Research Article

Huanglian Jiedu Decoction Exerts Antipyretic Effect by Inhibiting MAPK Signaling Pathway

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Aim. The aim of this study was to explore the antipyretic effect and potential mechanism of Huanglian Jiedu Decoction (HLJDD) on LPS-induced fever in rats. Materials and Methods. The fever rat model was established by LPS. Anal temperature of rats was measured every 1 hour after modeling. TNF-α, IL-6, PGE2, and cAMP in rat serum or hypothalamus tissue were detected by ELISA kit. In order to explore the potential active ingredients and mechanism of antipyretic effect of HLJDD, we predicted the underlying antipyretic mechanism by using network pharmacology and then verified its mechanism by Western Blotting. Results. The results showed that HLJDD can alleviate LPS-induced fever in rats. The expression levels of TNF-α, IL-6, PGE2, and cAMP in the treatment group were significantly lower than those in the model group. Western Blotting results showed that the protein expression of p-ERK, p-JNK, and p-P38 was significantly inhibited. Conclusion. The findings suggest that HLJDD has a good antipyretic effect on LPS-induced fever in rats, which may be closely related to the inhibition of MAPK signaling pathway.

1. Introduction

Fever is a complex physiological stress response characterized by a regulatory rise in body temperature in response to inflammation or infectious disease [1]. Normally, the body maintains a dynamic balance between heat production and heat loss. When this balance is upset, the body temperature becomes abnormal. Fever is a controlled increase in body temperature, a hypothalamic-mediated response caused by pathogenic injury or invasion [2, 3]. This reaction promotes the synthesis of endogenous heat-producing factors, such as TNF-α, IL-6, PGE2, and cAMP, which will cause a series of biochemical and physiological changes in the body and eventually lead to elevated body temperature [4–7]. Hyperthermia is a treatment method that heats the temperature of a specific part of the body or the whole body to above the normal body temperature, so as to achieve the therapeutic effect. Traditional Chinese medicine (TCM) has heat therapy, such as sweat steaming, moxibustion, and cupping. Modern research shows that the combination of hyperthermia and chemotherapy is more effective than chemotherapy alone in the treatment of cancer diseases [8]. Studies have also found that increased body temperature regulation may be beneficial to the improvement of human immunity and reduce the sensitivity to infectious diseases [9]. However, in addition to being good for the body, fever can also be harmful [10]. For example, uncontrolled fever is associated with worse outcomes in patients with sepsis or neuronal damage [11].

TCM is a great treasure house, and it has unique advantages in the treatment of fever diseases. Chinese medicine
classifies fever into two categories: fever due to external sensation and fever due to internal injury. External fever is caused by the feeling of external evil. Internal fever is caused by the imbalance of Yin, Yang, Qi, and blood in the internal organs. In external fever, the onset is rapid and the duration is short. In internal fever, the onset is slow and the duration is long, from weeks and months to years. According to the principles of TCM diagnosis and treatment, TCM treatment of fever includes the method of relieving symptoms and reducing fever, the method of dispelling dampness and reducing fever, and the method of nourishing Yin and reducing fever. Huanglian Jiedu Decoction (HLJDD), which originated from Medical Secrets of an Official (Wai Tai Mi Yao as named in Chinese) in the Tang Dynasty, was created by Tao Wang, a famous medical scientist in the Tang Dynasty. The original prescription of HLJDD is composed of Coptidis Rhizoma, Scutellariae Radix, Phellodendri Chinensis Cortex, and Gardeniae Fructus in the ratio of 3:2:2:3, which has the effect of clearing heat and removing toxicity. Clinical evidence suggests that HLJDD can be used in the treatment of a variety of diseases, which can relieve symptoms with a good clinical effect [12]. Pharmacological studies show that HLJDD has significant anti-inflammatory, antibacterial, and antiendotoxin activities [13, 14]. It can be used to treat high fever in children [15], sepsis, and other diseases [16].

Lipopolysaccharide (LPS), commonly known as endotoxin, is an important cell wall component of Gram-negative bacteria [17]. The proinflammatory effects of LPS play an important role in inhibiting bacterial infection. However, dysregulation of the host response to LPS may lead to systemic inflammation, such as sepsis [18]. In recent years, LPS is often used to establish animal models of fever [19–21]. Studies have shown that MAPK signaling pathway plays a key role in regulation of the production of proinflammatory mediators in LPS-induced inflammatory responses [22–24]. However, the effects of HLJDD in LPS-induced fever and the relationship between HLJDD and MAPK signaling pathways are still unclear.

