



Available online at SciVerse ScienceDirect

Chinese Herbal Medicines (CHM)

ISSN 1674-6384

Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

Original article

Antipyretic Effects of *Eupatorium chinense* and Its Mechanism

Yi-ping Jiang, Jiang-ping Xu*

Department of Neuropharmacology, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, China

ARTICLE INFO

Article history

Received: May 13, 2015

Revised: July 2, 2015

Accepted: August 1, 2015

Available online:

November 10, 2015

DOI:

10.1016/S1674-6384(15)60059-4

ABSTRACT

Objective To investigate the antipyretic effect of *Eupatorium chinense* and its mechanism. **Methods** The content of arginine vasopressin (AVP) in ventral septal area (VSA) and blood plasma, and cyclic adenosine monophosphate (cAMP) levels of hypothalamus and blood plasma were determined by enzyme linked immunosorbent assay. **Results** The body temperature (Tb) was decreased at 1 h after administration of *E. chinense* (3 and 6 g/kg) and Aspirin (0.3 g/kg) respectively, which was significantly different from the temperature of fever model group. The antipyretic effect of Aspirin and *E. chinense* lasted for longer time. Aspirin (0.3 g/kg) and *E. chinense* (3 and 6 g/kg) reduced the level of cAMP in hypothalamus of fever rats and increased AVP content in plasma. The changes in cAMP content in plasma of all drug treatment groups were not obvious. **Conclusion** *E. chinense* has strong antipyretic effect and may affect the production of AVP and cAMP in fever rats.

Key words

antipyretic; arginine vasopressin; cyclic adenosine monophosphate; *Eupatorium chinense*; ventral septal area

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1. Introduction

Eupatorium chinense L. (Compositae) (Liao et al, 2010) is mainly distributed in the south part of China. *Eupatorium* L. is a genus of flowering plants containing from 36 to 60 species, most of which are herbaceous perennial plants growing to 0.5–3 m tall, but a few are shrubs. The whole plant is usually harvested between May and June, and then air dried. The dried roots, called Guangdong Tunixi (Zhang et al, 2009), have been used medicinally for the treatment of coughing, amygdalitis, diphtheria, and other laryngological diseases in Guangdong province, China, for a long time (Editorial Committee of Chinese Materia Medica). Species of *Eupatorium* L. have been used in folk medicine, for instance, to excrete uric acid which

causes gout, numbers of sesquiterpenoids and sterols were reported from the whole plants. But they also contain toxic compounds that can cause liver damage (Itoh et al, 2009). Pharmacological studies showed that it had anti-inflammatory, analgesia, antiseptis, and anticancer effects.

Previously, several sesquiterpene lactones were isolated from *E. chinense* and the structures of the compounds contained in the fraction were determined. Parthenolide, a major sesquiterpene lactone, had been known to inhibit the growth of tumor cells. Especially, bioactive effect of parthenolide was mediated by preventing NF- κ B signaling (Yao et al, 2012). Thus, sesquiterpene lactones may be candidates of cancer preventive agents.

In Japan, it had been reported that species of *Eupatorium*

* Corresponding author: Xu JP E-mail: jpx@smu.edu.cn Tel: 86-20-6164 8236

L. had antibacterial, anti-inflammatory, anti-oxidant, and anti-tumor activities (Itoh et al, 2009).

Our previous work verified that *E. chinense* had the action of analgesia and anti-inflammation (Jiang et al, 2013). In this study, the animal models were established by sc injecting yeast suspension and then treated with the extract of *E. chinense*. Aspirin was used as the positive control for comparison. The antipyretic effect of *E. chinense* and its mechanism were investigated in this paper, thus to provide the theoretical basis for clinical application.

2. Materials and methods

2.1 Materials

Commercially available dry yeast was obtained from Guangdong Meishan-Marley Yeast Co., Ltd. Aspirin was obtained from Wuhan Yuancheng Biotechnology Co., Ltd. *Eupatorium chinense* L. was obtained from Zhejiang Jinhua region. Rat arginine vasopressin (AVP) ELISA determination reagent kit and rat cyclic adenosine monophosphate (cAMP) ELISA determination reagent kit were obtained from R & D Co., Ltd. Using 3.8% sodium citrate as anticoagulants, sodium citrate was obtained from Guangzhou Chemical Reagent Factory. Thermometer and medical thermostat water bath were obtained from Shanghai Tianmei Science Instrument Co., Ltd. Spectrum Microplate Spectrophotometer (Model 680) was obtained from America BIO-RAD. Pulverizer was obtained from Tester Tianjin Co., Ltd.

