Angelica dahurica extracts attenuate CFA-induced inflammatory pain via TRPV1 in mice

Chan Zhu

Nanjing University of Traditional Chinese Medicine - Xianlin Campus: Nanjing University of Chinese Medicine

Meiyuan Wang

Nanjing University of Chinese Medicine

Jun Guo

Nanjing University of Traditional Chinese Medicine: Nanjing University of Chinese Medicine

Shu-Lan Su

Nanjing University of Chinese Medicine

Guang Yu

Nanjing University of Chinese Medicine

Yan Yang

Nanjing University of Chinese Medicine

Yuan Zhou

Nanjing University of Chinese medicine

Zongxiang Tang (✉ tangzxlab@njucm.edu.cn)

Nanjing University of Chinese Medicine <https://orcid.org/0000-0003-2803-0984>

Research

Keywords: *Angelica dahurica*, inflammatory pain, complete Freund's adjuvant, TRPV1

Posted Date: January 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-151543/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: *Angelica dahurica*, belonging to the family Apiaceae, is a well-known herbal medicine. The roots of *Angelica dahurica* is commonly used for the treatment of headache, toothache, abscess, furunculosis, and acne. However, little is known about their analgesic molecular mechanism underlying pain relief. Here, we investigated the anti-nociceptive activity of *Angelica dahurica* extracts (ADE) in complete Freund's adjuvant (CFA)-induced inflammatory pain mice models, and its possible mechanism of the action associated with transient receptor potential vanilloid member 1 (TRPV1) was also explored.

Material and Methods: In this study, we used behavioral tests to assess the analgesic effect of the ADE on CFA-induced inflammatory pain mice models. TRPV1 protein activity in dorsal root ganglion (DRG) was assessed with calcium imaging assay. TRPV1 expression was detected with western blot and immunohistochemistry. Then we examined the constituents of ADE using combined ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF-MS).

Results: Our results showed that ADE effectively attenuated mechanical and thermal hypersensitivities in CFA-induced inflammatory pain model in mice. ADE also significantly reduced the activity and the protein expression of TRPV1 in DRG from CFA mice.

Conclusion: These findings suggest that ADE exhibits an analgesic effect in CFA inflammatory pain models by targeting TRPV1. Therefore, ADE might be an attractive and suitable analgesic agent for the management of chronic inflammatory pain.

Introduction

Pain is normally a transitory unpleasant sensation subsequent to a noxious or potentially injurious stimulus, acting as a warning system for tissue protection against injuries and is also a key diagnostic criterion for several acute and chronic medical conditions[1]. Chronic inflammatory pain, one of the most common types of pain, is induced by different chemical mediators released during an inflammatory process, such as bradykinin, prostaglandins, nerve growth factor (NGF) and serotonin (5-HT), which can eventually lead to peripheral and central sensitization. The sensitization of primary nociceptive neurons is a common denominator for all types of inflammatory pain that lead to a state of hyperalgesia and allodynia, typically represented by hypersensitivity to both mechanical and thermal stimuli[2].

Treatment of chronic inflammatory pain still remains a major challenge in clinical practice because of its heterogeneous etiology and the complex underlying pathophysiology mechanisms. Drugs acting on the opioid receptor system or showing non-steroidal anti-inflammatory mechanisms have been the only successful molecules over the last decades. However, non-steroidal anti-inflammatory drugs (NSAIDs) are limited by side effects (primarily gastrointestinal and cardiovascular) and insufficient efficacy against pain of higher intensity. Opioid analgesics may be used for severe inflammatory pain, whereas the risk of side effects, tolerance, and dependence restrict their use. Therefore, there is an urgent need to develop novel and efficient pain medicines that are lack of side effects to meet the needs of patients.

Traditional Chinese medicine (TCM) has been viewed as safe treatments and widely used to prevent and cure many diseases under the guidance of the theory of traditional Chinese medical science. *Angelica dahurica*(AD), belonging to the family Apiaceae, is a well known herbal medicine. The roots of AD (also known as Bai Zhi) are commonly used for the treatment of headache, toothache, abscess, furunculosis, and acne. Pharmacological studies have investigated the effects *Angelicae dahuricae* of anti-inflammatory, analgesic and antipyretic actions and acute toxicity as a guideline for clinic application[3]. However, little is known about their analgesic molecular mechanism underlying pain relief. In this study, we investigated the anti-nociceptive activity of *Angelica dahurica* extract (ADE) on cutaneous inflammation pain by CFA-induced mice, and its possible mechanism of the action associated with TRPV1 ion channels was also explored.