TCM has multicomponent, multitarget, and multipathway characteristics. The therapeutic mechanisms and material basis of many herbal medicines have not been elucidated. With the rapid development of bioinformatics and various medical databases, network pharmacology has strongly contributed to the understanding of the molecular mechanisms of TCM from a holistic and systemic perspective [25]. Meanwhile, it has great advantages in predicting the target of TCM components, discovering multitarget drugs and providing new insights for the study of TCM [26]. HLJDD is a classical Chinese medicine formula for relieving fever and is also commonly used in Chinese medicine clinics. Its antipyretic effect is remarkable, but its antipyretic mechanism has not been completely elucidated. In order to initially explore this problem, this study was conducted to comprehensively evaluate the antipyretic effect of HLJDD by establishing an animal model of fever, combined with modern bioinformatics technology, aiming to provide ideas for the development of new drugs for the efficient, safe, and rapid treatment of fever symptoms.

Meanwhile, it aimed to provide reasonable dosing guidance and lay an experimental foundation for the clinical application of HLJDD.

2. Materials and Methods

2.1. Drugs and Reagents. Coptidis Rhizoma (batch number: 20121701), Scutellariae Radix (batch number: 19101501), Phellodendri Chinensis Cortex (batch number: 19120601), and Gardeniae Fructus (batch number: 18091101) were purchased from Beijing Lyce Pharmaceutical Co., Ltd. (Beijing, China), and chemically authenticated by thin layer chromatography (TLC) in accordance with the instructions of Chinese Pharmacopoeia. The content was determined by high performance liquid chromatography (HPLC). The product inspection report numbers are CP-20-12-20, CP-19-10-07, CP-19-12-05, and CP-18-09-09, respectively. All detection results show that the quality of Coptidis Rhizoma, Scutellariae Radix, Phellodendri Chinensis Cortex, and Gardeniae Fructus is in full compliance with the regulations of the Chinese Pharmacopoeia version 2015. LPS from Escherichia coli 0111:B4 purified by phenol extraction was purchased from Sigma (batch number: 000010267).

2.2. Preparation of HLJDD. Weigh Coptidis Rhizoma, Scutellariae Radix, Phellodendri Chinensis Cortex, and Gardeniae Fructus at 3:2:2:3 and soak 30 min in pure water (1/10, w/v). Then it was extracted twice by heating (1 h at a time) [27]. After filtering and drying, the yield of HLJDD was 17.90%.

2.3. Ethics Statement. This study was carried out in accordance with the recommendations of the Guidelines for the Care and Use of Laboratory Animals of the Ministry of Science and Technology of China. All operations related to animal experiment are examined and approved by the Animal Ethics and Experimental Committee of the Fifth Medical Center of the PLA General Hospital (Approval ID: IACUC-2019-004).

2.4. Animals. 50 male Sprague-Dawley rats (weighing 180 g–200 g) were purchased from Spiffy Biotechnology Co., Ltd. (Beijing, China, Permission No. SCXK-(Jing) 2019-0010). They were fed adaptively for 7 days under the conditions of temperature 25 ± 0.5°C, relative humidity 55 ± 5%, and alternating light (12 h light/dark cycle) and with free access to sufficient food and water. The anal temperature of rats was measured for 3 consecutive days, and the average value was taken as the baseline temperature of rats.

2.5. Establishment of LPS-Induced Fever Model and Drug Administration. The animals were randomly divided into 5 groups (n = 10), including control group, LPS-induced fever model group (100 μg/kg, i.p.), HLJDD low dose group (1.58 g/kg), HLJDD medium dose group (3.15 g/kg), and HLJDD high dose group (6.30 g/kg). The administered medium dose of HLJDD was determined based on the
recommended human dose (30 g/60 kg/day). The HLJDD groups were orally given different doses of HLJDD 1 h before the injection of LPS. At the same time, the control group were orally given with the same volume of normal saline. 1 hour after drug administration, the model group and each HLJDD group were intraperitoneally injected LPS (100 µg/kg) to establish fever rat model. Then the anal temperature of the rats was measured every 1 hour. The last anal temperature was measured at 7 hours, the rats were euthanized and blood was taken through the abdominal aorta, and then the hypothalamus tissue was collected for subsequent studies.

