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Effects of Huanglian Jiedu Decoction (HLJDD) on fever induced by lipopolysaccharide and inflammation induced by carrageenan

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This study aimed to investigate the antifebrile and anti-inflammatory effect of Huanglian Jiedu Decoction (HLJDD). The rabbits received intravenous injection of lipopolysaccharide (LPS) after orally administered with HLJDD and the rectal temperatures of rabbits were monitored. The concentrations of interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) in serum were assayed using rabbit IL-1β and TNF-α enzyme-linked immunosorbent assay (ELISA) kits. Carrageenan-induced paw edema in rats and the anti-inflammatory effects of HLJDD were also explored. HLJDD treatment group resulted in a significant fall in body temperature in biphasic fever peak ($p < 0.05$), and there were no significant differences between HLJDD treatment group and Ibuprofen (IBR) treatment group in biphasic fever peak at time 1 and 3 h ($p > 0.05$). HLJDD treatment group reduced the concentrations of IL-1β in serum at time 1 and 3 h to control febrile responses. Moreover, paw edema of carrageenan-treated rats were significantly attenuated in rats pretreated with HLJDD. Mechanistic studies showed that HLJDD effectively decreased expressions of COX-2 and inducible nitric oxide synthase (iNOS) proteins. These results suggested that HLJDD would be a valuable candidate for further investigation as a new anti-arthritic drug.

Key words: Lipopolysaccharide, Huanglian Jiedu Decoction (HLJDD), anti-inflammatory, interleukin-1 beta, tumor necrosis factor alpha.

INTRODUCTION

Infection and inflammation result in a number of metabolic changes that are often characterized by negative energy balance, increased thermogenesis and anorexia (Johnson et al., 1998). In experimental animals, these changes can be induced by exposure to the bacterial cell wall product lipopolysaccharide (LPS), which suppresses appetite and triggers a number of other behavioral responses including sleepy, general malaise and fever as part of the brain-coordinated host defensive mechanisms. These responses are mediated by cytokines, such as interleukin-1 (IL-1) (Dinarello et al., 1987) and tumor necrosis factor (TNF), and are the most likely candidates for endogenous pyrogen. Nonsteroidal anti-inflammatory drugs (NSAIDs) and immunosuppressants are commonly prescribed in clinical medication.

However, some of them can cause serious adverse effects such as gastric mucosal damage, water and salt retention and carcinomas. Thus, alternative agents with less severe side effects are required, and botanical products are important candidates.

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Huanglian Jiedu Decoction (HLJDD), a traditional Chinese medicine (TCM), had been described in “Waitai Miyao” a classical piece of TCM literature of the Tang dynasty (about 752 a.d.), and consists of four herbs, namely, *Coptidis rhizoma*, *Scutellariae radix*, *Phellodendri cortex* and *Gardeniae fructus*. HLJDD has been used as a therapy for various clinical symptoms associated with gastrointestinal disorders, inflammation and cardio-vascular diseases for one thousand years (Ohta et al., 1999; Wang and Mineshita, 1996). It was found to exert a preventive effect on the development of stress-induced acute gastric lesions in rats due to an inhibitory action on tissue neutrophil infiltration. HLJDD had protective effects against the impairment of learning and memory induced by transient cerebral ischemia in rat (Xu et al., 2000).

In the present study, we investigated the effect of HLJDD on fever induced by the LPS model in rabbit and the role of HLJDD upon inflammation.

MATERIALS AND METHODS

Animals

Female New Zealand white rabbits weighing 2.0 to 3.0 kg and Ihara's cataract rat (ICR) male rat weighing 20 ± 2 g were obtained from the Laboratory Animal Services Center, Liaoning University of TCM, and maintained in plastic cages at 21 ± 2°C with free access to pellet food and water. They were kept on a 12 h light: 12 h dark cycle. All animals used in this experiment were cared according to the ethical regulations on animal research of our university.

Regents and preparation of herbal extract

LPS from *Escherichia coli* 0127:B8 was purchased from Sigma Aldrich (China Mainland). Rabbit IL-1β and TNF-α ELISA kits were purchased from Shanghai Lang-dun Biologic Technology Company. Preparation of herbal extract was defined according to ancient records. The ingredients of the HLJDD (1000 g) were *C. rhizoma* (300 g), *S. radix* (200 g), *P. cortex* (200 g) and *Gardeniae fructus* (300 g). The herbs and ibuprofen were purchased from the Tong Ren Tang Medicinal Materials Company of Shenyang, China. The herbs were immersed in distilled water (1,500 ml), and boiled for 20 min up to the volume of the material until 200 ml was left. The extract obtained was filtered, and then stored at -20°C until use. Ibuprofen was made in the third plant of Harbin Pharmaceutical Industry (Harbin, China), and the lot number is, 080135. Other drugs and reagents, carrageenan and Tween 80, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The HLJDD groups were orally administered with HLJDD 5.25 g/kg for rabbits after intravenous injection of LPS and in the carrageenan-induced paw edema rats were orally administered with HLJDD 18.75 g/kg for rats.

Chromatographic system and conditions

High performance liquid chromatography (HPLC) analysis was carried out on an Agilent 1100 series HPLC (Palo Alto, CA, USA) incorporating an ultra violet (UV) detector. The analytes were determined at room temperature on an analytical column (Diamonsil C18, 150 × 4.6 mm, i.d., 5 µm) (Dikma Technologies, Beijing, China). The mobile phase consisted of a mixture of acetonitrile-1% aqueous phosphoric acid (28:72, v/v). The mobile phase was passed under vacuum through a 0.45 µm membrane filter before use. The analysis was carried out at a flow rate of 1 ml/min with the detection wavelength set at 280 nm.

