



RESEARCH ARTICLE

Protective Effect of Huangteng Soluble Powder on Endogenous Pyrogen and Central Mediators of Fever Induced by Lipopolysaccharide in Chicken

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ABSTRACT

The objective of this study was to understand the effect of Huangteng Soluble Powder (HTSP) on endogenous pyrogen and central mediators of fever induced by Lipopolysaccharide (LPS) in chicken. The model was established, and 90 chickens were randomly assigned into 6 groups, including Group I (control), Group II (injected with LPS), Group III (administered with Banqing Granules once a day for 3 days after 5 h post injection of LPS), Group IV, V and VI (administered with HTSP once a day for 3 days after 5 h post injection of LPS). The temperature was measured at different time point, and samples were collected at 5 h post injection of LPS and 12 h, 24 h after the administration of HTSP. The results showed that HTSP significantly decreased the temperature of fever chicken in comparison to Group II. Compared with Group I, the levels of IL-1, IL-6, TNF- α in serum and Prostaglandin E₂ (PGE₂), cyclic adenosine monophosphate (cAMP) in hypothalamus in Group II significantly increased at 5 h post injection of LPS, the mRNA expression levels of PGE₂ and cAMP in Group II were significantly increased also. Compared with Group II, the levels of IL-1, IL-6 in serum and PGE₂, cAMP in hypothalamus in Groups III, IV, V and VI decreased after 12 h and 24 h, the levels of TNF- α in serum in Group III and V decreased after 12 h and 24 h, and the mRNA expression levels of PGE₂ and cAMP of Group V were significantly decreased at the end of the administration. In conclusion, HTSP can attenuate the LPS-induced fever by diminishing the levels of IL-1, IL-6 and TNF- α in serum and the levels of PGE₂ and cAMP in hypothalamus and inhibiting the mRNA expression of PGE₂ and cAMP.

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INTRODUCTION

As the intensive breeding degree of chicken increased, the stocking density increased, the air circulation of poultry houses was not enough, or the climate changed suddenly, resulting in that chickens are susceptible to disease, especially replacement of seasons such as winter to spring and autumn to winter. Catching exogenous wind-cold, poultry will be fever. Furthermore, it will be low feed-intake, depressed, gathered together, shivering and other symptoms. If no timely treatment, the immune system will be impacted causing susceptibility to be infection by pathogens and serious economic losses.

The pyrogen acts on the body to induce fever and thermoregulatory center of the body dysfunction (Roth

and Blatteis, 2014; Zhang *et al.*, 2017). Pyrogens can be classified into exogenous pyrogen and endogenous pyrogen (Jia *et al.*, 2013). Generally, some exogenous pyrogens (e.g., Lipopolysaccharide) do not act directly on the brain to cause fever, but by activating the mononuclear macrophages *in vivo* to produce and release endogenous pyrogen (EP) and then interact with both the thermoregulatory center of the body and central mediators of fever (PGE₂, cAMP, etc.) to cause fever (Bastos-Pereira *et al.*, 2015; Zampronio *et al.*, 2015). The well-accepted EPs include interleukin-1 (IL-1) and IL-1 receptor antagonist protein (IL-1ra), tumor necrosis factor (TNF- α), interleukin-6 (IL-6), interferons and so on (Dinarelli *et al.*, 1987; Zampronio *et al.*, 2015). These cytokines banded their receptors, stimulated the fever

reaction, mediated the inflammatory process, participated in the immune regulation and affected tissue metabolism (Schneiders *et al.*, 2015; Ye *et al.*, 2015).

HTSP, composed of two traditional Chinese herbs, *Scutellaria baicalensis* and *Ren Dong Teng* (*Honeysuckle* Stem), have the function in heat-clearing and damp-drying, purging fire and detoxifying toxicosis, expel the wind (China, 2015). Reportedly, *Scutellaria baicalensis* have been used for the treatment of inflammation, fever, dysentery, diarrhea, influenza, pneumonia, and have potential anticancer activities (Nhoek *et al.*, 2018; Xu *et al.*, 2018). For instance, the effective constituent of *baicalin* in *Scutellaria baicalensis* can effectively treat the fever induced by LPS in rats and rabbits (Tsai *et al.*, 2006; Ye *et al.*, 2015). *Ren Dong Teng* has good antibacterial, antipyretic, anti-inflammatory and antiviral effects (Xiong *et al.*, 2013; Fan *et al.*, 2015). Heretofore, there is a little known regarding the mechanisms of HTSP in curing poultry fever. In this study, we conducted to evaluate the protective effects of HTSP on fever induced by LPS in chicken. In addition, we tried to investigate the mechanism of HTSP on protecting the poultry from fever.

