vitro* cell model. We thus chose RAW 264.7 cells as a model for *in vitro* bioassay of LHQW quality.

Thus, we established an anti-inflammatory bioassay for LHQW. Methodology research showed that the new quality control method was reliable. We then compared the differences between bioassay and chemical evaluation for evaluating the marketed and destructed samples of LHQW. For marketed samples, bioassay can more sensitively reflect quality variations as compared to chemical evaluation. Additionally, bioassay confirmed that the destructed samples were significantly damaged during chemical evaluation. This finding shows that in marketed or destructed samples, the minimum content of forsythin reflects the quality of LHQW. Chemical evaluation mainly focuses on the authenticity of the sample quality, while bioassay focuses more on the sample quality (Xiong et al. 2014). In summary, bioassays not only sensitively reflect the quality variations of marketed samples but also effectively distinguish the quality of marketed and destructed samples.

Therefore, bioassays are helpful to select optimal samples for clinical implementation. At the same time, they contribute to monitoring the influencing factors during preparation production, carrying, storage, transportation and application, including tracing the source and batch of Chinese medicinal materials. This includes adopting moisture-proof packaging materials and outer packaging, control of temperature and light. In summary, bioassay is a relatively simple model, an easily quantified index and fills the gaps of single chemical indexes to evaluate quality. However, our research was not designed to determine the reproducibility, standardisation and accuracy of bioassay, which should be addressed in future studies.

Conclusions

LHQW, a complex TCM formula, has good potential in the treatment of viral respiratory diseases. However, a single quality control method may prove deficient and may not meet the clinical needs for evaluating the efficacy of LHQW. In this study, network pharmacology showed that IL-6 and macrophages played an important role in LHQW against inflammation. Based on this, an anti-inflammation bioassay was established to distinguish the quality variance of LHQW and to improve its quality assurance ability. This study uncovered the feasibility and robustness of using bioassays in the evaluation of TCM preparations.

Author contribution

All data were generated in-house. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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