Research Article

DOI: http://dx.doi.org/10.53043/2347-3894.acam90033

Antipyretic Effect of Sophora Flavescens (Kushen) Decoction on Dry Yeast-Induced Fever in Rats

Eerdunchaolu[1,2],Minghai Fu[1,2] and Sungbo Cho[2]*

[1]NMPA Key Laboratory of Quality Control of Traditional Chinese Medicine (Mongolian Medicine), Inner Mongolia University for Nationalities, Tongliao 028000, China

[2]School of Mongolian Medicine, Inner Mongolia University for Nationalities, Tongliao 028000, China

Asian Journal of Complementary and Alternative Medicine. Volume 10 Issue 02

Published on: 21/04/2022

***Author for Correspondence:** Sungbo Cho, School of Mongolian Medicine, Inner Mongolia University for Nationalities, Tongliao 028000, China, E-mail: blue0555@hotmail.com

Cite this article as: Eerdunchaolu, Fu M, Cho S. *Antipyretic Effect of Sophora Flavescens (Kushen) Decoction on Dry Yeast-Induced Fever in Rats.* Asian Journal of Complementary and Alternative Medicine, Vol 10(2), 43-49:2022.

ABSTRACT

Objective: The current study investigated the effect of Sophora flavescens, known as Kushen decoction (KSD) on body temperature and metabolism against fever induction in Wistar rats.

Methods: Firstly UPLC-Q-TOF/MSE analysis was carried out for identifying the KSD composition. Fever model was induced by dry yeast and, orally administered at dose of KSD (500 mg/kg) to the rats. The alteration of the rectal temperature (TR) was estimated to evaluate the positive effect of KSD. TR changes were recorded over time. In addition, the antipyretic effect was confirmed from the hypothalamus of rats through real-time PCR.

Results: The model and treatment groups showed significantly increased TR and the mRNA expression of IL-1 and IL-6 after 5 hours, even though the expression level of arginine vasopressin (AVP) and TNF-alpha were not altered. The TR tests indicated the administration of KSD to fevered rats attenuated yeast-induced fever and, significantly reduced PGE2 expression levels in hypothalamus. The mRNA expression results reveal that dry yeast-induced fever could be mediated by PGE2 production.

Conclusions: KSD could play the antipyretic effect by reducing PGE2 expression in the hypothalamus. Subsequently, KSD inhibited the development of yeast-induced fever.

Keywords: Sophora flavescens decoction; Antipyretic effect; PGE2; Chemical composition

INTRODUCTION

Fever is commonly caused by infection, injury and inflammation. The higher body temperature than normal rage is a physiological response to systemic inflammation and pathological infection. It is a result of the complex pathological process between the peripheral immune system and the brain, which is assisting a host defense response [1,2]. After contact with a pathogen such as bacteria, viruses, fungi or an inflammatory stimulus, macrophages and other immune cells are activated to produce the proinflammatory mediators (IL-1β, IL-6, TNF-α) [3,4]. The proinflammatory mediators have originally acted on the body temperature regulation. These cytokines further induce the expression of cyclooxygenase-2 (COX-2) enzyme in the brain, which increase the synthesis of, cAMP, arginine vasopressin (AVP), prostaglandin E2 (PGE2) at hypothalamus area and causing the increase of heat production or reduction of heat dissipation, and eventually elevating the body temperature [3,5]. Current medicinal approaches for fever have focused on inhibiting the activity of COXs. As antipyretic drugs, nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely used [6] even though it has reported gastrointestinal side effects and cardiovascular risks [7,8].

Recently, there is an increasing interest in the herbal medicines. Many medicinal plants and herbs might possess anti-inflammatory and antipyretic properties through inhibition of prostaglandin production or inhibiting the production and/ or activity of proinflammatory mediators [9-11]. Sophora flavescens (S. flavescens) is often used in traditional medicine prescription as a functional food ingredient for health problem including fevers. S. flavescens, also named 'KuShen' in China, is a popular medicinal plant in ease Asia

and, used in decoction forms for the treatment, the boiling extraction method. The form of decoction is the process of the molecule transference from the original structure into the solution. Previous studies reported KuShen includes lots of phytochemicals such as quinolizidine alkalonids, flavonoids and triterpenoids [12,13]. So far, it is unclear how the major components of KuShen in the decoction have positive functions on the antipyretic effect. Therefore, our present study investigated the possible involvement of pro-inflammatory cytokines in this mechanism.