Material And Methods

Preparation of plant extract

The roots of *Angelica dahurica* were purchased from Jiangsu Traditional Chinese Medical Hospital (Nanjing, China). The dried roots weighted 200 g were soaked in water 10 times of their total weight for 1 h, and then heated to refluxing for 2 h. Eight times of water was added for another 1.5 h refluxing after filtering. The filtered extraction solutions were combined and concentrated using a rotary evaporator at 60°C and then lyophilized. *Angelica dahurica* extracts (ADE) were then prepared into several desired dose concentration for pharmacological tests.

Analysis of ADE.

The chromatographic separation of ADE was performed on Acquity TM UPLC liquid chromatographic system (Waters, Milford, MA, USA). An Acquity TM UPLC BEH C18 column (50 mm × 2.1 mm, i.d., 1.7 μm) maintained at 30 °C with a mobile phase of acetonitrile (A) and 0.1% formic acid aqueous solution (B) using a gradient elution of 0-0.5 min, 5 %A; 0.5~7.5 min, 5 %A~30 %A ; 7.5~9.0 min, 30 %A~40 % A; 9.0~11.0 min, 40 % A~50 % A; 11.0~12.0 min, 50 % A~80%A; 12.0~14.0min, 80%A~80%A; 14.0~15.0min, 80%A~70%A; 15.0~16.0min, 70%A~60%A; 16.0~17.0min, 60%A~50%A; 17.0~18.0min, 50%A~35%A; 18.0~19.0min, 35%A~20%A; 19.0~20.0min, 20%A~5%A .

Mass spectrometric detection was applied with electrospray ionization (ESI) ion source . Mass spectra were acquired in the negative-ion mode over a range of m/z 100–1000. The capillary voltage and cone voltage were kept at 3.0 kV and 30 V, respectively. Cone gas flow (50 L/H) and desolvation gas flow (900 L/H) were kept constant throughout the study. The desolvation temperature and ion source temperature were operated at 400°C and 150°C, respectively. Scan time was 0.3s, Scan interval time was 0.02s.

Animals

Adult male C57BL/6 mice (aged 6-8weeks) from the Beijing Vital River Laboratory Animal Technology were used. All experiments involving animals were approved by the Nanjing University of Chinese

Medicine Animal Ethics Committee approval number ACU170903. The animals were housed in Nanjing University of Chinese Medicine Experimental Animal Center with a temperature-controlled colony room ($24 \pm 2^{\circ}\text{C}$), humidity (50%-60%), and a 12 h light/12 h dark cycle (lights on 08:00 to 20:00). Food and water were available *ad libitum*. All behavioral tests were performed between 09:00 and 17:00. All experimental procedures were approved by the Animal Care and Use Committee of Nanjing University of Chinese Medicine (Nanjing, China). Mice must adapt to laboratory conditions for at least 2 days prior to testing. TRPV1 knock out mice were kindly provided by Dr. Michael J. Caterina (Johns Hopkins University School of Medicine, Baltimore).

Behavioral assays

CFA-induced chronic inflammatory pain.

C57BL/6 mice received an intraplantar injection of 20 μl of CFA (Sigma-Aldrich, USA) to the right hind paw and were divided separately into four groups randomly with the equal number ($n=10$). Four groups of mice were orally administrated by syringe feeding with distilled water, diclofenac sodium (10mg/kg) or ADE (100mg/kg, 600mg/kg) daily for 14 days after CFA injection, respectively.