2.7.4. GO Enrichment and KEGG Pathway Analysis. The database (DAVID) (https://david.ncifcrf.gov/) has annotation, visualization, and integrated discovery capabilities. We therefore applied the DAVID database for gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis.
3.3. Prediction Results of Antipyretic Effect of HLJDD

3.3.1. Compound-Target Network and Analysis. Due to the characteristics of multiple components and multiple targets, TCM compounds show a variety of pharmacological activities. Therefore, we constructed a network to study the potential mechanism of TCM compounds treating diseases. As shown in Figure 3, there are 257 nodes with 792 edges. Among these active components, we screened 20 components with high degree value, such as quercetin (MOL000098, degree = 127), kaempferol (MOL000422, degree = 50), wogonin (MOL000173, degree = 37), and bai-calein (MOL002714, Degree = 29). These components with high degree value in the network are likely to be the main active components of HLJDD (Table 2).

3.3.2. The PPI Network Construction of the Underlying Antipyretic Targets. A total of 64 chemical ingredients and 193 targets of HLJDD were collected and 939 targets of fever were obtained from GeneCard database (Figure 4(a)). These 70 intersection targets may be potential targets of antipyretic effect of HLJDD. The intersection targets were imported into STRING database. After that, PPI network of the underlying antipyretic targets was constructed by Cytoscape3.8.0 with 63 nodes and 455 edges (Figure 4(b)).

3.3.3. GO Enrichment and KEGG Pathway Analysis. The top 10 significantly enriched terms in biological process (BP), cellular component (CC), and molecular function (MF) categories are shown in Figure 5, which indicated that HLJDD may exert its antipyretic effect by regulating positive regulation of nitric oxide biosynthetic process, cellular response to organic cyclic compound, extracellular space, and identical protein binding. In order to explore the potential pathways involved in the antipyretic effect of HLJDD, we conducted KEGG pathway analysis, as shown in Figure 6, with 15 top signaling pathways. Among these 15 signaling pathways, MAPK signaling pathway plays a crucial role.

3.4. Experimental Verification on the Mechanism of HLJDD against LPS-Induced Fever. To further evaluate the underlying antipyretic mechanism of HLJDD, MAPK signaling pathway was determined. As shown in Figure 7, the expression of ERK, p-ERK, JNK, p-JNK, P38, and p-P38 was detected and the results of Western Blotting were quantified by ImageJ. The results showed that, compared with the control group, the protein expression levels of p-ERK, p-JNK, and p-P38 were significantly increased after LPS injection ($P < 0.05$ or $P < 0.01$). HLJDD could downregulate their protein expression levels, which indicated that HLJDD has antipyretic effect on LPS-induced fever in rats through MAPK signaling pathway suppressing.

Table 1: Antibodies information.

| Antibodies          | Dilution | Manufacturers          | Cat. No. |
|---------------------|----------|------------------------|----------|
| Rabbit anti-Erk1/2  | 1:1000   | Cell Signaling Technology | 4695     |
| Rabbit anti-p-Erk1/2| 1:2000   | Cell Signaling Technology | 4370     |
| Rabbit anti-JNK     | 1:1000   | Cell Signaling Technology | 9252     |
| Rabbit anti-p-JNK   | 1:1000   | Cell Signaling Technology | 4668     |
| Rabbit anti-p38     | 1:1000   | Cell Signaling Technology | 8690     |
| Rabbit anti-p-p38   | 1:1000   | Cell Signaling Technology | 4511     |
| Rabbit anti-GAPDH   | 1:10000  | Proteintech            | 10494-1-AP|
| Goat anti-Rabbit IgG| 1:10000  | ZENBIO                 | 511203   |

Figure 1: Changes of anal temperature in each group. The temperature at 0 h was the baseline temperature of rats, and the anal temperature was measured every 1 hour after LPS modeling. Control: as a blank control group; Model: intraperitoneal injection of LPS 100 μg/kg; HLJDD L: HLJDD low dose group (1.58 g/kg); HLJDD M: HLJDD medium dose group (3.15 g/kg); HLJDD H: HLJDD high dose group (6.30 g/kg).

4 Evidence-Based Complementary and Alternative Medicine
4. Discussion

Our study shows that HLJDD has a good antipyretic effect on LPS-induced fever in rats, and this effect may be carried out by inhibiting the MAPK signaling pathway.

HLJDD is a classic Chinese medicine prescription for clearing heat and detoxifying [28]. Previous studies have shown that HLJDD significantly reduces the levels of inflammatory factors such as IL-2, TNF-α, and IFN-γ and inflammatory mediators such as PGE₂ and NO and suppresses immune and inflammatory responses [29, 30]. We studied the pharmacological effects of HLJDD (composed of Coptidis Rhizoma, Scutellariae Radix, Phellodendri Chinensis Cortex, and Gardeniae Fructus at 3 : 2: 2: 3) on LPS-induced fever in rats. At present, there are many methods to replicate the rat model of fever; the common ones are dry yeast, 2,4-dinitrophenol, and lipopolysaccharide. LPS, the outer membrane of Gram-negative bacteria, is a common febrifuge in animal experiments and stimulates macrophages and neutrophils to produce the endogenous pyrogens [31]. In this experiment, the rat fever model was established by intraperitoneal injection of LPS (100 μg/kg). HLJDD L: HLJDD low dose group (1.58 g/kg); HLJDD M: HLJDD medium dose group (3.15 g/kg); HLJDD H: HLJDD high dose group (6.30 g/kg).