MATERIALS AND METHODS

Plant extract preparation

A powdered concentrate of KSD was provided by Affiliated Hospital of Inner Mongolia University for Nationalities, Tongliao, China. A 1000 g of powder was macerated in 5000 mL of distilled water for 1h. Then the mixture was boiled for 30min. After cooling, the supernatant (decoction) was collected and filtered with filter paper. After filtration, the decoction was evaporated to dryness under rotary evaporators. The yield of the extraction was 8.6%.

UPLC-MS analysis

Preparation of sample solutions: KSD powder (0.5 g) was sonicated in 100 ml of methanol for 30 min followed by centrifugation for 10 min at 3000 rpm. The supernatant was diluted with methanol, then filtered through 0.22 μm membrane and a 2 μl aliquot was injected into the UPLC system for analysis.

Liquid-Phase Conditions: The analysis was performed on a Waters Acquity UPLC System. The column was a Waters Acquity BEH - C18 (100 mm x 2.1 mm, 1.7 μm) operated at 40°C. The elution solvents were aqueous 0.1% formic acid (A) - acetonitrile (B). Samples were eluted using a linear gradient from 5% to 10% B in the first 0-5 min, followed by a linear gradient from 10% to 30% B from 5-20 min, 30% to 55% B from 20-45 min, and then 55% to 5% B from 45-50 min. The flow rate was 0.3 mL/min.

Mass Spectrometry Conditions: Mass spectrometry was performed with a Xevo G2S TOF (Waters MS Technologies, UK) using a mass spectrometer with ESI operating in negative ion mode for scanning. The scanning range was m/z 100 to 1000. The capillary voltage was 2.5 kV, and the sampling cone voltage was 40V. The ion source temperature was 100°C, and the atomization temperature was 280°C. The desolvation flow rate was 650 L/h. L-enk was the lock mass, and sodium formate was used to correct the mass axis. Spectral data were examined using MassLynx V4.1.

UPLC-Q-TOF/MSE analysis: The chemical structures of 18 components were characterized based on their retention behavior and MS information, and from reference to databases such as Scifinder and Chemspider, as well as the general literature.

Animals

The study was conducted using male Wistar rats (200-220 g), housed at 23 ± 1 °C and 40-60% humidity under a 12-h light dark cycle (lights on at 06:00 AM) with free access to food and water. The animals were habituated for at least one week before the experiment began. The experiments described here were approved by the Animal Ethics Committee (approval number: NM-LL-2021-06-15-1) of Inner Mongolia University for Nationalities in compliance with the Provisions and General Recommendation of the Chinese Experimental Animals Administration Legislation.

Dry yeast induced fever in rats and drug administration

In the first experiment, we assessed the fever model induced by commercially available dry yeast (production license number QS420528010005, China) in rats. Twenty rats were divided into two groups containing ten in each. Animals were trained for two consecutive days for habituation to rectal temperature (T_R) measurement using a lubricated thermocouple inserted into the rectum. After measuring the initial T_R in the experimental day, animals were subcutaneously injected with 1g/kg of dry yeast as a 20% suspension in 0.9% saline solution (Engelhardt et al., 1995). T_R changes were recorded and evaluated every 30 min up to 15 h. The pattern of TR changes in the yeast-induced fever rats were associated with immature and mature phase in TMM theory.

In the second experiment, we assessed whether KSD prevents yeast-induced fever in rats. The animals were injected with dry yeast (20% yeast at 1g/kg) or vehicle (0.9% NaCl) to induce fever. Meanwhile, previously injected with yeast rats immediately received oral administration of KSD (500 mg/kg) or vehicle (5% Tween 80 in 0.9% NaCl). In the end, animals were anesthetized and decapitated at 5h and 9h after drug administration. The brain tissue was then collected for gene expression analysis.