Von Frey mechanical assay

Mechanical sensitivity in mice were tested using a set of Von Frey filaments (0.008-2 g). Mice were placed on a raised wire mesh grid ($6 \times 6 \text{ mm}^2$ apertures) under plastic chambers. The filament was applied to the plantar surface at a vertical angle for up to 2-3 s from the bottom. Fifty percent withdrawal threshold values were determined using the up-down method. Baseline von Frey measurements were obtained before drug administration or prior to CFA injection and subsequent measurements were taken at 2, 4, 6, 8 h and every two days post-injection until the 14th day. Behavioral assessments were conducted during the light cycle at approximately the same time each day. All behavioral analyses were performed blindly.

Radiant heat assay

Nociceptive thermal sensitivity was measured by focusing a beam of light on the plantar surface of the hind paw to generate radiant heat. Hind paw withdrawal latency was measured by the method of Hargreaves *et al.*[4]. Mice were placed in elevated chambers on a plexiglas floor and were allowed to acclimated to the testing room for 30 minutes prior to testing. The radiant heat source (Ugo Basile Plantar Test 37370) was applied to the center of the plantar surface of the hind paw with at least 3 min intervals. The average withdrawal latency of the trials was recorded as the response latency. Baseline latency was determined before drug administration or prior to CFA injection.

Cultures of dissociated DRG neurons

Immunostaining of DRG neurons.

Mice were anesthetized with pentobarbital and perfused with 20 ml 0.1M phosphate buffer solution (PBS; pH 7.4; 4°C) followed with 25 ml fixative (4% formaldehyde in PBS; 4°C). Dorsal root ganglia (DRG) of spine levels L4-L6 was dissected from the perfused mice and then post-fixed in fixative at 4°C for 30 min. DRG was cryoprotected in 30% sucrose for more than 24 hr and were sectioned with a cryostat at 10 µm and mounted on slides. Sections were immediately processed for detection of target protein or stored at -20 °C for future use. Sections were incubated in the following solutions: (1) blocking solution (containing 3% fetal bovine serum, 0.1% Triton X-100, and 0.02% sodium azide in PBS) for 1 h at room temperature; (2) primary antibodies (TRPV1 rabbit monoclonal antibody, dilution 1 : 200, proteintech, 22686-1-AP, Neuronal monoclonal antibody, dilution 1 : 500, abcam, ab104225) in blocking solution at 4°C overnight; (3) PBS, 3 × 10 min each; (4) secondary antibodies [Alexa Fluor 555-labeled Donkey Anti-Rabbit IgG(H+L), 1 : 300, Beyotime, A0453; Alexa Fluor 488-labeled Goat Anti-Rabbit IgG(H+L), 1 : 300, Beyotime, A0423] in blocking solution for 1 h at room temperature; (5) PBS, 3 × 10 min. Tissue sections were examined with a Carl Zeiss Axio Zoom.V16 fluorescence microscope. To calculate the positive cells in DRGs, every two slices were captured and counted. The number of fluorescence positive DRG neurons was counted and calculated.

Calcium imaging.

The ipsilateral L4-L6 DRG neurons were loaded with fura-2-acetomethoxyl ester (Thermo Fisher Scientific) for 25 min in the dark at 37°C in accord with previous studies[6]. After being washed 3 times with PBS, the glass coverslips were placed into a chamber and perfused with a calcium imaging buffer containing 137mM NaCl, 5.4mM KCl, 1.2mM MgCl₂, 1.2mM NaH₂PO₄, 1mM CaCl₂, 10mM glucose, and 20mM HEPES (pH 7.4). Ca²⁺ influx was detected by Fura-2 excitation at 340 and 380 nm by a high-speed continuously scanning monochromatic light source (Polychrome V, TILL Photonics, Gräfeling, Germany). 100nM capsaicin was added at the indicated time points. Cells were imaged under Olympus IX57 microscope. All calcium imaging assays were performed by an experimenter blind to the groups.

Western blot analysis.

Mice were sacrificed by cervical dislocation under Isoflurane anesthesia 14 days after the ADE and diclofenac sodium administration. DRG neurons from mice were lysed with RIPA Lysis Buffer (Beyotime, P0013B, China) and protein concentrations were determined using a BCA Protein Assay Kit (Pierce). Proteins (50 µg) were separated in 10% SDS PAGE gel, and transferred to PVDF membranes. After 60 minutes blocking, the membranes were incubated with primary antibody (TRPV1 mouse monoclonal antibody, dilution 1: 1000, abcam, ab203103) at 4°C overnight. After washing with TBST, the membrane was incubated with goat anti-mouse antibody (HRP labeled) diluted with 5% non-fat dried milk in TBST and detected with ECL reagents (Millipore). Blots were scanned with gel and blot imaging system (Biorad Gel Doc XR+ System) and band densities were compared with Image Lab software. TRPV1 protein levels were normalized against beta-actin and all experiments were done for three times.