Measurement of changes in body temperature

The rectal temperatures of rabbits were monitored by a copper-constantan thermocouple for 6 h after an intravenous injection of LPS, and the rectal temperature change (ΔT) was calculated by subtracting the temperature before the injection from the temperature at each time point. Before the formal experiment, the body temperatures of rabbits were monitored for 2 days and the basic temperatures were noted. On the day of the body temperature experiment, animals were minimally restrained in conventional rabbit stocks, at an ambient temperature of 21 ± 1°C between 09:00 and 16:00. Throughout the experiment, the rectal temperature was measured every 20 min with a copper-constantan thermocouple. The rectal temperature in each animal was allowed to stabilize for at least 90 min before any injections were made. The intravenous injection of LPS was made into the marginal ear vein.

Drug treatment and tissue preparation

All rabbits (n = 60) were randomly divided into three parts of experiments, and the rabbits (n = 20) of each part experiment were separated into four groups: Control (n = 5, orally administered with 0.9% saline solution only), LPS (n = 5, received intravenous injection of LPS only, 200 ng/kg), HLJDD + LPS (n = 5, received intravenous injection of LPS 30 min after orally administered HLJDD, 5.25 g/kg), Ibuprofen + LPS (n = 5, received intravenous injection of LPS 30 min after orally administered with ibuprofen 10 mg/kg). To determine the suitable dosage of HLJDD, the conversion ratio of dosage between person and animals were performed as described elsewhere (Xu, 2009), and pilot studies were performed where HLJDD dosage 5.25 g/kg for rabbits and 18.75 g/kg for rats were used as suitable middle dosage, respectively.

In the first series of experiments, the body temperatures were observed for 6 h only to prove the antifebrile effect of HLJDD. In the second and third series of experiments, we observed the body temperature for 1 and 3 h, respectively. Then the blood samples were collected in tubes from the ear center artery of rabbits and centrifuged after depositing for 2 h. The blood serum was stored at -80°C for IL-1β and TNF-α activity assays. In carrageenan-challenged paw edema of rats, paw soft tissues were removed from individual rat and homogenized in Radioimmunoprecipitation assay (RIPA) buffer and incubated at 37°C on a rotator for 2 h. After washing the plate, biotinylated antibodies were added (100 µl per well) and plates were incubated at 37°C on a rotator for 1 h. Then the plates were incubated for 0.5 h with streptavidin-horseradish peroxidase at a dilution of 1: 20000, and the antibodies were detected with 3,3′,5,5′-tetramethylbenzidine (TMB) dissolved in dimethyl sulfoxide to a concentration of 1% in a solution containing 0.1 M citric acid, 0.1 M sodium acetate (pH 6), and 0.016% H_2O_2 for 30 min. The reaction was stopped by the addition of 1.5 M H_2SO_4. Plates were read using a wavelength (450 nm) on a microplate reader (BIO-RAD iMark^{TM}, Made in Japan 2010, Serial No.12843), and total cytokine concentrations were calculated using the standard curve prepared from recombinant cytokines. The lower limit of detection for the cytokines based on the standard curves ranged from 10 to16 pg/ml. The ELISA for rabbit IL-1β detects both precursor and mature IL-1β but not TNF-α.

Similarly, the ELISA for rabbit TNF-α does not cross-react with rabbit IL-1β. The protocol of the ELISA for rabbit IL-1β is similar to that for rabbit TNF-α. The sensitivities of the assays were: IL-1β, 10 pg/ml and TNF-α, 16 pg/ml.

Enzyme-linked immunosorbent assay (ELISA)

The concentrations of IL-1β and TNF-α in serum were assayed using a commercially available rabbit IL-1β and TNF-α ELISA kits (R&D Systems, Inc., Minneapolis, Minn. Catalog No. F2019; Catalog No. F2041), following the manufacturer's instructions. The plasma samples and working standards were added (100 µl per well) in duplicate and concentrations of cytokines at the site of inflammation compared to those in the circulation, some samples required large dilutions.

Induction and assessment of acute inflammation in rat hind paws by carrageenan

The assay was conducted as previously described by Winter et al. (1962). Oral administration was conducted with 18.75 g/kg doses of HLJDD, the reference drug (Ibuprofen, 10 mg/kg), or the control, at 1 h prior to inflammation induction. At induction of the paw edema, each rat was injected with 0.05 ml freshly prepared carrageenan (1% w/v) in physiological saline (0.9% w/v NaCl) into subplantar tissues of the right hind paw. The left hind paws without injection were used as controls. The volumes (ml) of both hind paws of each animal were measured using a plethysmometer (YLS-7A, Anhui, China) at 1 h before inflammation induction and the time intervals of 1, 2, 3, 4, 5 and 6 h after induction. The rates of increase in paw volume (paw edema) of the right hind paws of rats were calculated by the following equation:

The increase rate (%) = (A - B) / B × 100
where A represents the paw volumes at different time points after injection, and B represents the paw volume before injection. The mean values of the treated animals were compared with the mean values of the control animals, and results were analyzed using statistical methods.