Real time PCR analysis

Real time PCR assays were developed to study the expression of fever related genes in rats' brain. The sequences of the primers used are listed in supplementary Table 1. In brief, the methodology consisted of the total RNA extraction from the tissue samples using a TRIZOL-chloroform method. Purification and cDNA synthesis were performed using Qiagen RNeasy column and Qiagen Reverse transcription kit. The

Table 1: Sequences of primers, fragment sizes.

Gene	GB.accession#		Primer Sequence 5'→3'	Amplicon
GAPDH	NM_017008.4	F	TGCTGAGTATGTCGTGGAG	288
		R	GTCTTCTGAGTGGCAGTGAT	
IL-1	NM_031512.2	F	TGTCCGTGTGTATGGGATGAA	141
		R	TCATCAAAATGATGTGCTTGTGCT	
IL-6	NM_012589.2	F	CCCAACTTCCAATGCTCTCCT	142
		R	AGCACACTAGGTTTGCCGAG	
PGE2	NM_031088.1	F	AAAAGTGGGACCTCCGAGC	115
		R	CAACAGAGGACTGAGCGCAT	
TNF-a	NM_012675.3	F	GTGATCGGTCCCAACAAGGA	141
		R	CGCTTGGTGGTTTGCTACG	
AVP	NM_016992.2	F	AGCGATGAGAGCTGCGTG	134
		R	GCCAGCTGTACCAGCCTAAG	

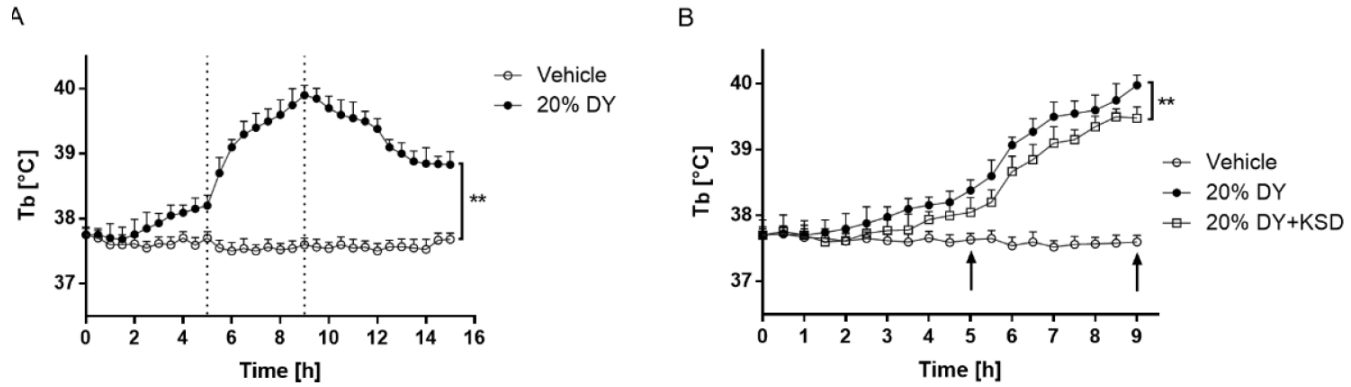

Figure1: Rectal temperature of rats in each group. A. effect of subcutaneous administration of vehicle (0.9% NaCl) or dry yeast (20% yeast at 1g/kg) on rectal temperature change (TR) over time. B. Effect of the subcutaneous administration of saline (0.9% NaCl), vehicle (5% Tween 80 in 0.9% NaCl), and KSD (500 mg/kg) on dry yeast (20% yeast at 1g/kg). Dry yeast was injected at time zero. The arrow indicates time of animal sacrifice. Values represent mean ± SEM change. ** indicates P<0.01 compared with dry yeast-treated or vehicle group.

real time qPCR assays were carried out using SYBR green in a ViiATM 7 Real-Time PCR system (Applied Biosystems, Life Technologies). The relative gene expression levels were estimated using the Pfaffl method (Pfaffl, 2001) that taking into account the cycle threshold (CT) values of both the candidate genes and of the two-reference gene GAPDH. The single products/bands of the predicted size of PCR products from the specific primers were performed on Agarose gels (2%) electrophoresis.