MATERIALS AND METHODS

Animals and Treatment: One hundred and thirty 30-day-old healthy three-yellow chickens (body weights 550±50g), 50% females and 50% males, purchased from Rongchang County Guili farm. All chickens were maintained in a strictly sterilized chicken coop, treated at a controlled temperature (20~25°C) and relative humidity (56%~65%), fed with standard laboratory chow and water. The animal experiments were performed according to the instructions (SYXK20110001) of the Medical Ethics Committee for the use of Experimental Animals at Southwest University.

Drugs: Huangteng Soluble Powder (HTSP): 50g/bag, drinking water administration, once a day for 3 consecutive days, and produced by Luoyang Ruihua Animal Health Products Co., Ltd, No. 20150227; Banqing Granules: 100g/bag, 0.5g/100ml in water once a day for 3 consecutive days, produced by Jiangxi Chinese Patent Medicine Raw Materials Co., Ltd, No. 2015072201.

FT-IR characteristics of HTSP: The effective active components of HTSP were analyzed by the Fourier transform infrared (FT-IR) spectroscopy. Briefly, take 0.2 mg (0.201 mg) HTSP and 2 mg (2.001 mg) potassium bromide into an agate mortar, mix, tablet, and then placed in the Fourier transform infrared spectrometer for testing. Select 2000 and 400 cm⁻¹ as the starting and ending points of the spectral baseline and calculate the height ratio of the characteristic peaks (Zhang *et al.*, 2010; Liu *et al.*, 2012).

Fever model establishment: According to recent reports (De Boever *et al.*, 2008; Lieboldt *et al.*, 2017), using different doses of LPS combined with body temperature detection to select the best LPS dose for fever model. Finally, according to the optimal LPS dose (150 µg/kg) that has been screened, every chicken in the experimental group received the LPS 150 µg/kg with intraperitoneal injection. The chickens were fasted 6 h and drank water normally before the attack. The positive chickens in fever model were used for further experiment.

Animal grouping and treatment: The chickens were randomly divided into 6 groups (15 chickens in each group): Group I (control group without any treatments), Group II (only challenge without drugs), Group III (After challenged 5h, drinking water administration of Banqing Granules 0.5g/100ml once a day for three consecutive days), Group IV, Group V, Group VI (After challenged 5h, drinking water administration of HTSP 1.0g/100ml, 0.5g/100ml, 0.25g/100ml once a day for three consecutive days). During the test, the body temperature of each group was measured at 5h after challenged, 2, 7, 12, 24, 36, 48 and 72h after administration (drugs), and recorded in detail. Blood was collected via wing vein to obtain serum before the test and at 5h after challenged, 12h and 24h after administration (drugs). Hypothalamus was collected at the end of the third day, and then frozen immediately in liquid nitrogen, stored at -80°C.

IL-1, IL-6, TNF-α, PGE₂ and cAMP levels: The concentration of IL-1, IL-6 and TNF-α in the chicken serum, cAMP and PGE₂ in the chicken hypothalamus were detected by using ELISA kit and calculated by standard curve, and the absorbance (OD) was measured at 450 nm using a Microplate Reader according to the ELISA kit instructions.

Analysis of relative mRNA expression of PGE₂ and cAMP: Total RNA was extracted with Trizol reagents according to manufacturer's instruction (Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China). Then cDNA was prepared with Primer-Script™ reagent kit (TakaRa Biotechnology Co. Ltd, Dalian, China). The gene-specific primers of cAMP, PGE₂ and GAPDH were synthesized by TakaRa Biotechnology Co. Ltd (Dalian, China) and are listed in Table 1. Cycling conditions were 95°C for 5min, then 40 cycles of following: 95°C for 30s, 63°C for 30s. The 2^{-ΔΔCt} method was used to analyze Quantitative Real-time PCR data.