Western blot analysis of iNOS, COX-1, and COX-2 protein expressions

Protein levels of iNOS, COX-2, and COX-1 were assessed by Western blot analysis as previously described (Li et al., 2008). Paw soft tissues were removed from individual rat and homogenized in RIPA buffer containing 50 mM Tris-HCl (pH 7.5), 250 mM NaCl, 5 mM EDTA, 1 mM EGTA, 1 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 20 µg/ml leupeptin, 20 µg/ml aprotinin, 0.1% Triton X-100, and 1% SDS for 1 h. After centrifugation at 15,000 × g at 4°C for 10 min, the supernatants were collected, and the protein amount in each sample was measured by a Bio-Rad DC kit (Bio-Rad, Hercules, CA). The equal amount of sample (50 µg of protein) was subjected to electrophoresis on either 10 or 12% SDS-polyacrylamide gel. Following the electrophoresis, protein blots were transferred to a nitrocellulose membrane. The membrane was blocked with 5% nonfat milk in TBST solution, and incubated with the corresponding primary antibodies in the blocking solution at 4°C for 12 h. After washing three times with TBST solution (10 mmol/L Tris-HCl, pH 7.5, 100 mmol/L NaCl, and 0.1% Tween 20), the membrane was incubated, with horseradish peroxidase-conjugated secondary antibody diluted with TBST solution (1:1000) at room temperature for 1 h. The detected protein signals were visualized by the enhanced chemiluminescence reaction system according to manufacturer's recommendation (Amersham Biosciences, Indianapolis, IN).

Statistical analysis

All results were confirmed in at least three separate experiments. Data were expressed as mean ± standard error of mean (SEM). A one-way analysis of variance (ANOVA) was used for multiple comparisons. A value of $p < 0.05$ was considered significant.

RESULTS

Effects of HLJDD on body temperature during LPS induced fever in rabbits

In the first series of experiment, we injected LPS intravenous at time 1 h after orally administering with HLJDD or ibuprofen, and the changes of body temperature of rabbits were in the Figure 1. Figure 2 showed changes in rectal temperature in the four different groups. Injection of LPS (200 ng/kg) induced a biphasic fever, in which the first peak occurred at 1 h and the second peak occurred at 3 h after injection. In contrast, HLJDD treatment group resulted in a significant fall in body temperature in both peaks ($p < 0.05$), and treatment with ibuprofen also attenuated both peaks of the fever ($p < 0.05$). There were no significant differences between HLJDD treatment group and Ibuprofen treatment group in both peaks ($p > 0.05$). Control animals administered only saline failed to show any change in body temperature.

The concentrations of IL-1β and TNF-α in serum after intravenous injection of LPS for 1 h in the four different groups

In order to discuss the mechanism of HLJDD in reducing the first peak of the body temperature in LPS induced fever, the concentrations of IL-1β and TNF-α in serum were measured at time 1 h, and the results showed that the level of IL-1β had significant differences between Control and LPS groups ($p < 0.05$). Both HLJDD and Ibuprofen treatment groups resulted in a significant fall in the concentrations of IL-1β in serum comparing with that of only treated with LPS ($p < 0.05$). Besides, Ibuprofen treatment group resulted in a significant fall in the concentrations of TNF-α in serum comparing with that of only treated with LPS group ($p < 0.05$). HLJDD did not reduce the concentrations of TNF-α in serum comparing with the LPS group ($p > 0.05$). Ibuprofen could reduce both the concentrations of IL-1β and TNF-α in serum to lower the first peak of the body temperature in LPS induced fever, while HLJDD reduced the concentrations of IL-1β in serum to lower the first peak of the fever (Table 1).

The concentrations of IL-1β and TNF-α in serum after intravenous injection of LPS for 3 h in the four different groups

The blood samples were collected at time 3 h after injecting LPS, and the concentrations of IL-1β and TNF-α were assayed (Table 2). The concentrations of IL-1β in