Statistical analysis

The data were analysed by one-way ANOVA using GraphPad Prism 7 (Graph Pad Software, Inc). Carcass P2 and loin muscle depth were analysed using the hot carcass weight (HCW) as a covariant. Linear and quadratic polynomial contrasts were used to evaluate the effects of the dose response. All reported means are least square means. Comparisons among groups were assessed using LSD Multiple Comparisons test. Linear regression and Pearson correlation analysis was performed using GraphPad Prism 7 (Graph Pad Software, Inc). In double-choice preference test, the average preference rate of different treatments was compared to the neutral value (50%) by using the Student's t test. The significance level and tendency were considered at $P \leq 0.05$ and $P \leq 0.10$.

RESULTS

Dry yeast induced fever and the effects of KSD on dry yeast-induced pyrexia

The effect of KSD on body temperature was investigated using yeast-induced pyrexia mice model. Subcutaneous injection of dry yeast evoked a significant elevation of rectal temperature (TR) 5hours later, and reached the peak within 9h (P<0.01), then declined gradually during time period observed (Figure 1A). The results showed that yeast could cause significant increase of fever. The rectal temperature (TR) of young rats over time is shown in Figure 1B. The elevation of TR was significantly suppressed by KSD after 5 hours from the onset of the pyrexia.

Phytochemical Analysis of Sophora flavescens Decoction by UPLC-Q/TOF-MS

In this study, UPLC-Q/TOF-MS analysis was conducted on KSD to confirm its biological composition. All compounds were tentatively assigned by matching the empirical molecular formula of each constituent with those of the published compounds and/or by elucidating the quasi-molecular ions and fragment ions. A total of 18 of them were identified (Table 2). Typical base peak intensity (BPI) chromatogram of the KSD is presented in Figure 2, with the properties of identified compounds listed in Table 2. The chemical structures of seven representative compounds are illustrated in Figure 2. The majority of them had been reported as the main bioactive compounds of KSD.

The effects of KSD on inhibiting production of pyrogenic factors

The Real-time PCR method detected the mRNA expression in the hypothalamus of rats in each experimental group. The expression results are shown in Figure 3. The mRNA expression level of IL-1β, IL-6, PGE2, TNF-α and AVP in different groups were shown in Figure 3. The level of IL-1β, and IL-6 in both model and KSD group were all increased at 5 hours ($p<0.05$). And compared with model group, ones of KSD group had higher expression ($p<0.01$). Whereas, the expression level of those two were significantly decreased at 9 hours ($p<0.05$). The AVP and TNF-α expression was not altered in all groups. The expression level of PGE2 in model group was increased at 9 hours, while the expression of PGE2 was dramatically reduced in KSD group.

Table 2: The identified phytochemical compounds in as Sophora flavescens (KSD) decoction detected by UHPLC-MS.

No.	Identification	RT (minute)
1	Monobenzyl phthalate	2.986
2	3-Carboxy-4-methyl-5-propyl-2-furanpropanoic acid	6.731
3	Acacetin or Biochanin A	9.784
4	Biochanin A	10.205
5	Baicalein	11.574
6	Pectolinarin	14.545
7	Reserpine	15.084
8	3-Hydroxy-3'-methoxyflavon	15.387
9	3',4'-Dihydroxyflavone	16.642
10	5,7-Dihydroxy-2'-methoxyflavone	17.197
11	7-Hydroxy-2'-methoxyflavone	23.941
12	Xanthohumol	26.111
13	Kushenol N	29.781
14	Kurarinone	31.755
15	Kotanin	33.606
16	Dihomo-alpha-linolenic acid	34.849
17	Blasticidin	35.302
18	sophoraflavanone G	36.978
RT means retention time		