2.2 Herbal medicine immersion and decocting method

The dried stems and leaves of *E. chinense* (344 g) were weighed accurately, which was collected from Jinhua District, Zhejiang province during May and June, and taken into stainless steel pot after grinding. The herb was soaked with distilled water for 1 h before decocting, and added water of six times the weight of the herbs, heated to boiling, then mildly heated for 30 min, and the filtrate was filtered and collected. Water of four times the weight of the herbs was added, heated to boiling, then mildly heated for 30 min, and the filtrate was filtered and collected. The two parts of filtrate were combined, concentrated on a rotary evaporator to obtain 282 mL of liquid extract with content of 4 g/mL, and stored at 4 °C. On the day of use, the liquid extract was diluted to suitable concentration with distilled water.

2.3 Animals and housing

Wistar rats (male, weighing 180–200 g) were provided by the animal house of Southern Medical University. Animals were housed in groups, eight rats per cage, and maintained in a room with controlled temperature of (24 ± 1) °C, 12 h light/dark cycle (lights on at 6:00 AM), and standard laboratory chow and tap water *ad libitum* (Ferreira et al, 2012).

The rats were allowed to habituate to the housing

facilities for at least one week before the experiment. All animal treatments were performed strictly in accordance with the National Institutes of Health Guide of the Care and Use of Laboratory Animals. The experiments were carried out under the approval of the Committee of Experimental Animal Administration of the University.

2.4 Temperature measurements and fever induction

Body temperature (Tb) was measured by gently inserting a lubricated thermometer (external diameter of 3 mm) into the rectum at 4.0 cm for 1 min. During the temperature measurement, the rat was gently held by hand, without removing from its home cage. This procedure was performed at least twice two days prior to the experiments, thus to avoid the rectal temperature changes after handling. On the day of the experiments, the Tb of each animal was stabilized for 1 h and the baseline temperature was determined for three times, at 30 min intervals, and the average temperature was calculated. Only the animals with stable Tb in the range of 36.4–37.4 °C were used to investigate the effect of drug application. All the animals were used only once in this experiment (Hu et al, 2008).

Febrile response was induced and monitored over time according to Tomazetti et al (2005). Briefly, immediately after measuring the basal Tb at 8:00, the animals were sc injected with 20% yeast suspension (10 mL/kg) in the back. Tb was measured at 30 min intervals and recorded Tb every hour for 8 h. Made one measurement once at every time point and recorded the values displayed.

At the 5 h point (fever peak), removed the rats whose Tb raise was less than 0.6 °C, then randomly divided the qualified rats into different groups.

2.5 Grouping and drug administration

The qualified rats were randomly divided into five groups ($n = 6$): normal group (normothermia), model group (sc with 20% yeast, ig NS); Aspirin group (sc with 20% yeast, ig 0.3 g/kg Aspirin); *E. chinense* groups (sc with 20% yeast, ig 3 and 6 g/kg *E. chinense*).

For the *E. chinense* groups, the dosage strength of *E. chinense* was determined according to previous summary on *E. chinense* (Li et al, 2012) and previous experiment results (Jiang et al, 2013).

Tb was measured at 30 min intervals, temperature was recorded at the time points of 6, 7, and 8 h, and the difference of temperature was calculated at 5 h.

2.6 Measurement of AVP and cAMP concentration

After the last temperature measurement, the rats were anaesthetized and decapitated to obtain hypothalamus and ventral septal area (VSA), preserved in the refrigerator at –80 °C after liquid nitrogen freezing. Blood plasma was collected by abdominal aortic method (anticoagulation reagent: blood = 1:9) and centrifuged at 3600 g for 30 min, the extract plasma

was stored in the refrigerator at $-80\text{ }^{\circ}\text{C}$. The contents of AVP and cAMP were measured using a commercially available reagent kit from R & D Systems.

2.7 Statistical analysis

The results were presented as $\bar{x} \pm s$. The variations in Tb were expressed as changes from the initial value (ΔTb , $^{\circ}\text{C}$). The data were analyzed by two-way analysis of variance (ANOVA) with treatment as one factor and the sampling period as the other factor (repeated measurement). Differences were considered to be significant when $P < 0.05$ and statistically significant when $P < 0.01$.

3. Results

3.1 Antipyretic activity of *E. chinense* on established dry yeast induced fever

Whether *E. chinense* (3 and 6 g/kg) has the effect on fever induced by dry yeast was investigated. Statistical analysis of Tb change over time revealed that *E. chinense* could significantly attenuate the dry yeast-induced fever.