![Graphs showing effect of HLJDD on serum and hypothalamus tissue biochemical indexes of fever rats.](image)

Figure 2: Effect of HLJDD on serum and hypothalamus tissue biochemical indexes of fever rats. (a-b) Expression of TNF-α and IL-6 in serum of fever rats. (c-d) Expression of PGE₂ and cAMP in hypothalamus tissue of fever rats. ** <0.01 versus control group. ## <0.01 versus model group, * <0.05 versus model group. Control: as a blank control group; Model: intraperitoneal injection of LPS 100 μg/kg; HLJDD L: HLJDD low dose group (1.58 g/kg); HLJDD M: HLJDD medium dose group (3.15 g/kg); HLJDD H: HLJDD high dose group (6.30 g/kg).
aglycone, baikalein, are the main components in pharmacodynamic components of HLJDD. Baicalin and its kaempferol, wogonin, and baikalein might be the main network pharmacology analysis, we found that quercetin, core targets and predict the possible pathways. through construct a component target network, and then screen themainpharmacodynamiccomponentsofHLJDD,further

Figure 3: Herb-compound-target network of HLJDD (the ellipses represent components of HLJDD, the 3 yellow ellipses represent Coptidis Rhizoma components, the 27 blue ellipses represent Scutellariae Radix components, the 15 pink ellipses represent Phellodendri Chinensis Cortex components, the 9 orange ellipses represent Gardeniae Fructus components, the 10 purple ellipses represent the shared components in the four herbs of HLJDD, and the green round rectangles represent the 193 potential targets of HLJDD).