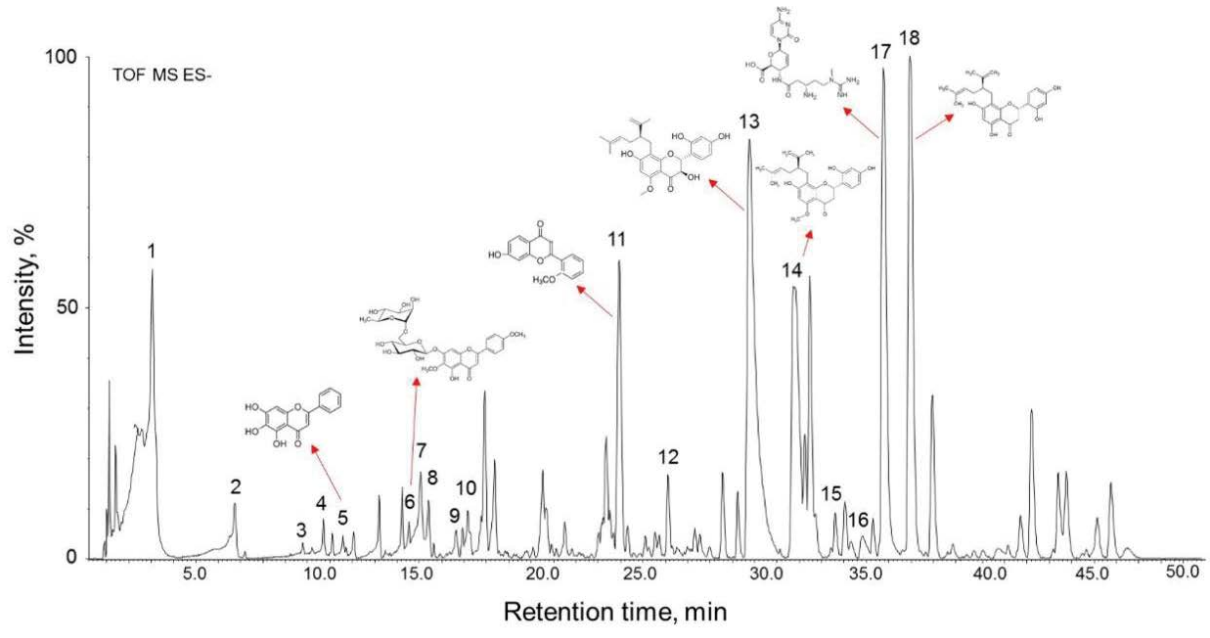

Figure 2: Representative base peak intensity (BPI) chromatograms of Sophoraflavescens decoction analysed by UHPLC-MS.