Data analysis.

All statistical calculations were performed using the GraphPad Prism 6.0 software. Data are presented as means $\pm$ SEMs. Groups were compared by a two-tailed, unpaired Student's t test. The designation "n" represents the number of animals analyzed. Differences were considered statistically significant for $P < 0.05$. Representative data are from experiments that were replicated biologically at least three times with similar results.

Results

2.1 ADE attenuates mechanical and thermal hyperalgesia in CFA-induced chronic inflammatory pain mice model.

To evaluate the analgesic effect on the chronic pain of ADE, the CFA-induced chronic inflammatory model was adopted. Chronic inflammatory pain is characterized by sensitization of nociceptors resulting in hyperalgesia and allodynia. It can happen with wounds, burns, and infection, evoking a complex series of cellular responses that together is proposed to drive painful hyperalgesic states. Mechanical and thermal hyperalgesia, increased sensitivity to mechanical and heat stimulus, is a hallmark of inflammatory pain[7]. In our study, baseline values for the paw thermal and mechanical pain threshold were measured before any treatment. Then, CFA was injected subcutaneously into the plantar surface of the right hind paw for 20ul per mouse. The control group was treated with distilled water. The positive control group received diclofenac sodium (10mg/kg) orally. Other two groups received ADE at respective doses of 100 and 600 mg/kg. As expected, CFA injection induced hypersensitivity to mechanical and heat stimuli in the ipsilateral hind paw during the experimental period(Fig. 1A,B). The paw withdrawal threshold in response to mechanical stimuli decreased significantly at 2 h after CFA injection. From 24h after CFA injection to the last time point assessed (14 days), Oral administration of ADE (100mg/kg and 600mg/kg) significantly reduced the CFA induced mechanical hyperalgesia compared to administration of distilled water, the effects of which were superior to oral administration of diclofenac sodium (10mg/kg). Administration of ADE (100 and 600 mg/kg) and diclofenac sodium also attenuated thermal hyperalgesia in CFA-injected mice as shown in Fig. 1B, mean paw response latency was approximately 3.5 s in con group and which was increased to approximately 8.5 s in treated groups. This finding indicates that oral administration of ADE can indeed alleviate the mechanical and thermal hyperalgesia in CFA-induced mice model.

2.2 ADE decreased TRPV1 activity in isolated DRG neurons from CFA mice.

To determine how TRPV1 involve in the analgesia effects of ADE, the activity of TRPV1 in primary cultured lumbar DRG(L4-L6) neurons isolated from CFA mice in four groups were examined. We utilized calcium imaging and detected the intracellular fluorescent calcium level in DRG neurons in response to capsaicin. Representative traces illustrate that capsaicin elicited calcium influx responses in DRG neurons were showed in Fig. 2D. The percentage of capsaicin-responsive neurons in diclofenac sodium, ADE-M and ADE-H treated mice was significantly reduced by 56.8%, 47.3%, 48.7% respectively as compared to Con group (Fig. 2B). Additionally, the amplitude of the capsaicin responses DRG neurons

from diclofenac sodium, ADE-M and ADE-H group mice was diminished by 35.6%, 20.8%, 18.8% respectively (Fig. 2C). These results showed that ADE can decrease TRPV1 activity in isolated sensory neurons from CFA mice.