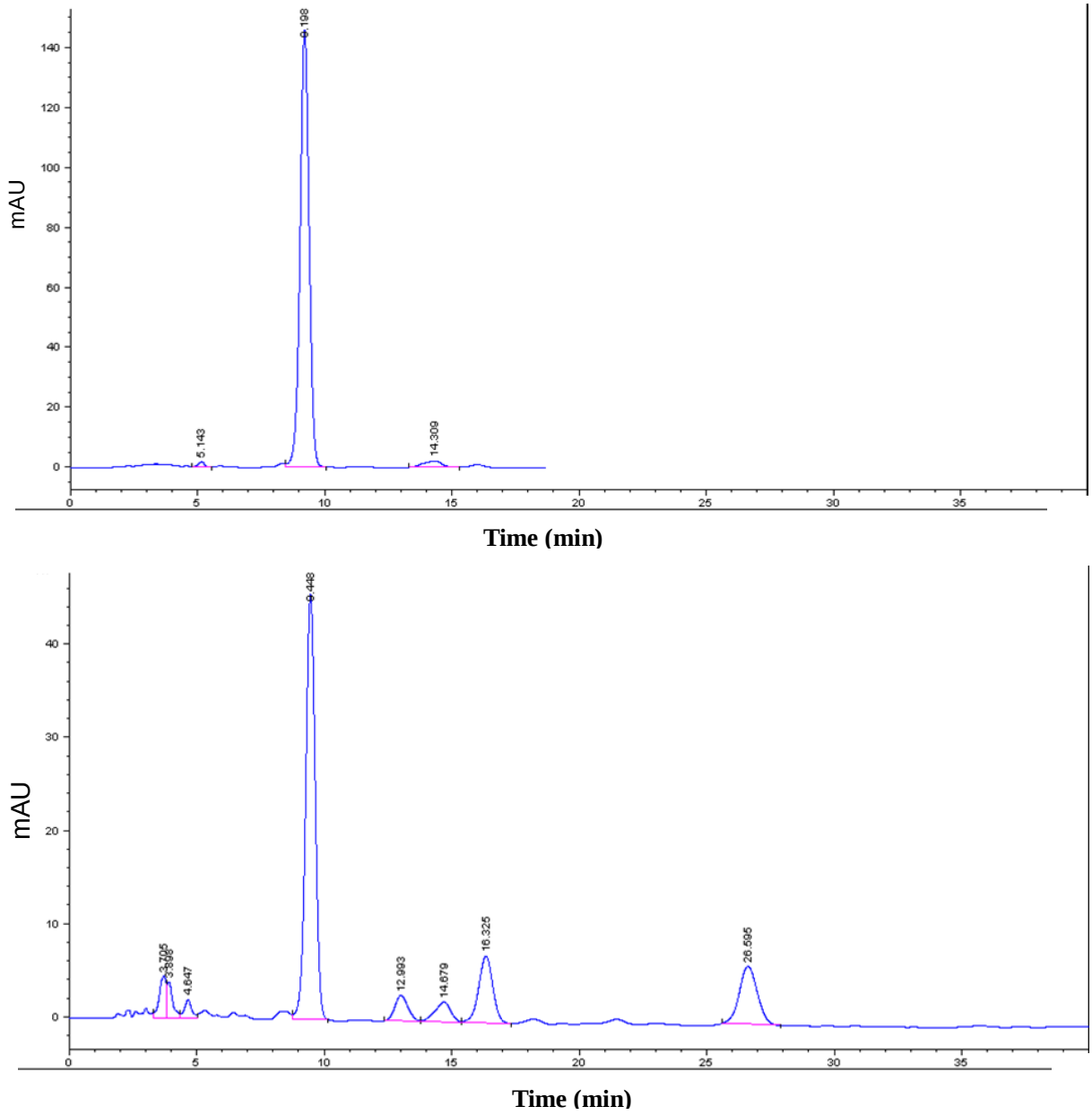

Figure 1. A. HPLC chromatogram of Baicalin. B. HPLC chromatogram of HLJDD.

serum of HLJDD and Ibuprofen treatment groups were significantly decreased comparing with the concentrations of LPS group ($p < 0.05$). There was no significant change on the concentrations of TNF-α in serum between LPS and Control groups ($p > 0.05$). HLJDD treatment group resulted in a significant decrease in the concentrations of IL-1β comparing with the group that only treated with LPS ($p < 0.05$), but there was no remarkable difference in the concentrations of TNF-α comparing with Ibuprofen treatment group ($p > 0.05$). From our experiment, we found that IL-1β in serum played the important role in the second peak of LPS induced fever.

Inhibition of the paw edema of rat by oral treatment of HLJDD

Figure 3 shows the effect of HLJDD on inhibition of the acute paw edema in rats evoked by carrageenan injection into the subplantar tissues of the right hind paws. It can be seen that the maximum phlogistic response of carrageenan was observed at 4 to 6 h after the injection