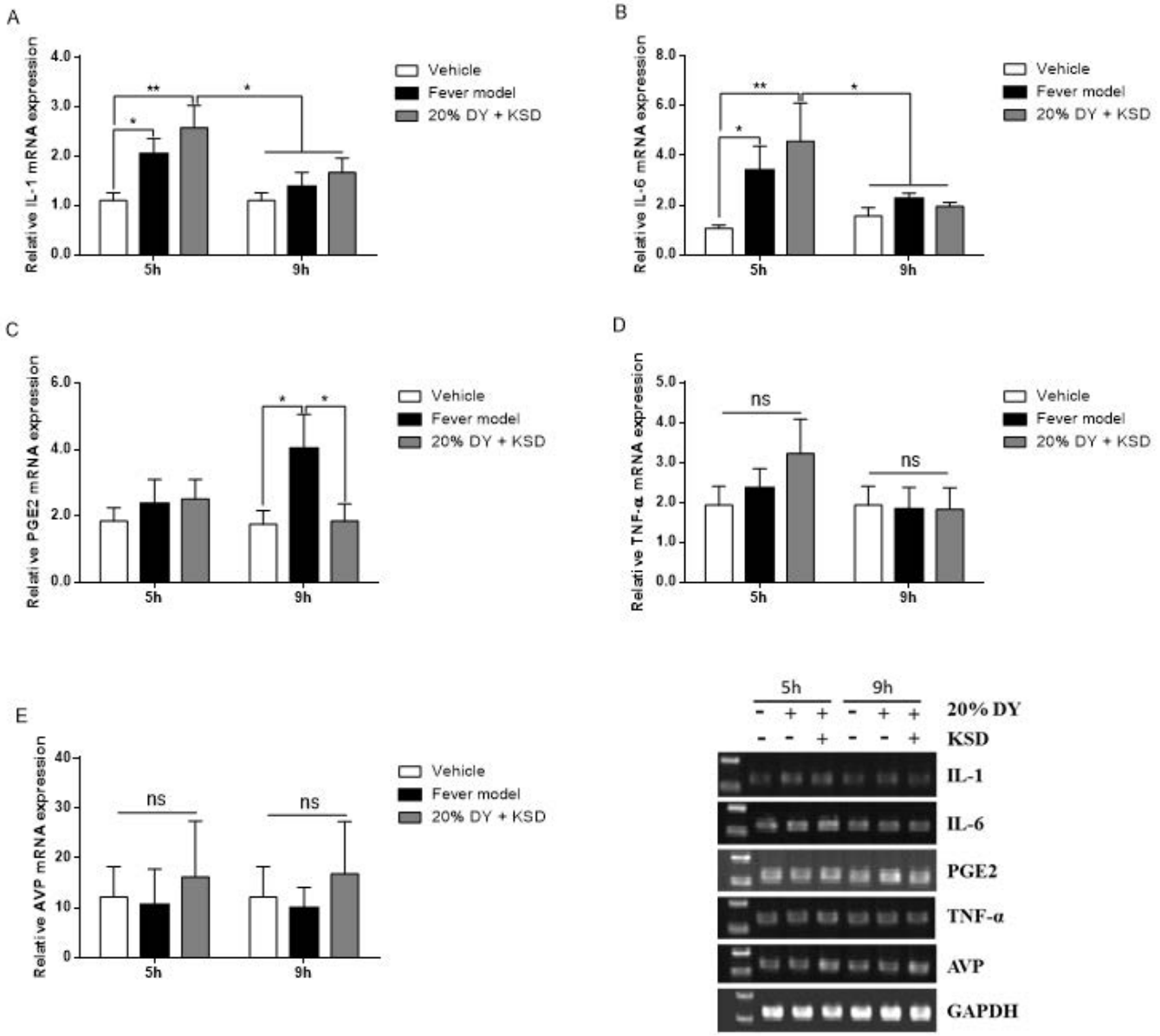

Figure 3: Effects of Sophora flavescens decoction on the RNA expression levels of the inflammatory mediators. A-E. relative mRNA expression level of TNF-alpha, IL-1β, and IL-6, PGE2, AVP in rats' brains under dry yeast induced fever. (n=5, *P < 0.05, **P < 0.01 versus control or model group).

DISCUSSION

In the present study, to identify protective effects of KuShen decoction (KSD) on the dry yeast induced pyrexia, we evaluated if the specific compound has antifungal activity and/or alteration of the mRNA expression level of leukocyte and cytokine in hypothalamus. we first identified 38 compounds in KSD by UPLC-Q/TOF-MS and then investigated that the compounds could prevent the increase of body temperature induced by dry yeast. For the investigation of plants extracts and, synthesis of drugs regarding its antipyretic effect, the pyrogens, which induce fever in animal such as dry yeast, lipopolysaccharides (LPS) and 2, 4-dinitrophenol are often used as a fever model in rat [14,15]. Especially, the dry yeast induced fever model is preferred due to its' good stability, reproducibility and availability [16]. As expected, our model group clearly indicated the increased rectal temperature after 5 hours and, the highest temperature was reached at 9 hours after the dry yeast injection.

The dry yeast-induced fever coincided with a dramatic increase in mRNA expression level of IL-1 and IL-6 at 5 hours, yet those were not differentially expressed at 9 hours (Figure 3). These results indicated that KSD had different

time of callback effects on IL-1β, IL-6, and PGE2 in fevered rats. The external pyrogens like bacterial endotoxins trigger the release of endogenous pyrogens including IL-1, IL-6 and TNF-α induce fever and other inflammatory response, which impair homeostasis of the immune system [17]. In this study, the dry yeast effectively activated the cytokine network, which stimulates IL-1 and IL-6. These IL-1b and IL-6 as primary mediators are involved in acute phase response and additional stimulation of central thermoregulatory organ, hypothalamus [18,19]. The inflammatory signals in the peripheral organs and tissues stimulate a cross communication between the CNS and the immune system, which promotes the CNS to balance the immune system [20,21]. Then, antipyretic mechanisms are initiated to balance the released pro-inflammatory cytokines. The mechanisms stimulate the production of anti-inflammatory cytokines such as internalization of endotoxins-receptor complexes, down regulation of cytokine and the circulation of IL-1 and TNF receptors and its antagonists [22,23]. The expression of IL-1b, and IL-6 in the hypothalamus indicated the fever development in this study, but the inhibitory effect of KSD on cytokine production or release was not shown in the mRNA expression, which implies that KSD may not directly inhibit dry yeast-induced fever by decreasing pyrogenic cytokine levels.