2.3 ADE decreases TRPV1 expression in DRG neurons isolated from CFA mice

Next, to test whether the ADE affect the TRPV1 expression, the TRPV1 expression in primary cultured DRG neurons was examined. CFA mice were orally administrated with ADE (100, 600mg/kg, resp.), *Diclofenac* (10mg/kg), or water daily for 14 days. Double immunostaining of TRPV1 and NeuN (a mature neuronal marker) on transverse sections of lumbar DRGs (L4-L6) (Fig. 3A) shows that the percentage of the TRPV1 positive neurons was significantly decreased in DRGs from CFA mice after administration of diclofenac and ADE (Con, $34.7\% \pm 1.5\%$; diclofenac, $28 \pm 1.3\%$; ADE-M, $20.5 \pm 0.9\%$; ADE-H, $26.7 \pm 1.5\%$ (Fig. 3B). Western blotting showed the protein expression of TRPV1 was significantly decreased in ADE-M and diclofenac groups as compared to Con group (Fig. 3C).

2.4 UPLC/Q – TOF – MS analysis and identification the constitution of ADE

Identification of the ADE was based on retention time, mass assignment and the fragments of corresponding production for the structural identification from the UPLC/Q-TOF-MS analysis platform. In this study, scopoletin, oleic acid, osthole and byak-angelicin were identified by comparing the retention time and mass data with those of the authentic standards. The MS spectra of fragment ions of ADE were shown in Fig. 4. The four compounds were summarized in Tables 1.

Discussion

There is a growing interest in the utilization natural products that present fewer side effects for prevention and treatment of pain. *Angelica Dahurica* has been traditionally used as a single herb or in combination with other herbs for relieving headache, toothache, supraorbital pain and rheumatism[8]. To date, the root has been reported to have wound healing effect[9], anti-asthmatic effect[10], antitumor effect[11] and antioxidant activity[12]. Furthermore, recently studies indicated that the extract of *Angelica Dahurica* possess anti-inflammatory and analgesic effect[13]. However, its analgesic molecular mechanism remains unclear.

Our previous study indicated that ADE can potently attenuate heat, capsaicin, and formalin-induced acute pain[14]. To further confirm the analgesia effects of ADE on chronic pain, we established a chronic inflammatory pain model by intraplantar injection of CFA in mice. A characteristic symptom of this model of nociception is that it displays hyperalgesia to mechanical and heat stimulation. Our results showed that oral administration of ADE at doses of 100mg/kg and 600mg/kg daily could remarkably reduce mechanical and thermal hyperalgesia caused by intraplantar CFA injection. Specially, the antinociceptive

effect of ADE treatment on mechanical hyperalgesia was evident when compared to administration of diclofenac sodium(Fig. 1A,B), which is a non-steroidal anti-inflammatory drug used for clinical treatment.

TRPV1 appears to play a critical role in the transduction of noxious chemical and thermal stimuli by sensory nerve endings in peripheral tissues. Recent evidence supporting the role of TRPV1 in inflammatory pain comes from studies reporting that TRPV1 is essential for the analgesia in a mouse model of inflammatory pain[15]. In mouse models, genetic deletion of TRPV1 essentially eliminated thermal hyperalgesia induced by inflammation in two independent TRPV1 knock-out lines[16, 17]. Considering the close connections of TRPV1 with hot, capsaicin induced pain and inflammatory pain, it is reasonable to think that the analgesic effect of ADE may be mediated by TRPV1. In principle, there are two mechanisms by which TRPV1 activity can increase and thus induce thermal hypersensitivity: higher expression levels, and increased sensitivity/activity. Indeed, Increased expression of TRPV1 at the RNA and protein levels has been both reported in inflammatory models[18]. In our present study, calcium imaging assay exhibited that ADE exerted analgesia effects by decreasing TRPV1 activity in isolated DRG neurons from CFA mice (Fig. 2). Then, the double labeling experiments indicate a decrease of the percentage of TRPV1-positive neurons in ADE treated group (Fig. 3A,B). Also, ADE significantly reduced the protein expression of TRPV1 in DRGs(Fig. 3C). These results presented here clearly point to an interaction with TRPV1 channels as a possible mechanism of action of ADE. If this is a direct or indirect interaction, through other intracellular signaling pathways, remains to be elucidated. There is evidence that regarding to disease related changes in receptor expression, inflammatory mediators such as cytokines, prostaglandin, bradykinin, glutamate, serotonin, and nerve growth factor all have been shown to increase the phosphorylation state of TRPV1, thereby increasing channel activity[19–21]. Specifically, direct phosphorylation of TRPV1 protein by PKC and PKA increases the channel probability and decreases channel desensitization, respectively, and phosphorylation of TRPV1 by the tyrosine kinase src enhances TRPV1 channel trafficking to the cell surface[22–24].