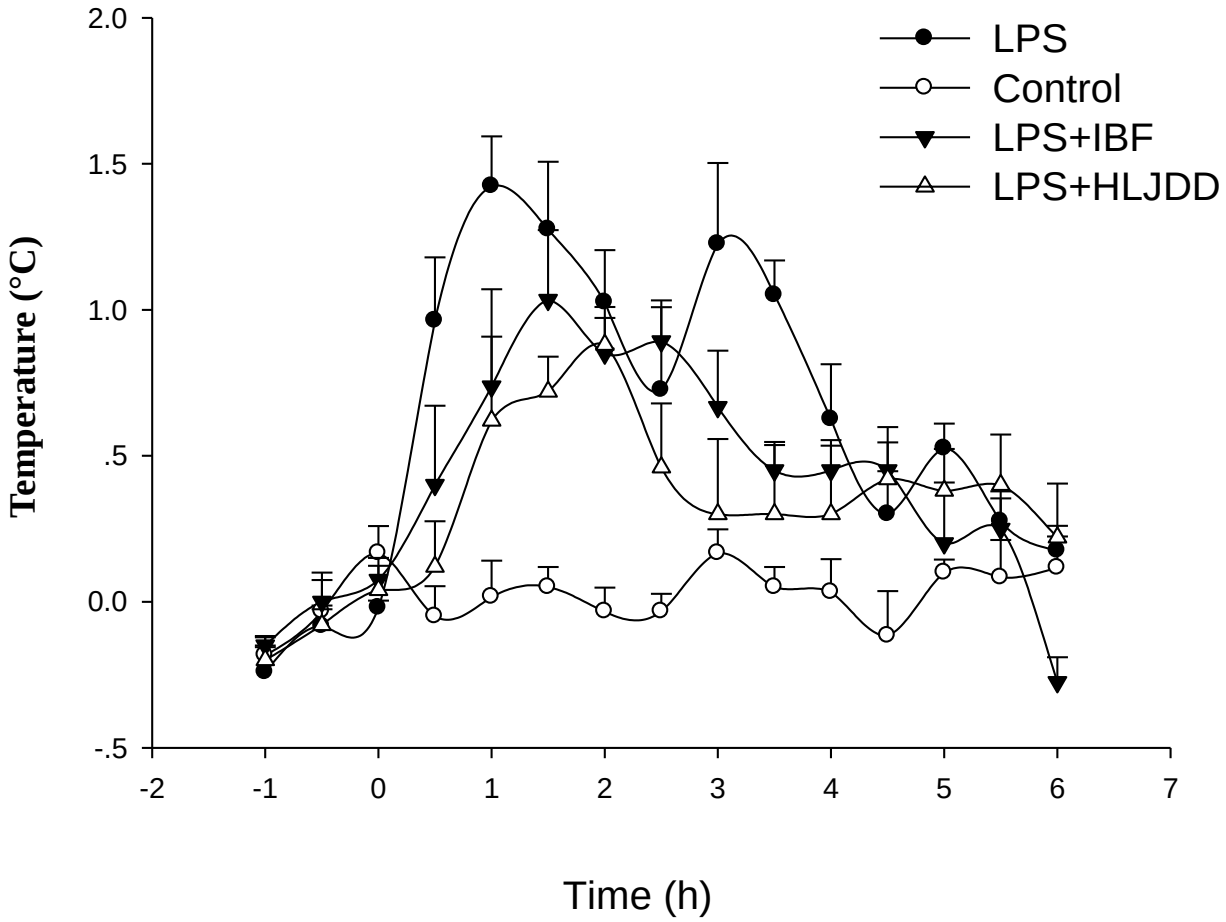

Figure 2. The changes of body temperature in different groups. Rabbits were injected of LPS intravenously 30 min after orally administered with HLJDD or Ibuprofen and the Control animals were orally administered with 0.9% saline solution only.
●.LPS (n = 5); ○.Control (n = 5); ▼.LPS + Ibuprofen (n = 5); △.LPS + HLJDD (n = 5). Each point represents mean ± S.E.M. At time 1 h LPS vs Control, $p < 0.05$; LPS vs LPS + HLJDD, $p < 0.05$; LPS vs LPS + Ibuprofen, $p < 0.05$; and at time 3 h LPS vs Control, $p < 0.05$; LPS vs LPS + HLJDD, $p < 0.05$; LPS vs LPS + Ibuprofen, $p < 0.05$.
There were no significant differences between HLJDD treatment group and Ibuprofen treatment group in biphasic fever peak at time 1 h and 3 h ($p > 0.05$).

in the control animals. The paw volumes from HLJDD-treated animals with dosages of 18.75 g/kg, at 1, 2, 3, 4, 5 and 6 h after induction of paw edema showed remarkable decrease in comparison with the data of non-treated animals at the same time points ($p < 0.05$), and there were no significant differences between the HLJDD-treated and the Ibuprofen-treated animals ($p > 0.05$). These results indicated that the anti-acute inflammatory effect of HLJDD in rats were effective.

Inhibition of iNOS, COX-1 and COX-2 protein expressions in the paw tissues of rats by treatment of HLJDD

It can be seen in Figure 4 that HLJDD dose-dependently attenuated the protein expression of COX-2 in carrageenan-injected paw tissues. Reduction was achieved by treatment with HLJDD at dosages of 18.75, 9.38, and 4.69 g/kg, respectively. Figure 4 also showed that the protein expression of iNOS in carrageenan-injected paw tissues was dose-dependently attenuated in HLJDD treated rats. HLJDD at 18.75/kg can significantly inhibit the iNOS protein expression. Ibuprofen at a dosage of 10 mg/kg also demonstrated significant inhibition in iNOS protein expression. Examination of COX-1 protein expression showed that the level of expression was not suppressed by administration of HLJDD at any dosage. These results suggested that HLJDD may have a selective inhibitory effect on COX-2 protein expression in the paw edema tissues of rat. However, Ibuprofen at a dosage of 10 mg/kg demonstrated significant inhibition on