On the other hand, in our study, pre-treatment with KSD inhibited PGE2 expression in the brain tissues. We confirm that at 9 hours KSD attenuated expression level of prostaglandin E2 (PGE2) in the hypothalamus. KSD seems to play an important role in reduction of PGE2 expression. It is well known that PGE2 plays a very important role in central temperature regulation. The dry yeast induced pathogenic fever is activated by the high synthesis of prostaglandin [24]. Prostaglandins such as PGE2 are considered the main messenger of dry yeast-induced fever [25]. Thus, inhibiting PGE2 production had antipyretic effect. The results indicated KSD prevented the fever induced by the hypothalamic PGE2. PGE2 production is mainly catalyzed by cyclooxygenase (COX) enzymes including two distinct isoforms, COX-1 and COX-2 [26]. The pro-inflammatory mediators (or cytokines) such as IL-1, IL-6 and TNF-a promote cerebrovascular cells to stimulate COX-2 expression, which result in initiating fever [27]. In details, COX-2 enzyme induced by the cytokines in the brain could transform arachidonic acid to the formation of PGE2. The PGE2 disturbs the heat loss and heat retention [28,29]. As mentioned above, the mRNA expressions of IL-1, Il-6 and TNF-a were not significantly different between model and treatment group. Thus, the antipyretic effect of KSD might not be associated with interfering with pro-inflammatory mediators, but might straightly inhibiting COX-2 activity. It is well-known the paracetamol exerts the antipyretic action from paracetamol prevented the increase of PGE2 in cerebrospinal fluid and blocking the activity of COX-2. It is reasonable to conclude that mechanisms down-regulation of the PGE2 mediate the antipyretic effect. Consequently, the results suggested that KSD has antipyretic effect against yeast-induced fever, and indicate that this effect appears to be due to inhibition of PGE2 production.

CONCLUSION

The present study reported that the administration of Sophora flavescens (Kushen) decoction has a role of Antipyretic effect in rats. The elevation of rectal temperature was significantly suppressed by KSD after 5 hours. In the mRNA expression, the level of IL-1b, and IL-6 in hypothalamus was not decreased but, PGE2 expression was significantly decreased by KSD. These findings provide scientific evidence for antipyretic effect of KSD. Thus, further studies require the isolation and characterization of the active compounds of KSD responsible for PGE2 expression and antipyretic effect.

REFERENCES

1. Zeisberger E (1999) From humoral fever to neuroimmunological control of fever. Journal of Thermal Biology 24: 287-326.
2. Szelényi J (2001) Cytokines and the central nervous system. Brain Research Bulletin 54: 329-338.
3. Blomqvist A, Engblom D (2018) Neural Mechanisms of Inflammation-Induced Fever. The Neuroscientist 24: 381-399.
4. Roth J, Blatteis CM (2014) Mechanisms of fever production and lysis: lessons from experimental LPS fever. Compr Physiol 4: 1563-604.
5. Saper CB, Breder CD (1994) The Neurologic Basis of Fever. New England Journal of Medicine 330: 1880-1886.
6. Charlier C, Michaux C (2003) Dual inhibition of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-inflammatory drugs. European Journal of Medicinal Chemistry 38: 645-659.
7. Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, et al. (2005) Cardiovascular Events Associated with Rofecoxib in a Colorectal Adenoma Chemoprevention Trial. New England Journal of Medicine 352: 1092-1102.
8. Chan FKL, Graham DY (2004) Prevention of non-steroidal anti-inflammatory drug gastrointestinal complications – review and recommendations based on risk assessment. Alimentary Pharmacology & Therapeutics 19: 1051-1061.
9. Arul V, Miyazaki S, Dhananjayan R (2005) Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of Aegle marmelos Corr. Journal of Ethnopharmacology 96: 159-163.