Tb of the normal group remained essentially unchanged as time prolonged (Table 1), Tb of the model control group was significantly raised compared with the normal group ($P < 0.01$), and continued to raise at the point of 6, 7, and 8 h (0.92 ± 0.6 , 1.2 ± 0.5 , and 1.3 ± 0.5), with significant difference.

Aspirin (0.3 g/kg) had significant antipyretic effect, differences at the point of 6, 7, and 8 h were (-0.62 ± 0.1 , -0.88 ± 0.1 , and -0.97 ± 0.1), separately.

E. chinense (3 g/kg) had weak antipyretic effect, with differences of -0.27 ± 0.1 and -0.38 ± 0.2 at 6 and 7 h,

respectively, and had significant antipyretic effect at 8 h, with difference of -0.58 ± 0.2 .

E. chinense (6 g/kg) had strong antipyretic effect after drug administration, with difference of -0.42 ± 0.1 , -0.63 ± 0.2 , and -0.88 ± 0.2 at 6, 7, and 8 h, respectively.

As indicated by the results, Aspirin (0.3 g/kg) and *E. chinense* (3 and 6 g/kg) had significant antipyretic effect and lasted for 3 h. *E. chinense* (6 g/kg) had similar antipyretic effect to Aspirin (0.3 g/kg).

3.2 Contents of AVP and cAMP in plasma, VSA, and hypothalamus of fever rats

The contents of AVP in VSA and the content of cAMP in hypothalamus of the rats in model group were all significantly higher than those in the normal group ($P < 0.01$). In all drug treatment groups, the content of cAMP in hypothalamus and the content of AVP in VSA were all significantly lower than those in the model group; but the content of cAMP in plasma was essentially unchanged (Table 2).

4. Discussion

Fever is part of defense response known as “acute phase reaction”, which occurs during inflammatory process induced by several origins, including the currently reported yeast-induced peritonitis.

The model of fever rat is prepared by sc injection with yeast, and the region begins to rot, triggers strong inflammation, and ultimately causes fever. This simulates pathological state of fever and is used to investigate the antipyretic effect of heat-drug.

The results indicate that 3 and 6 g/kg *E. chinense* have

Table 1 Effect of *E. chinense* on yeast-induced febrile response of fever rats

Groups	Doses / (g·kg ⁻¹)	Basal body temperature / $^{\circ}\text{C}$	Fever peaks / $^{\circ}\text{C}$		ΔTb / $^{\circ}\text{C}$	
			5 h	6 h	7 h	8 h
normal		38.3 ± 0.1	38.4 ± 0.1	0.13 ± 0.1	0.12 ± 0.1	0.13 ± 0.1
model		38.4 ± 0.2	39.3 ± 0.3	$0.92 \pm 0.6^{\Delta}$	$1.20 \pm 0.5^{\Delta}$	$1.30 \pm 0.5^{\Delta}$
Aspirin	0.3	38.5 ± 0.1	39.5 ± 0.2	$-0.62 \pm 0.1^*$	$-0.88 \pm 0.1^*$	$-0.97 \pm 0.1^*$
<i>E. chinense</i>	6.0	38.5 ± 0.1	39.5 ± 0.2	$-0.42 \pm 0.1^*$	$-0.63 \pm 0.2^*$	$-0.88 \pm 0.2^*$
<i>E. chinense</i>	3.0	38.5 ± 0.2	39.4 ± 0.3	$-0.27 \pm 0.1^*$	$-0.38 \pm 0.2^*$	$-0.58 \pm 0.2^*$

$^{\Delta}P < 0.01$ vs normal group; $^*P < 0.01$ vs model group

GraphPad Prism 5.0 showed that the febrile effect in the model group was significant. The antipyretic effect of 0.3 g/kg Aspirin was outstanding and the Tb of 0.3 g/kg Aspirin group returned to near normal 3 h after the drug administration. *E. chinense* (3 and 6 g/kg) showed antipyretic effect 0.5 h after administration and the effect lasted for 3 h

Table 2 Effect of *E. chinense* on contents of AVP and cAMP in plasma, VSA and hypothalamus of fever rats induced by dried yeast