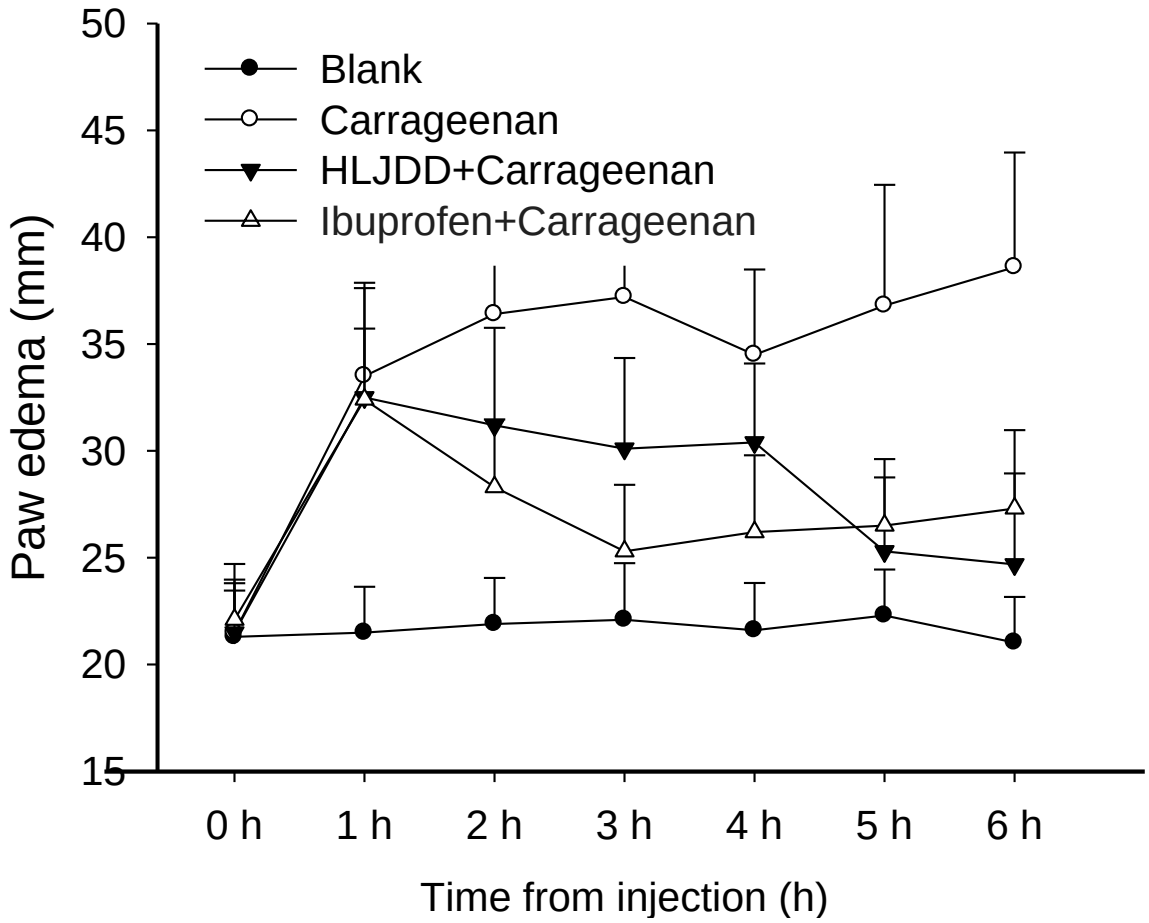

Figure 3. The inhibition of carrageenan-evoked paw acute inflammation of rat by HLJDD. The paw volumes from HLJDD-treated animals with dosages of 18.75 g/kg, at 1, 2, 3, 4, 5 and 6 h after induction of paw edema showed remarkably decrease in comparison with the data of non-treated animals at the same time points ($p < 0.05$).

both COX-2 and COX-1 protein expressions.

DISCUSSION

Fever is a regulated rise in body temperature and one of the most common responses to infection, injury or trauma. Administration of bacterial endotoxin LPS is widely used as a laboratory model of fever. IL-1 is thought to be an endogenous pyrogen during LPS-induced fever. The pro-inflammatory cytokine IL-1 is a pivotal mediator of local and systemic responses to infection and inflammation, of which fever is the most widely studied experimentally and clinically (Dinarello, 1996; Kluger, 1991). Rabbit TNF injection also elicited biphasic fever in rabbits, the second phase of which was found to be mediated by the similar endogenous pyrogen, and endogenous TNF played an important role in eliciting a febrile response to endotoxin (Kawasaki et al., 1989).

We made a biphasic fever model in rabbits using a high dosage of LPS (200 ng/kg) (Chen et al., 2008), that was conformed as experiments had been made by others, and the first phase of biphasic fever appeared at about time 1 h and the second phase of biphasic fever appeared at about time 3 h, that was similar as the investigation reported before (Nakamori et al., 1994). The main findings of this study were that treatments with the crude extract of HLJDD could remarkably reduce the biphasic fever induced by LPS. Ibuprofen, a specific cyclo-oxygenase inhibitor, not only reduced the first phase of biphasic fever clearly, but also affects the latter fever peak. This result of ibuprofen antifebrile action was consistent with the investigation reported before that it could result in a significant fall in body temperature in both fever peaks (Sobrado et al., 1983), and the reasons for this might be the different dosages of ibuprofen that the rabbits were administrated or the difference of animal species which are needed for further investigation.

The present results showed that IL-1β and TNF-α were increased in serum during biphasic fever induced by intravenous injection of LPS (200 ng/kg). In addition, at time 3 h, there were no differences in TNF-α level in the