Statistical analysis: SPSS22.0 statistics software is used for data processing. All data were analyzed by the SPSS statistical software, using a one-way ANOVA and expressed as mean±standard error (SE). P<0.05 was considered as significant statistical difference.

Table 1: Primer sequences used in quantitative real time PCR assays

Gene	Forward primer (5'-3')	Reverse primer (3' -5')	Size (bp)	Accession No.
cAMP	GATCCCGTTCTGGTCCAAC	GCCCCATTTATTCATTGAGC	123	NM_001024830
PGE ₂	TGCTGGATGAGAAGGAGACA	GGGAGATGAGGTGAACAAGC	108	XM_015302168
GAPDH	GCACGCCATCACTATCTT	GGACTCCACAACATACTCAG	82	NM_001289746.1

RESULTS

FT-IR characteristics of HTSP: HTSP is composed of two Chinese herbal medicines-*Scutellaria baicalensis* and *Ren Dong Teng*. Flavonoids are the main active ingredients of *Scutellaria baicalensis*, and chlorogenic acid and galuteolin are the main active ingredients of *Ren Dong Teng*. Fig. 1 showed that the absorption peaks were around 1735.87, 1619.83, 1509.75, 1453.22, 1402.64, 1316.36 and 1247.93 cm^{-1} which were mainly absorption peaks of flavonoids. The absorption peaks around 3363.31, 2922.98, 1619.83, 1111.07 and 1054.55 cm^{-1} were mainly absorption peaks of chlorogenic acid and galuteolin. In conclusion, it is showed that HTSP contains qualified *Scutellaria baicalensis* and *Ren Dong Teng*.

Validation of fever chicken model: The change of body temperature in different doses of LPS in chicken is shown in Fig. 2. Obviously, the group with the fastest onset of action and the longest duration of maintenance was the LPS dose 3 (150 $\mu\text{g}/\text{kg}$ LPS). As a consequence, the fever model dose of 150 $\mu\text{g}/\text{kg}$ LPS was selected.

Effect of HTSP on body temperature of fever chicken induced by LPS: The test results showed that the basal body temperature of the chicken was in the normal range (40 to 42°C) before the test, and there was no significant difference between each group ($P>0.05$). As shown in Fig. 3. After 5 h of LPS injection, compared with the Group I, the body temperature of all the test groups increased significantly ($P<0.01$). Drug administration started after 5 h of LPS injection. After 2 h of administration, compared with the Group II, the body temperature in Group III and Group IV decreased after 7 h of administration ($P<0.05$); the body temperature in Group V decreased significantly after 7 h of administration ($P<0.01$); and the body temperature in Group VI decreased significantly after 12 h of administration ($P<0.01$). These results showed that the fever model was reliable and that both of Group IV and V could effectively protect the chicken from the LPS-induced fever.

Effects of HTSP on the levels of IL-1, IL-6 and TNF- α in the chickens' serum: The results in Fig. 4 shows that the concentration of IL-1 in the chickens' serum of each experimental Group (Group II to VI) increased significantly ($P<0.05$) at the period 2 in comparison to Group I. Compared to the Group II, the concentration of IL-1 in the chickens' serum of Group III, Group IV, Group V and Group VI was decreased at the period 3 and significantly decreased ($P<0.05$) at the period 4.

As shown in Fig. 5, the content of IL-6 in the chickens' serum of each experimental Group (Group II to VI) increased significantly ($P<0.05$) at the period 2 in comparison to Group I. Compared with the Group II, the levels of IL-6 in the chickens' serum of Group IV, Group V and Group VI decreased significantly ($P<0.05$) at the period 3, as well as there was no significant difference ($P>0.05$) at the period 4.

Fig. 6 shows that the content of TNF- α in the chickens' serum of each test group (Group II to VI) increased significantly ($P<0.05$) at the period 2 in comparison to Group I. Compared with the Group II, the

levels of TNF- α in the chickens' serum of Group III and Group VI decreased at the period 3, and the Group III and Group V decreased at the period 4, whereas there was no significant difference ($P>0.05$).