Finally, we applied UPLC/Q – TOF – MS to analyses the main analgesia active ingredients in ADE. In the negative ion mode, scopoletin, oleic acid, osthole and byakangelicin were identified from ADE. Scopoletin is an anti-oxidant, hepatoprotective, and anti-inflammatory activities reagent. Scopoletin was reported to have anti-inflammatory activity in λ -carrageenan (Carr)-induced paw edema mouse model of inflammation and can significantly inhibited formalin-induced pain in the late phase[25]. Scopoletin also can ameliorate anxiety-like behaviors in complete Freund's adjuvant-induced mouse model[26]. Osthole was also found to significantly decrease acetic acid and formalin-induced hyperalgesia and carrageenan-induced paw oedema[27]. Oleic acid exhibits an expressive anti-inflammatory effect in croton oil-induced irritant contact dermatitis in mice[28]. Recently study indicates that byakangelicin inhibits IL-1 β -induced mouse chondrocyte inflammation in vitro and ameliorates murine osteoarthritis in vivo[29]. In summary, all of the four compounds that we identified from ADE were reported to have anti-inflammatory or analgesic effects, which can partly explain why ADE has a good effect in attenuating chronic inflammation pain. Furthermore, these compounds may also have synergistic effects in vivo.

Conclusion

In conclusion, our data in this study demonstrated for the first time that ADE produced a remarkable inhibition in chronic inflammatory pain models in mice and the possible mechanism of its analgesic action is associated with the inhibition of activities and protein expression of TRPV1. These findings suggest that ADE might be an attractive and suitable therapeutic agent for the management of chronic inflammatory pain.

Abbreviations

TRPV1: Transient receptor potential vanilloid member 1; CFA: Complete freund's adjuvant; DRG: Dorsal root ganglion; NSAIDs: Non-steroidal anti-inflammatory drugs; TCM: Traditional Chinese medicine; AD: Angelica dahurica; ADE: Angelica dahurica extract; UPLC/Q-TOF-MS: ultra-performance liquid chromatography-quadrupole time-of-light mass spectrometry;

Declarations

Acknowledgements

Not applicable.

Authors' contributions

ZXT, CZ, and YY conceived and designed the study. ZXT and CZ wrote the main manuscript text. MYW, JG, YZ, GY performed the animal experiments and collected data. CZ, JG and SLS analyzed the data. CZ and JG prepared the figures and tables. All authors read and approved the final manuscript.

Funding

This work was supported by the National Science Foundation of China Grants 31471007 and 31771163 (to Z.T.). This work was also supported by the Hunan Cooperative Innovation Center for Molecular Target New Drug Study, and Postdoctoral Science Foundation of China (M601863).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The experiment was approved by the ethical committee of Nanjing University of Chinese Medicine (No. ACU170903).

Consent to publish

The authors consent for the publication of this manuscript.

Competing interests

All authors declared no competing interests.