10. Olajide OA, Awe SO, Makinde JM, Ekhelar AI, Olusola A, et al. (2000) Studies on the anti-inflammatory, antipyretic and analgesic properties of Alstonia boonei stem bark. Journal of Ethnopharmacology 71: 179-186.

11. Chattopadhyay D, Arunachalam G, Mandal AB, Mandal SC (2002) Evaluation of antipyretic activity of leaf extracts of Mallotus peltatus (Geist) Muell. Arg. var acuminatus: A folk medicine. Phytomedicine 9: 727-730.

12. Huang R, Liu Y, Zhao LL, Chen XX, Wang F, et al. (2017) A new flavonoid from Sophora flavescens Ait. Natural Product Research 31: 2228-2232.

13. Li JJ, Zhang X, Shen XC, Long QD, XU CY, et al. (2020) Phytochemistry and biological properties of isoprenoid flavonoids from Sophora flavescens Ait. Fitoterapia 143: 104556.

14. Trongsakul S, Panthong A, Kanjanapothi D, Taesotikul T (2003) The analgesic, antipyretic and anti-inflammatory activity of Diospyros variegata Kruz. Journal of Ethnopharmacology 85: 221-225.

15. Gao X, Huang C, Geng T, Chen X, Wang J, et al. (2020) Serum and urine metabolomics based on UPLC-Q-TOF/MS reveals the antipyretic mechanism of Reduning injection in a rat model. Journal of Ethnopharmacology 250: 112429.

16. Zhang F, Wang D, Li X, Li Z, Chao J, et al. (2013) Metabolomic study of the fever model induced by baker's yeast and the antipyretic effects of aspirin in rats using nuclear magnetic resonance and gas chromatography–mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 81-82: 168-177.

17. Prajitha N, Athira S, Mohanan P (2019) Comprehensive biology of antipyretic pathways. Cytokine 116: 120-127.

18. Bluthé RM, Michaud B, Poli V, Dantzer R (2000) Role of IL-6 in cytokine-induced sickness behavior: a study with IL-6 deficient mice. Physiology & Behavior 70: 367-373.

19. Klir JJ, McClellan JL, Kluger MJ (1994) Interleukin-1 beta causes the increase in anterior hypothalamic interleukin-6 during LPS-induced fever in rats. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 266: R1845-R1848.

20. Tracey KJ (2002) The inflammatory reflex. Nature 420: 853-859.

21. Martelli D, Yao ST, Mckinley MJ, McAllen RM (2014) Reflex control of inflammation by sympathetic nerves, not the vagus. The Journal of Physiology 592: 1677-1686.

22. McCarthy PL (1994) 8 Down-regulation of cytokine action. Baillière's Clinical Haematology 7: 153-177.

23. Rothwell NJ (2013) Cytokines in the Nervous System. 2013: Springer Science & Business Media.

24. Moltz H (1993) Fever: Causes and consequences. Neuroscience & Biobehavioral Reviews 17: 237-269.

25. Ataŏglu H, Dogan MD, Mustafa F, Akarsu ES (2000) Candida albicans and Saccharomyces cerevisiae cell wall mannans produce fever in rats: Role of nitric oxide and cytokines. Life Sciences 67: 2247-2256.

26. Vane J (1994) Towards a better aspirin. Nature 367: 215-216.

27. Steiner AA, Chakravarty S, Rudaya AY, Herkenham M, Romanovsky AA (2006) Bacterial lipopolysaccharide fever is initiated via Toll-like receptor 4 on hematopoietic cells. Blood 107: 4000-4002.

28. Inoue W, Matsumura K, Yamagata K, Takemiya T, Shiraki T, et al. (2002) Brain-specific endothelial induction of prostaglandin E2 synthesis enzymes and its temporal relation to fever. Neuroscience Research 44: 51-61.

29. Ivanov AI, Pero RS, Scheck AC, Roamnovsky AA (2002) Prostaglandin E2-synthesizing enzymes in fever: differential transcriptional regulation. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 283: R1104-R1117.