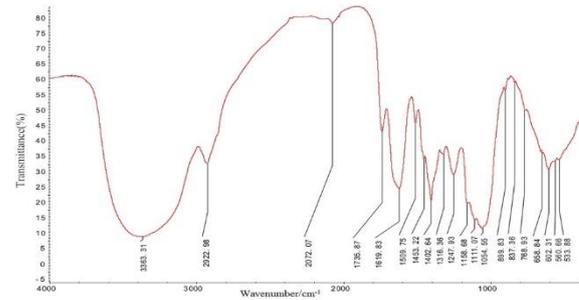


Fig. 1: Fourier Transform Infrared Spectroscopy spectrum of HTSP.

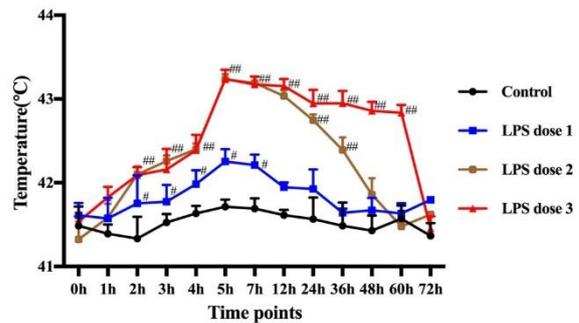


Fig. 2: Temperature change of chicken in each group after injection with different doses of LPS. ### $P<0.01$ vs Control group, # $P<0.05$ vs Control group.

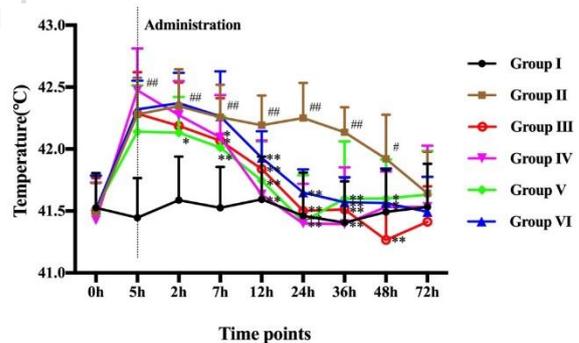


Fig. 3: Effect of HTSP on body temperature of LPS-induced fever in Chickens. ### $P<0.01$ vs group I, # $P<0.05$ vs group I, ** $P<0.01$ vs group II, * $P<0.05$ vs group II.

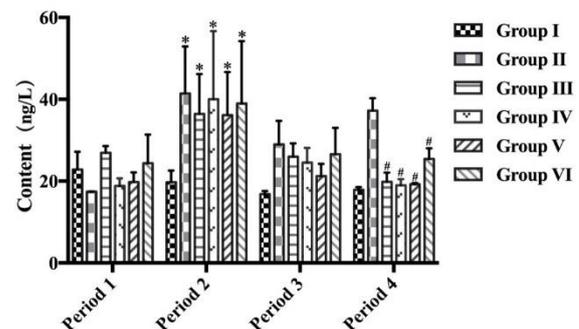


Fig. 4: Changes of the IL-1 content in chickens' serum. Period 1: before the test, Period 2: after 5 h of LPS injection, Period 3: after 12 h of administration, Period 4: after 24 h of administration. * $P<0.05$ vs group I, # $P<0.05$ vs group II. The same as below.

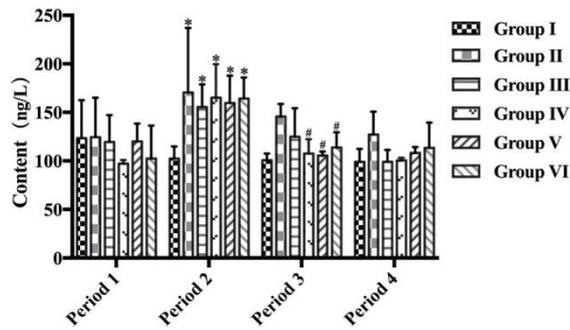


Fig. 5: Changes of the IL-6 content in chickens' serum.