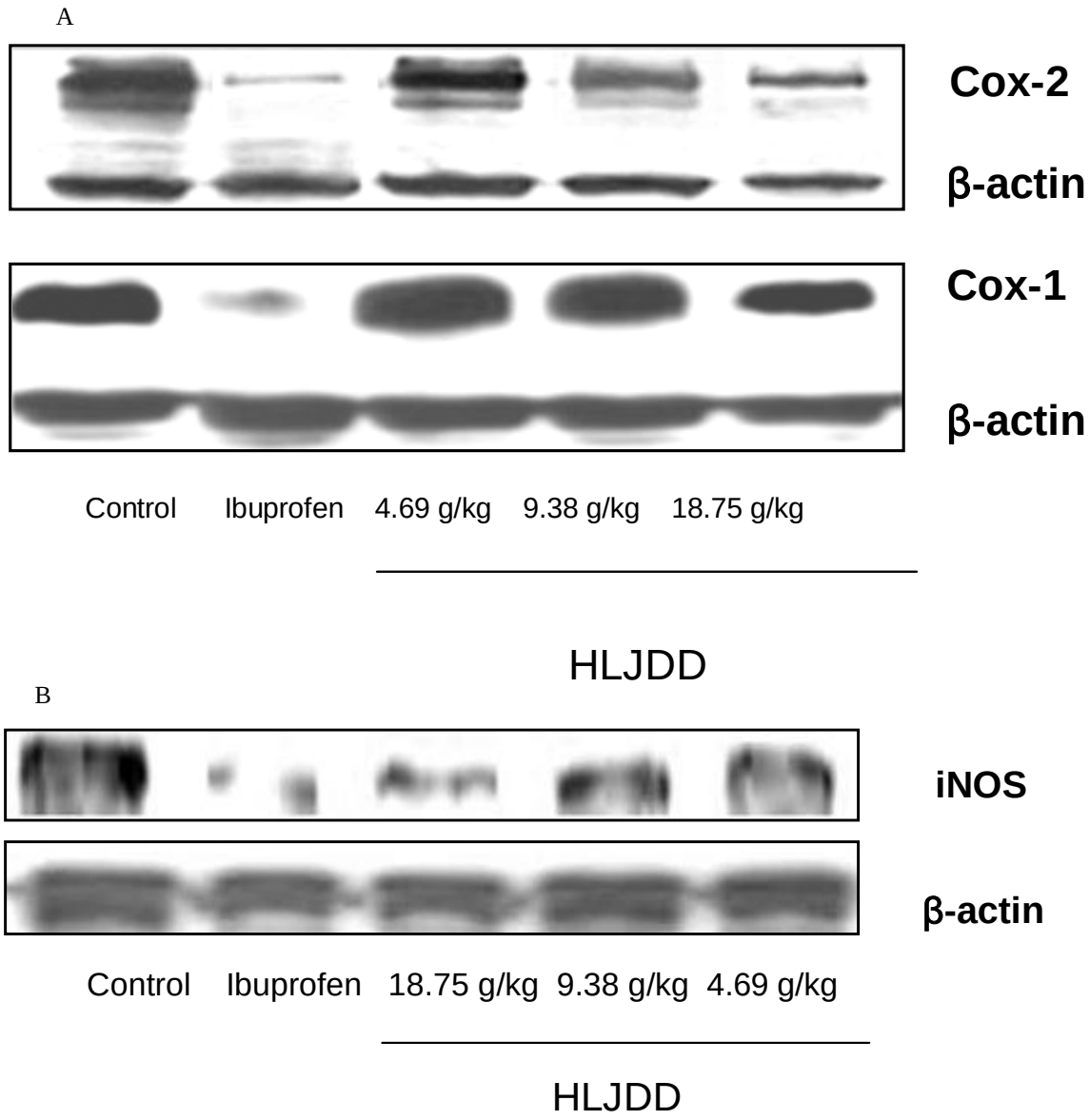

Figure 4. A. Effects of HLJDD on COX-1 and COX-2 protein expressions in carrageenan-injected paw tissues of rats. HLJDD at dosages of 4.69 g/kg, 9.38 g/kg, and 18.75 g/kg was orally administrated 1 h before the carrageenan injection. At 4 h after the injection, paws were removed. Then COX-1 and COX-2 protein expressions in the paw tissues were detected by Western blot analysis, using β-actin as the internal control. HLJDD dose-dependently attenuated the protein expression of COX-2, while the COX-1 protein expression was not suppressed by administration of HLJDD at any dosage. Ibuprofen at a dosage of 10mg/kg demonstrated significant inhibition on both COX-2 and COX-1 protein expressions. B. Effects of HLJDD on iNOS protein expressions in carrageenan-injected paw tissues of rats. HLJDD at dosages of 4.69 g/kg, 9.38 g/kg, and 18.75 g/kg was orally administrated 1 h before the carrageenan injection. At 4 h after the injection, paws were removed. Then iNOS protein expression in the paw tissues was detected by Western blot analysis, using β-actin as the internal control. HLJDD at 18.75/kg can significantly inhibit the iNOS protein expression. Ibuprofen at a dosage of 10 mg/kg also demonstrated significant inhibition in iNOS protein expression.

serum between LPS and Control groups. Therefore, it was possible that the early peak fever was induced by the indirect action of IL-1β and TNF-α produced in serum by intravenous injection of LPS, and the latter fever peak appeared at 3 h which was mediated by LPS induced endogenous IL-1β in serum. The previous study had shown that endogenous TNF activity was detected in 1 h blood in an endotoxin dose dependent manner coincident with the early peak fever but was not detected in 2.5 h blood (Kawasaki et al., 1989). HLJDD reduced the concentrations of IL-1β in serum to control the febrile responses at time 1 h; and the levels of IL-1β in serum

Table 1. Concentrations (ng/ml) at time 1 h (mean ± SEM).

Test group	IL-1β	TNF-α
LPS	11.00±4.24	95.15±8.05
Control	3.72±0.53*	92.47±16.27#
IBR+LPS	2.94±1.76*	83.74±5.41*
HLJDD+LPS	5.11±3.06*	106.39±7.49#

The concentrations of IL-1β, TNF-α in serum at time 1 hour after the injection of LPS in the different groups were detected. Data are expressed as mean ± S.E.M. (n = 5). Compared with LPS group, *p < 0.05; #p > 0.05.

Table 2. Concentrations (ng/ml) at time 3 h (mean ± SEM).

Test group	IL-1β	TNF-α
LPS	8.66±0.92	81.28±1.52
Control	6.79±0.61*	76.54±3.88#
IBR+LPS	4.91±0.46*	97.61±1.38#
HLJDT+LPS	5.66±2.17*	79.00±9.99#

The concentrations of IL-1β and TNF-α in serum at time 3 hour after the injection of LPS in the different groups were detected. Data are expressed as mean ± S.E.M. (n = 5). Compared with LPS group, *p < 0.05; # p > 0.05.

were remarkably decreased in HLJDD group than that of in LPS group at time 3 h. But ibuprofen failed to reduce the rising concentrations of TNF-α in serum in the latter fever peak, and the concentrations of TNF-α in serum between HLJDD and Ibuprofen groups had significant differences ($p < 0.05$). The reasons might be the dissimilar anti-inflammation mechanism between ibuprofen and HLJDD.

To compare with ibuprofen, according to TCM selective treatment based on the differential diagnosis, the TCM prescription HLJDD has some advantages in reducing fever induced by various diseases. Salvemini et al. (1993) proposed that NO stimulates COX activity in macrophages through direct interaction with active sites of the COX enzyme. Inhibition of NO production by the suppression of the enzyme activity of iNOS is one of the pathways for anti-inflammatory effect (Cai et al., 2005; Salvemini et al., 1993). Furthermore, they reported that NO produced by iNOS is involved in maintenance of the carrageenan evoked inflammatory response, while peripheral or central administration of iNOS inhibitors could effectively inhibit carrageenan-induced hyperalgesia in rats (Salvemini et al., 1996).

In the present work, the anti-inflammatory and analgesic effects of HLJDD in carrageenan induced rat paw edema were also studied. The possible effective mechanisms were investigated with regard to the iNOS, COX-1, and COX-2 protein expressions in paw tissues. The anti-inflammatory effect of these drugs is believed to result from their ability to inhibit the formation of prostaglandins by cyclooxygenases (COXs). Two isoforms of cyclooxygenase, COX-1 and COX-2, have been identified. COX-1 constitutively expressed in normal tissues functions as necessarily physiological activities including protection of the gastric mucosal lining; while COX-2 is overproduced in the sites of inflammation (Smith et al., 1998). In clinical use, some NSAIDs have been proven to block both COX-1 and COX-2 activities resulting in induction of gastric ulcerization and kidney failure. Moreover, the marketing of new COX-2 inhibitors which emphasized the advantages of not blocking the necessary COX-1 pathway and gastrointestinal problems from the use of those drugs are still being reported (Silverstein et al., 2000).