cytopskeleton proteins and enzymes, and is closely related to the occurrence of many diseases such as inflammation and tumor [33]. The MAPK pathway has four major branching routes, including ERK, JNK, p38/MAPK, and ERK5. Each MAPK signaling pathway has a relatively independent function. An important role of MAPK signaling pathway is to regulate cellular responses in response to changes in the extracellular environment. ERK pathway is mainly involved in cell proliferation and differentiation, while JNK pathway and p38MAPK pathway are mainly involved in cellular inflammatory response, stress response, and apoptosis [34]. Since MAPK signaling pathway is a classical inflammatory pathway, in addition, we referred to the predicted results of this network pharmacology and selected MAPK signaling pathway, using WB experiments as experimental validation. It was found that the administration of HLJDD reduced the expression levels of phosphorylated proteins of ERK, JNK, and p38 in different dose groups. In particular, the high dose group of HLJDD significantly inhibited the expression of related proteins in MAPK signaling pathway. In summary, HLJDD inhibits the protein expression level in MAPK signaling pathway, thus playing the role of antipyretic and relieving inflammation.
| Molecule ID | Molecule name | OB (%) | DL | Degree | Molecule structure | Herb |
|-------------|--------------|--------|----|--------|--------------------|------|
| MOL000098   | Quercetin    | 46.43  | 0.28 | 127    | ![Quercetin structure](image) | Coptidis Rhizoma, Scutellariae Radix, Phellodendri Chinensis Cortex, Gardeniae Fructus |
| MOL000422   | Kaempferol   | 41.88  | 0.24 | 50     | ![Kaempferol structure](image) | Gardeniae Fructus |
| MOL000173   | Wogonin      | 30.68  | 0.23 | 37     | ![Wogonin structure](image) | Scutellariae Radix |
| MOL002714   | Baicalein    | 33.52  | 0.21 | 29     | ![Baicalein structure](image) | Scutellariae Radix |
| MOL000358   | Beta-sitosterol | 36.91 | 0.75 | 27     | ![Beta-sitosterol structure](image) | Coptidis Rhizoma, Scutellariae Radix, Phellodendri Chinensis Cortex, Gardeniae Fructus |
| MOL000790   | Isocorypalmine | 35.77 | 0.59 | 24     | ![Isocorypalmine structure](image) | Phellodendri Chinensis Cortex |
| MOL000449   | Stigmasterol | 43.83  | 0.76 | 23     | ![Stigmasterol structure](image) | Coptidis Rhizoma, Scutellariae Radix, Phellodendri Chinensis Cortex, Gardeniae Fructus |
| MOL001455   | (S)-Canadine | 53.83  | 0.77 | 23     | ![Canadine structure](image) | Phellodendri Chinensis Cortex |
| MOL002903   | (R)-Canadine | 55.37  | 0.77 | 22     | ![Canadine structure](image) | Coptidis Rhizoma |
| MOL002670   | Cavidine     | 35.64  | 0.81 | 21     | ![Cavidine structure](image) | Phellodendri Chinensis Cortex |
| Molecule ID   | Molecule name                  | OB (%) | DL  | Degree | Molecule structure | Herb                                      |
|--------------|--------------------------------|--------|-----|--------|-------------------|-------------------------------------------|
| MOL000787    | Fumarine                        | 59.26  | 0.83| 20     | ![Fumarine](image1) | *Phellodendri Chinensis Cortex*            |
| MOL002928    | Oroxylin A                       | 41.37  | 0.23| 19     | ![Oroxylin A](image2) | *Scutellariae Radix*                       |
| MOL001689    | Acacetin                        | 34.97  | 0.24| 19     | ![Acacetin](image3)  | *Scutellariae Radix*                       |
| MOL003095    | 5-Hydroxy-7-methoxy-2-(3,4,5-trimethoxyphenyl)chromone | 51.96  | 0.41| 16     | ![5-Hydroxy-7-methoxy-2-(3,4,5-trimethoxyphenyl)chromone](image4) | *Gardeniae Fructus*                        |
| MOL002651    | Dihydrotanshinone II A           | 43.76  | 0.4 | 15     | ![Dihydrotanshinone II A](image5) | *Phellodendri Chinensis Cortex*            |
| MOL008206    | Moslosooflavone                  | 44.09  | 0.25| 15     | ![Moslosooflavone](image6) | *Scutellariae Radix*                       |
| MOL000228    | Alpinetin                        | 55.23  | 0.2 | 15     | ![Alpinetin](image7) | *Scutellariae Radix*                       |
| MOL002662    | Rutaecarpine                     | 40.3   | 0.6 | 14     | ![Rutaecarpine](image8) | *Phellodendri Chinensis Cortex*            |
| MOL002904    | Berlambine                       | 36.68  | 0.82| 14     | ![Berlambine](image9) | *Coptidis Rhizoma*                        |
| MOL00785     | Palmatine                        | 64.6   | 0.65| 13     | ![Palmatine](image10) | *Coptidis Rhizoma, Scutellariae Radix, Phellodendri Chinensis Cortex, Gardeniae Fructus* |
| Molecule ID  | Molecule name                  | OB (%) | DL    | Degree | Molecule structure | Herb                                                                 |
|-------------|--------------------------------|--------|-------|--------|--------------------|----------------------------------------------------------------------|
| MOL012266   | Rivularin                      | 37.94  | 0.37  | 13     |                    | *Scutellariae Radix*                                                  |
| MOL002934   | Neobaicalein                   | 104.34 | 0.44  | 13     |                    | *Scutellariae Radix*                                                  |
| MOL001454   | Berberine                      | 36.86  | 0.78  | 12     |                    | *Coptidis Rhizoma, Scutellariae Radix, Phellodendri Chinensis Cortex, Gardeniae Fructus* |
| MOL000552   | 5,2’-Dihydroxy-6,7,8-trimethoxyflavone | 31.71  | 0.35  | 12     |                    | *Scutellariae Radix*                                                  |
| MOL002927   | Skullcapflavone II             | 69.51  | 0.44  | 12     |                    | *Scutellariae Radix*                                                  |

Figure 4: The network and analysis. (a) Distribution of HLJDD targets and the disease targets. (b) The PPI network. The higher the degree value is, the darker and larger the nodes in the graph will be.
Figure 5: Top 10 GO terms of hub genes.
Figure 6: Top 15 KEGG pathway of hub genes.

Figure 7: Continued.
5. Conclusion

In this study, we investigated the antipyretic effects of HLJDD at the overall animal level by constructing a febrile rat model and combined with network pharmacology techniques to detect the serum levels of the pyrogenic factors IL-6 and TNF-α and the biochemical indicators PGE2 and cAMP in the hypothalamus of the model animals. The expression of ERK, p-ERK, JNK, p-JNK, P38, and p-P38, which are related proteins of MAPK signaling pathway, was analyzed in the hypothalamus. The results of this study suggest that HLJDD has antipyretic effect on LPS-induced fever in rats, and its potential mechanism may be related to the inhibition of MAPK signaling pathway. This study lays a theoretical foundation for further study of HLJDD in the treatment of fever.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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