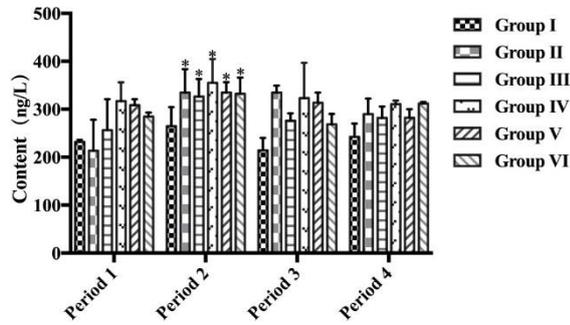


Fig. 6: Changes of the TNF-α content in chickens' serum.

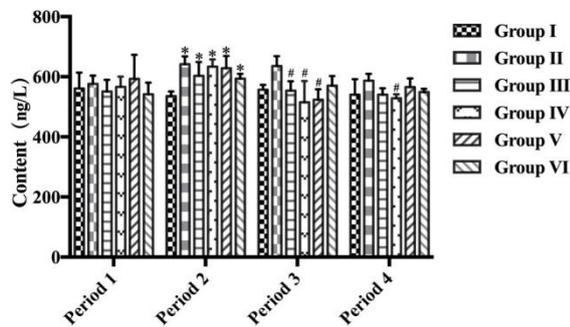


Fig. 7: Changes of the PGE₂ content in chickens' serum.

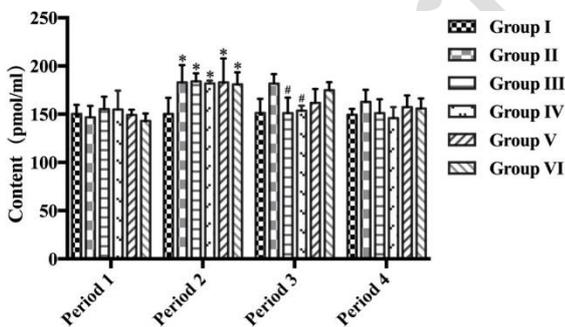


Fig. 8: Changes of the cAMP content in chickens' serum.

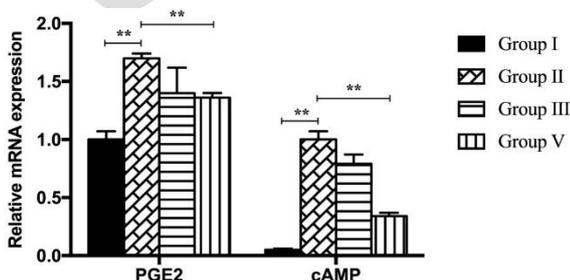


Fig. 9: The effect of HTSP on PGE₂, cAMP mRNA expression in chicken's hypothalamus **P<0.01.

Effects of HTSP on the levels of PGE₂ and cAMP in the chickens' Hypothalamus: As shown in Fig. 7, compared with the group I, the levels of PGE₂ in the chickens' hypothalamus of each experimental Group (Group II to VI) significantly increased (P<0.05) at the period 2. Compared with the group II, the levels of PGE₂ in the chickens' hypothalamus of Group III, Group IV and Group V decreased significantly (P<0.05) at the period 3. At the period 4, the levels of PGE₂ in the chickens' hypothalamus of Group IV, Group V and Group VI were decreased, in addition, Group V decreased significantly (P<0.05) in comparison to group II.

In Fig. 8, the levels of cAMP in the chickens' hypothalamus of each experimental group (Group II to VI) significantly increased (P<0.05) at the period 2 in comparison to Group I. Compared to Group II, the levels of cAMP in the chickens' hypothalamus of Group IV and Group V decreased at the period 3 and 4, moreover, there was significantly decreased (P<0.05) at the period 3.

Effects of HTSP on mRNA expression of PGE₂ and cAMP in the chickens' hypothalamus: As shown in Fig. 9, compared with the Group I, PGE₂ and cAMP mRNA expression of Group II were significantly increased (P<0.01). Compared with the Group II, PGE₂ and cAMP mRNA expression of Group V were significantly decreased (P<0.01) at the end of the administration.