References

1. Montes-Sandoval L. An analysis of the concept of pain. *J Adv Nurs*. 1999;29:935-41.
2. Kidd BL, Urban LA. Mechanisms of inflammatory pain. *Br J Anaesth*. 2001;87:3-11.
3. Li H, Dai Y, Zhang H, Xie C. [Pharmacological studies on the Chinese drug radix *Angelicae dahuricae*]. *Zhongguo Zhong Yao Za Zhi*. 1991;16:560-2, 576.
4. Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain*. 1988;32:77-88.
5. Kim AY, Tang Z, Liu Q, Patel KN, Maag D, Geng Y, Dong X. Pirt, a phosphoinositide-binding protein, functions as a regulatory subunit of TRPV1. *Cell*. 2008;133:475-85.
6. Liu Q, Tang Z, Surdenikova L, Kim S, Patel KN, Kim A, et al. Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus. *Cell*. 2009;139:1353-65.
7. Chiu IM, von Hehn CA, Woolf CJ. Neurogenic inflammation and the peripheral nervous system in host defense and immunopathology. *Nat Neurosci*. 2012;15:1063-7.
8. Sarker SD, Nahar L. Natural medicine: the genus *Angelica*. *Curr Med Chem*. 2004;11:1479-500.
9. Yang WT, Ke CY, Wu WT, Harn HJ, Tseng YH, Lee RP. Effects of *Angelica dahurica* and *Rheum officinale* Extracts on Excisional Wound Healing in Rats. *Evid Based Complement Alternat Med*. 2017;2017:1583031.
10. Lee MY, Seo CS, Lee JA, Lee NH, Kim JH, Ha H, Zheng MS, Son JK, Shin HK. Anti-asthmatic effects of *Angelica dahurica* against ovalbumin-induced airway inflammation via upregulation of heme oxygenase-1. *Food Chem Toxicol*. 2011;49:829-37.
11. Kim YK, Kim YS, Ryu SY. Antiproliferative effect of furanocoumarins from the root of *Angelica dahurica* on cultured human tumor cell lines. *Phytother Res*. 2007;21:288-90.
12. Wang GH, Chen CY, Tsai TH, Chen CK, Cheng CY, Huang YH, Hsieh MC, Chung YC. Evaluation of tyrosinase inhibitory and antioxidant activities of *Angelica dahurica* root extracts for four different probiotic bacteria fermentations. *J Biosci Bioeng*. 2017;123:679-84.
13. Wang C, Sun J, Li H, Yang X, Liu H, Chen J. In vivo anti-inflammatory activities of the essential oil from *Radix Angelicae dahuricae*. *J Nat Med*. 2016;70:563-70.
14. Guo J, Chen D, Zhu C, Tang ZX. Analgesic effect and analgesic mechanism of *Anhelica dahurica* extracts. *Journal of Guang Xi Normal University (Nature Science Edition)*, 2019,37(4):103-110..

15. Liao HY, Hsieh CL, Huang CP, Lin YW. Electroacupuncture Attenuates CFA-induced Inflammatory Pain by suppressing Nav1.8 through S100B, TRPV1, Opioid, and Adenosine Pathways in Mice. *Sci Rep*. 2017;7:42531.
16. Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeit KR, Koltzenburg M, Basbaum AI, Julius D. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science*. 2000;288:306-13.
17. Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, et al. Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature*. 2000;405:183-7.
18. Ji RR, Samad TA, Jin SX, Schmoll R, Woolf CJ. p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. *Neuron*. 2002;36:57-68.
19. Sikand P, Premkumar LS. Potentiation of glutamatergic synaptic transmission by protein kinase C-mediated sensitization of TRPV1 at the first sensory synapse. *J Physiol*. 2007;581:631-47.
20. Sugiura T, Tominaga M, Katsuya H, Mizumura K. Bradykinin lowers the threshold temperature for heat activation of vanilloid receptor 1. *J Neurophysiol*. 2002;88:544-8.
21. Brandt MR, Beyer CE, Stahl SM. TRPV1 Antagonists and Chronic Pain: Beyond Thermal Perception. *Pharmaceuticals (Basel)*. 2012;5:114-32.
22. Bhawe G, Hu HJ, Glauner KS, Zhu W, Wang H, Brasier DJ, Oxford GS, Gereau RW 4th. Protein kinase C phosphorylation sensitizes but does not activate the capsaicin receptor transient receptor potential vanilloid 1 (TRPV1). *Proc Natl Acad Sci U S A*. 2003;100:12480-5.
23. Morenilla-Palao C, Planells-Cases R, García-Sanz N, Ferrer-Montiel A. Regulated exocytosis contributes to protein kinase C potentiation of vanilloid receptor activity. *J Biol Chem*. 2004;279:25665-72.
24. Vellani V, Mapplebeck S, Moriondo A, Davis JB, McNaughton PA. Protein kinase C activation potentiates gating of the vanilloid receptor VR1 by capsaicin, protons, heat and anandamide. *J Physiol*. 2001;534:813-25.
25. Chang TN, Deng JS, Chang YC, Lee CY, Jung-Chun L, Lee MM, Peng WH, Huang SS, Huang GJ. Ameliorative Effects of Scopoletin from *Crossostephium chinensis* against Inflammation Pain and Its Mechanisms in Mice. *Evid Based Complement Alternat Med*. 2012;2012:595603.
26. Luo L, Sun T, Yang L, Liu A, Liu QQ, Tian QQ, Wang Y, Zhao MG, Yang Q. Scopoletin ameliorates anxiety-like behaviors in complete Freund's adjuvant-induced mouse model. *Mol Brain*. 2020;13:15.
27. Singh G, Bhatti R, Mannan R, Singh D, Kesavan A, Singh P. Osthole ameliorates neurogenic and inflammatory hyperalgesia by modulation of iNOS, COX-2, and inflammatory cytokines in mice. *Inflammopharmacology*. 2019;27:949-60.
28. Pegoraro NS, Camponogara C, Cruz L, Oliveira SM. Oleic acid exhibits an expressive anti-inflammatory effect in croton oil-induced irritant contact dermatitis without the occurrence of toxicological effects in mice. *J Ethnopharmacol*. 2020:113486.