Conclusion

The effects of TCM prescriptions were multi-target and complex, but at least in part. The present study showed that the prescription could evidently reduce the increased concentrations of IL-1β, and have selective inhibitory effect on COX-2 protein expression. All these results indicated that the HLJDD would be a valuable candidate for further investigation as a novel anti-arthritic botanical drug.

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ABBREVIATIONS

HLJDD, Huanglian Jiedu Decoction; **IBR,** ibuprofen; **TCM,** Traditional Chinese Medicine; **LPS,** lipopolysaccharide; **IL-1β,** interleukin-1 beta; **TNF-α,** tumor necrosis factor alpha; **NSAIDs,** nonsteroidal anti-inflammatory drugs; **ELISA,** enzyme-linked immunosorbent assay; **COX,** cyclooxygenase; **iNOS,** inducible nitric oxide synthase.

REFERENCES

Cai X, Zhou H, Wong YF, Xie Y, Liu ZQ, Jiang ZH, Bian ZX, Xu HX, Liu L (2005). Suppressive effects of QFGJS, a preparation from an anti-arthritic herbal formula, on rat experimental adjuvant-induced arthritis. Biochem. Biophys. Res. Commun. 337:586-594.

Chen CC, Hsiang CY, Chiang AN, Lo HY, Li CI (2008). Peroxisome proliferator-activated receptor gamma transactivation-mediated potentiation of glucose uptake by Bai-Hu-Tang. J. Ethnopharmacol. 118:46-50.

Dinarello CA (1996). Biologic basis for interleukin-1 in disease. Blood. 87:2095-2147.

Dinarello CA, Ikejima T, Warner SJ, Orencole SF, Lonnemann G, Cannon JG, Libby P (1987). Interleukin 1 induces interleukin 1. I. Induction of circulating interleukin 1 in rabbits in vivo and in human mononuclear cells in vitro. J. Immunol. 139:1902-1910.

Johnson RN, McDonald HR, Schatz H (1998). Rash, fever and chills

after intravenous fluorescein angiography. Am. J. Ophthalmol. 126: 837-838.
Kawasaki H, Moriyama M, Ohtani Y, Naitoh M, Tanaka A, Nariuchi H (1989). Analysis of endotoxin fever in rabbits by using a monoclonal antibody to tumor necrosis factor (cachectin). Infect Immun. 57:3131-3135.
Kluger MJ (1991). Fever: role of pyrogens and cryogens. Physiol. Rev. 71:93-127.
Li HB, Yi X, Gao JM, Ying XX, Guan HQ, Li JC (2008). The mechanism of hyperoside protection of ECV-304 cells against tert-butyl hydroperoxide-induced injury. Pharmacol. 82:105-113.
Nakamori T, Morimoto A, Yamaguchi K, Watanabe T, Murakami N (1994). Interleukin-1 beta production in the rabbit brain during endotoxin-induced fever. J. Physiol. 476:177-186.
Ohta Y, Kobayashi T, Nishida K, Sasaki E, Ishiguro I (1999). Preventive effect of Oren-gedoku-to (Huanglian-Jie-Du-Tang) extract on the development of stress-induced acute gastric mucosal lesions in rats. J. Ethnopharmacol. 67:377-384.
Salvemini D, Misko TP, Masferrer JL, Seibert K, Currie MG, Needleman P (1993). Nitric oxide activates cyclooxygenase enzymes. Proc. Nat. Acad. Sci. 90:7240-7244.
Salvemini D, Wang ZQ, Wyatt PS, Bourdon DM, Marino MH, Manning PT, Currie MG (1996). Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paws inflammation. Br. J. Pharmacol. 118:829-838.
Silverstein FE, Faich G, Goldstein JL, Simon LS, Pincus T, Whelton A, Makuch R, Eisen G, Agrawal NM, Stenson WF, Burr AM, Zhao WW, Kent JD, Lefkowith JB, Verburg KM, Geis GS (2000). Gastrointestinal toxicity with celecoxib vs. nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: A randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. JAMA 284:1247-1255.
Smith CJ, Zhang Y, Koboldt CM, Muhammad J, Zweifel BS, Shaffer A, Talley JJ, Masferrer JL, Seibert K, Isakson PC (1998). Pharmacological analysis of cyclooxygenase-1 in inflammation. Proc. Natl. Acad. Sci. 95:13313-13318.
Sobrado J, Moldawer LL, Bistrian BR, Dinarello CA, Blackburn GL (1983). Effect of ibuprofen on fever and metabolic changes induced by continuous infusion of leukocytic pyrogen (interleukin 1) or endotoxin. Infect. Immun. 42:997-1005.
Wang LM, Mineshita S (1996). Preventive effects of unsei-in and oren-gedoku-to, Chinese traditional medicines, against rat paw oedema and abdominal constriction in mice. J. Pharm. Pharmacol. 48:327-331.
Winter CA, Risley EA, Nuss CW (1962). Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med. 111:544-547.
Xu J, Murakami Y, Matsumoto K, Tohda M, Watanabe H, Zhang S, Yu Q, Shen J (2000). Protective effect of Oren-gedoku-to (Huang-Lian-Jie-Du-Tang) against impairment of learning and memory induced by transient cerebral ischemia in mice. J. Ethnopharmacol. 73:405-413.
Xu SY, Bian RL, Chen X (2009). Method of Pharmacological Experiments. People's Medical Publishing House.