DISCUSSION

According to the theory of Traditional Chinese Veterinary Medicine, fever is the main clinical symptom of all kinds of epidemic febrile disease. It is mainly caused by cold evil invasion, the decline of body resistance, or climate change. It is a systemic reaction of the body to the disease. And it is the reaction of the between vital qi of the body and disease. In addition, the therapeutic principle is based on relieve heat and save the Yin. In this study, the characters of *Scutellaria baicalensis* with curative effect on heat dampness, cooling blood miscarriage, and detoxifying are bitter and cold, and it converges to heart, lung, gallbladder, large intestine channel (Muluye *et al.*, 2014). Furthermore, the characters of *Ren Dong Teng* with curative effect on clears heat and toxin, dispels wind damp, unblocks the channels and cools the blood are sweet and cold, and it converges to heart and lung channel. The compatibility of *Scutellaria baicalensis* and *Ren Dong Teng* has good effects of heat-clearing and detoxifying, dispelling wind and relieving the exterior (Xie and Preast, 2013). Therefore, it is reasonable for HTSP to treat chicken fever caused by LPS, which is consistent with the theory of Traditional Chinese Veterinary Medicine.

The gram-negative bacterial lipopolysaccharide (LPS), composed of cell-specific polysaccharide, lung-specific core polysaccharide and lipid A3, is a component contained in the outer membrane of gram-negative bacterial cell wall and a representative bacterial pyrogen and also a classic model for inducing fever in experimental animals (Akhtar *et al.*, 2012). According to De Boever's research, the endotoxin inflammation fever model of broiler chickens characterized by fever and cytokine expression has been successfully established in

the laboratory (De Boever *et al.*, 2008). Moreover, the fever is that the body stimulated by the heat activator such as LPS after a series of signal transduction, such as TLR4 signal pathway (Guo *et al.*, 2016), generates endogenous pyrogens, acts on the thermoregulatory center of the body of the preoptic area in the anterior hypothalamus (AH/POA) and releases the central mediators of fever. Thus, the set-point of body temperature are moved up. It has been proposed that LPS can effectively stimulate the organism to produce endogenous pyrogens IL-1 β , IL-6 and PGE₂, as well as Baihu Decoction can effectively reduce the levels of IL-1 and TNF- α in rabbit serum and hypothalamus (de Boever *et al.*, 2010; Jia *et al.*, 2013). More recently, Emílio-Silva *et al.* demonstrated that LPS could increase the levels of IL-1 β , IL-6, TNF- α and PGE₂ in plasma of rats (Emílio-Silva *et al.*, 2017). The proinflammatory cytokine, IL-1, is a key medium for local and systemic infection and inflammatory response. Intraperitoneal, intraventricular, intraventricular and hypothalamic injection of recombinant IL-1 can induce a variety of animals to cause fever (Zampronio *et al.*, 2015). The formation of proinflammatory mediums, IL-1 and TNF- α , is increased in infected and injured tissues which stimulate the hypothalamus to cause the increase of body temperature by enhancing the synthesis of PGE₂ in the preoptic area/anterior hypothalamus (Saper and Breder, 1994). In the process of animal fever caused by exogenous pyrogens such as LPS, the expression of PGE₂ mRNA and the levels of PGE₂ in the preoptic area (POA) are increased, and a large number of cAMP-positive cells could be observed (Ivanov *et al.*, 2002; Steiner and Branco, 2003). In 2015, Liu Xin and colleagues showed that the antipyretic effect is usually a characteristic function of drugs or compounds that have inhibitory effects on the biosynthesis or release of prostaglandins, that the shell and soft tissue of the *Thais luteostoma* can effectively reduce the levels of IL-1, IL-2, IL-6, TNF- α , PGE₂ and cAMP, and that the mechanism of antipyretic effect may be through inhibition the release of the levels of PGE₂ and cAMP (Liu *et al.*, 2015).

In this study, the antipyretic effect of HTSP was evaluated for the first time. The results showed that the body temperature of the chickens in each experimental Group (Group II to VI) increased and peaked after 5 h of LPS injection, and the body temperature of chickens in Group III, Group IV, Group V and Group VI was close to Group I after 12h and 24h of administration, furthermore, the body temperature of chickens in Group IV and Group V decreased the fastest. This shows that HTSP has a great antipyretic effect on chicken fever LPS-induced. The levels of IL-1, IL-6 and TNF- α in chickens' serum as well as the levels and the expression of mRNA of PGE₂ and cAMP in chickens' hypothalamus in each experimental group (Group II to VI) were significantly higher than Group I after 5 h of LPS injection. It is suggested that LPS can effectively stimulate the organism to produce endogenous pyrogens, IL-1, IL-6 and TNF- α , so that the levels are increased, which directly or indirectly stimulates the release of central mediators of fever (PGE₂ and cAMP) in the thermoregulatory center of the body, leading to the change of the set-point of body temperature of the thermoregulatory center of the body. This is consistent with the reports of Li-Long Jia and S. DE