Groups	Doses / (g·kg ⁻¹)	AVP / (pg·mL ⁻¹)		cAMP / (nmol·L ⁻¹)	
		VSA	plasma	hypothalamus	plasma
normal		14.78 ± 0.83	2.08 ± 0.57	1.39 ± 0.38	2.79 ± 2.05
model		$24.21 \pm 1.39^{\Delta}$	3.01 ± 1.67	$10.20 \pm 2.56^{\Delta}$	2.66 ± 1.67
Aspirin 0.3 g/kg	0.3	$15.52 \pm 1.08^{**}$	$8.40 \pm 2.86^{\Delta**}$	$3.73 \pm 0.85^{**}$	3.03 ± 0.72
<i>E. chinense</i> 6 g/kg	6.0	$16.47 \pm 2.10^{**}$	$7.52 \pm 3.86^{\Delta**}$	$4.83 \pm 1.16^{**}$	4.34 ± 3.26
<i>E. chinense</i> 3 g/kg	3.0	$19.41 \pm 3.61^{**}$	$5.94 \pm 1.29^{\Delta*}$	$5.57 \pm 0.74^{**}$	3.23 ± 2.18

$^{\Delta}P < 0.01$ vs normal group; $^*P < 0.05$ $^{**}P < 0.01$ vs model group

antipyretic effect and the effect is positively related to the dosage. The results show the significant difference compared with the model group.

Modern physiology and pathophysiology believe that the mature human and mammals can keep their Tb constant by automatic control by the Tb regulation center, thus to maintain the normal conduct of all life activities. When pyrogen invades the body, the thermoregulation set-point raises, and consequently, the heat production increases and the heat dissipation decreases, and the equilibrium at a new thermoregulation set-point is achieved (Jin et al, 2000). Thermoregulation involves multiple parts of the central nervous system in the process of heating. Among them, the preoptic anterior hypothalamic area (POA-AH) is the temperature regulating center. POA-AH contains temperature-sensitive neurons and plays the role of integrating temperature information. Pyrogen acts on POA-AH, causes fever, and at the same time the medium of heat can be detected, such as prostaglandins, cAMP, etc.

As the data show, most domestic scholars argue that the central medium of fever is cAMP (Hu et al, 1990). For example, iv injection with endotoxin in rabbit causes dual phase heat, and the cAMP content in hypothalamus and cerebrospinal fluid are significantly increased, positively related to temperature rise significantly.

Besides, the body parts, such as amygdale, ventral septal, nucleus arciformis, etc., have negative influence on Tb when there is fever, and AVP plays the most important function. For example, when there is fever, AVP content in amygdale and ventral septal increases. Trace amount of AVP introduced in ventral septal could inhibit the ET fever. Also, trace amount of AVP injected into amygdale in two sides could inhibit interleukin 1 fever (Cooper et al, 1987; Li et al, 1994), and release AVP in ventral septal in rats with fever, thus to lower the temperature (Zhang et al, 2001).

On the contrast, amygdale, VSA, and arcuate nucleus have the negative influence on the thermoregulation, where, AVP plays the leading role: AVP is increased in VSA and amygdala during the febrile phase.

Trace amount of AVP injected into VSA could inhibit the ET-induced heating, and trace amount of AVP injected into bilateral amygdala could also inhibit interleukin 1 fever, and AVP releasing in the VSA of fever rats could lower the body temperature. Therefore, the thermoregulation center is constituted by positive regulating center (mainly POA-AH) and negative regulating center (mainly VSA and nucleus lateralis nervi vestibuli), and nucleus lateralis nervi vestibuli and VSA are closely related to POA-AH anatomically and functionally. The peripheral pyrogen stimulant transfers into the central, initiates the positive and negative thermoregulation mechanisms: positive regulator raises the temperature, negative the regulator limits temperature raise, so as to determine the raise, extent of thermoregulation set-point, the febrile extent, and the duration (Ye et al, 2011).

Due to the thermoregulation center is a complicated functional system, therefore, this experiment chooses cAMP as the positive regulator and AVP as negative regulator to

study the antipyretic effect of *E. chinense*, hoping to better understand this folk medicine.

In this experiment, Tb of rats raised after dry yeast injection, cAMP in hypothalamus and AVP in septal central area were increased when compared with those in the normal group, The Tb of rats was lowered, and the content of cAMP in hypothalamus and AVP in septal central area were decreased after the administration with *E. chinense* when compared with the model group, at the same time, the contents of cAMP and AVP in plasma did not change significantly with temperature.

Like other antipyretic, one of the mechanisms of *E. chinense* is to reduce the content of cAMP in hypothalamus and release AVP in septal central area. But the mechanism why *E. chinense* can reduce cAMP content in hypothalamus and release the content of AVP in septal central area and how it can influence blood metabolic of cAMP and AVP remains to be further investigated.

Acknowledgements

We thank Huang ZL for providing *E. chinense* herbal medicine.

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