29. Zhang T, He L, Yang W, Wang Y, Peng J, Sun P, Yang Q, Jia Y, Zhao K, Qian Y. Byakangelicin inhibits IL-1 β -induced mouse chondrocyte inflammation in vitro and ameliorates murine osteoarthritis in vivo. *Int Immunopharmacol.* 2020;85:106605.

Tables

Table 1 The identification results of the constituents of ADE

No.	Retention time	Name of the compound	Chemical Structure	Molecular weight	MS-(<i>m/z</i>)
1	0.848	Scopoletin		192.168	191.0204 [M-H] ⁻
2	13	oleic acid		340.5405	339.2344 [M-H] ⁻ 116.9304 [M-CH(CH ₂) ₇ CH ₃ -2H] ⁻
3	14.501	osthole		244.29	243.9005 [M-H] ⁻
4	15.217	Byak-angelicin		334.31	333.2289 [M-H] ⁻ 243.9007 [M-C(CH ₃) ₂ OH-OCH ₃] ⁻

Figures

Figure 1

Effects of ADE on the CFA-induced chronic inflammatory pain mice model. Mice were treated with distilled water (control) , diclofenac (positive control) ADE-M 100mg/kg and ADE-H 600mg/kg daily after injection of CFA, and then mechanical allodynia (A) and thermal hyperalgesia (B) were tested among the groups . Each point represents the mean $\pm$ SEM of the paw withdrawal threshold in response to mechanical or thermal stimuli. (* $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$ means comparison with control group, $n=10$)

Figure 2

Effect of ADE on the activity of TRPV1. (A) Representative Fura-2 ratio metric images of cultured DRG(L4-L6). Arrow indicates the DRG neurons in response to capsaicin. The color of the neurons switching from green to red indicates an increase in Ca²⁺ influx. (B) Percentage of DRG neurons responding to capsaicin in ipsilateral L4-L6 DRG neurons isolated from different group mice. (C) Activity of ipsilateral L4-L6 DRG neurons responding to capsaicin in neurons isolated from different group mice. (D) Representative traces illustrate that capsaicin elicited Ca²⁺ influx responses in ipsilateral L4-L6 DRG neurons. Each trace

corresponds to the change in fluorescence ratio in a single neuron of cultured DRG neurons. (*p < 0.05 , **p < 0.01***p < 0.001 means comparison with control group respectively. Scale bar:50μm, n=3)

Figure 3

The Effects of ADE on TRPV1 expression in the DRG. (A) Double immunostaining of TRPV1 (red)and NeuN(green) on transverse sections of ipsilateral L4-L6 DRGs from mice of four groups. Arrow and arrowhead indicate neurons with or without TRPV1 expression respectively. (B)Quantitative data of TRPV1+ neurons of four groups were listed. (C) Expression of TRPV1 in ipsilateral L4-L6 DRGs of mice from four groups was assayed by western blotting. (*p < 0.05 , **p < 0.01***p < 0.001 means comparison with control group, respectively. Scale bar:50μm. n=3)

Figure 4

MS spectra of fragment ions of ADE. (A)scopoletin, (B) oleic acid, (C) osthole and (D) byakangelicin