BOEVER (de Boever *et al.*, 2010; Jia *et al.*, 2013). Nevertheless, After the administration of HTSP, the levels of IL-1, IL-6 and TNF- α in chickens' serum as well as the levels and the expression of mRNA of PGE₂ and cAMP in chickens' hypothalamus in Group IV and Group V significantly decreased. Additionally, it has been reported that IL-1 itself is known to be crucial inducers of PGE₂, and at the same time, PGE₂ and cAMP can promote the transcription of IL-1 (Zaslona *et al.*, 2017). Therefore, it is presumed that the mechanism may be by inhibiting the stimulation of exogenous pyrogens (IL-1, IL-6 and TNF- α) to the animal body and inhibiting the mRNA expression and the levels of PGE₂ and cAMP in the hypothalamus, so that the set-point of body temperature is lowered and thus play an antipyretic effect.

Conclusions: In conclusion, HTSP can decrease the levels of IL-1, IL-6 and TNF- α in chicken's serum as well as the levels and the expression of mRNA of PGE₂ and cAMP in chicken's hypothalamus and has good antipyretic effect on chicken fever LPS-induced.

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Authors contribution: JL conceived and designed the research. BLL, TTY, CFL and ZQW did the experiment and analyzed the data. JL, BLL and TTY wrote this paper, JL finally approved the version.

REFERENCES

- Akhtar R, He YQO, Larson CB, *et al.*, 2012. Differential stimulatory activities of smooth and rough brucella abortus lipopolysaccharide in murine macrophages. *Pak Vet J* 32:339-44.
- Bastos-Pereira AL, Leite MCG, Fraga D, *et al.* 2015. Central mediators involved in the febrile response induced by polyinosinic-polycytidylic acid: Lack of involvement of endothelins and substance p. *J Neuroimmunol* 278:100-7.
- China MoHotPsRo, 2015. *Scutellaria baicalensis*, Ren Dong Teng. In: Pharmacopoeia of the people's republic of china. 10th Ed., China Medical Science Press, Beijing, China pp:179-283.
- De Boever S, Neirinckx EA, Meyer E, *et al.* 2010. Pharmacodynamics of tepoxalin, sodium-salicylate and ketoprofen in an intravenous lipopolysaccharide inflammation model in broiler chickens. *J Vet Pharmacol Ther* 33:564-72.
- De Boever S, Beyaert R, Vandemaele F, *et al.*, 2008. The influence of age and repeated lipopolysaccharide administration on body temperature and the concentration of interleukin-6 and igm antibodies against lipopolysaccharide in broiler chickens. *Avian Pathol* 37:39-44.
- Dinarello CA, Ikejima T, Warner SJC, *et al.*, 1987. Interleukin-1 induces interleukin-1. I. Induction of circulating interleukin-1 in rabbits in vivo and in human mononuclear-cells invitro. *J Immunol* 139:1902-10.
- Emílio-Silva MT, Mota CMD, Hiruma-Lima CA, *et al.*, 2017. Antipyretic effects of citral and possible mechanisms of action. *Inflammation* 40:1735-41.
- Fan L, Lin C, Duan W, *et al.*, 2015. Rapid and quantitative determination of 10 major active components in *Ionicera japonica* thunb. by ultrahigh pressure extraction-hplc/dad. *High Press Res* 35:57-68.
- Guo Y, Xu J, Wang L, *et al.*, 2016. Leptospiral lipopolysaccharide-induced cytokine production is dependent on toll-like receptor 2 in bovine cells. *Pak Vet J* 36:280-5.

- Ivanov AI, Pero RS, Scheck AC, *et al.*, 2002. Prostaglandin e(2)-synthesizing enzymes in fever: Differential transcriptional regulation. *Amer J Physiol Regulat Integr Compar Physiol* 283:R1104-17.
- Jia LL, Li R, Ma J, *et al.*, 2013. Effects of bai-hu decoction on fever induced by lipopolysaccharide. *Kaohsiung J Med Sci* 29:128-32.
- Lieboldt MA, Frahm J, Halle I, *et al.* 2017. Haematological and febrile response to escherichia coli lipopolysaccharide in 12-week-old cockerels of genetically diverse layer lines fed diets with increasing l-arginine levels. *J Anim Physiol Anim Nutr (Berl)* 101:743-54.
- Liu SL, Chen JB, Zhou Q, *et al.*, 2012. Analysis of the harvest seasons of scutellaria baicalensis georgi by tri-step identification of infrared spectroscopy and principal component analysis. *Spectrosc Spect Anal* 32:2669-73.
- Liu X, Tang YP, Liu R, *et al.*, 2015. Antipyretic and anti-inflammatory activities of thais luteostoma extracts and underlying mechanisms. *Chin J Nat Med* 13:192-8.
- Muluye RA, Bian Y and Alemu PN, 2014. Anti-inflammatory and antimicrobial effects of heat-clearing chinese herbs: A current review. *J Trad Complem Med* 4:93-8.
- Nhoek P, Chae H-S, Masagalli J, *et al.* 2018. Discovery of flavonoids from scutellaria baicalensis with inhibitory activity against pcsk 9 expression: Isolation, synthesis and their biological evaluation. *Molecules* 23:504.
- Roth J and Blatteis CM, 2014. Mechanisms of fever production and lysis: Lessons from experimental lps fever. *Comp Physiol* 4:1563-604.
- Saper CB and Breder CD, 1994. The neurologic basis of fever. *New Engl J Med* 331:1309-9.
- Schneiders J, Fuchs F, Damm J, *et al.* 2015. The transcription factor nuclear factor interleukin 6 mediates pro- and anti-inflammatory responses during lps-induced systemic inflammation in mice. *Brain Behav Immun* 48:147-164.
- Steiner AA and Branco LGS, 2003. Preoptic pge2-camp and no-cgmp pathways in fever. *Faseb J* 17:27-8.
- Tsai CC, Lin MT, Wang JJ, *et al.*, 2006. The antipyretic effects of baicalin in lipopolysaccharide-evoked fever in rabbits. *Neuropharmacology* 51:709-17.
- Xie H and Preast V, 2013. Traditional chinese veterinary medicine: Fundamental principles. Chi Institute.
- Xiong JH, Li SC, Wang WJ, *et al.*, 2013. Screening and identification of the antibacterial bioactive compounds from lonicera japonica thunb. Leaves. *Food Chem* 138:327-33.
- Xu J, Yu Y, Shi R, *et al.*, 2018. Organ-specific metabolic shifts of flavonoids in scutellaria baicalensis at different growth and development stages. *Molecules* 23:428-47.
- Ye L, Tao YH, Wang YM, *et al.*, 2015. The effects of baicalin on the tlr2/4 signaling pathway in the peripheral blood mononuclear cells of a lipopolysaccharide-induced rat fever model. *Int Immunopharmacol* 25:106-11.
- Ye L, Tao Y, Wang Y, *et al.*, 2015. The effects of baicalin on the tlr2/4 signaling pathway in the peripheral blood mononuclear cells of a lipopolysaccharide-induced rat fever model. *Int Immunopharmacol* 25:106-11.
- Zampronio AR, Soares DM and Souza GE, 2015. Central mediators involved in the febrile response: Effects of antipyretic drugs. *Temperature (Austin)* 2:506-21.
- Zasłona Z, Pålsson-McDermott EM, Menon D, *et al.*, 2017. The Induction of Pro-IL-1 β by Lipopolysaccharide Requires Endogenous Prostaglandin E₂ Production. *J Immunol* 198:3558-64.
- Zhang CH, Zhang GJ and Sun SQ, 2010. Study on the identification of radix scutellariae and extract using fourier transform infrared spectroscopy and two-dimensional ir correlation spectroscopy. *Spectr Spectr Anal* 30:1774-9.
- Zhang S, Wang D, Dong S, *et al.*, 2017. Itraq-based quantitative proteomic analysis reveals bai-hu-tang enhances phagocytosis and cross-presentation against lps fever in rabbit. *J Ethnopharmacol* 207:1-7.