Anti-inflammatory, Antinociceptive Activity of an Essential Oil Recipe Consisting of the Supercritical Fluid CO₂ Extract of White Pepper, Long Pepper, Cinnamon, Saffron and Myrrh in vivo

Yuanbin Zhang¹, Xinfang Wang¹, Ling Ma¹, Lin Dong^{1,2}, Xinhui Zhang^{1,2}, Jing Chen^{1,2} and Xueyan Fu^{1,2*}

¹ School of Pharmacy, Ningxia Medical University, Yinchuan 750004, China

² Ningxia Engineering and Technology Research Center for Modernization of Hui Medicine, Yinchuan 750004, China

Abstract: This study was designed to investigate the anti-inflammatory and antinociceptive activities of essential oil recipe (OR) in rodents. The anti-inflammatory activity was evaluated by inflammatory models of dimethylbenzene (DMB)-induced ear vasodilatation and acetic acid-induced capillary permeability enhancement in mice whereas the antinociceptive activity was evaluated using acetic acid-induced writhes and hot plate test methods in mice. Additionally, the chemical composition of OR has been also analyzed by gas chromatography and mass spectrometry (GC/MS). 37 compounds, representing 74.42% of the total oil content, were identified. β -Selinene (7.38%), aromadendrene (5.30%), β -elemene (5.22%), cis-piperitol (5.21%), cis- β -guaiene (4.67%), ylangene (3.70%), 3-heptadecene (3.55%), δ -cadinene (3%) and β -cadinene (2.87%) were found to be the major constituents of the oil. Oral pretreatment with OR (62.5-1000 mg/kg) not only decreased the DMB-induced ear vasodilatation but also attenuated capillary permeability under acetic acid challenge in mice. OR significantly reduced the writhing number evoked by acetic acid injection. All test samples showed no significant analgesic activity on the hot plate pain threshold in mice. These data demonstrated that the OR inhibits inflammatory and peripheral inflammatory pain. These results may support the fact that the essential oil of traditional Hui prescription played a role in the inflammation of stroke.

Key words: anti-inflammatory activity, antinociceptive activity, white pepper, long pepper, cinnamon, saffron, myrrh

1 INTRODUCTION

Traditional Chinese Hui Medicine (TCHM), an integration of the traditional Chinese medicine and the Arab medicine, skilled at curing bones sully and stroke. For six hundreds of years in China, TCHM recipes such as Zha Li Nu Si Fang, Ha Hei Li Li Fang and Mu Xiang You Fang listed in the Chinese Hui medical classic Hui Hui Yao Fang have excellent achievements in the treatment of stroke. Some prescriptions, including aromatic drugs, have been proven to improve neural function and carotid artery atherosclerosis in clinical applications and animal experiments¹⁻⁵. The theory of TCHM believed that aromatic drugs could induce resuscitation with fragrance and guide active compounds to achieve brain⁶⁻⁸. This theory has not been wealthy confirmed by a numerous fundamental studies because of the

limitation of ancient technology and backward regional economy, although it sounded very reasonable. A recent study from our laboratory revealed aromatic drugs enriched in essential oils, white pepper, long pepper, cinnamon, saffron and myrrh served as the main active ingredients in traditional Chinese Hui stroke prescriptions^{9, 10}. These five herbs (like monarch drugs in Chinese medicine) become the object of this study because of the pretty high frequency and concentration in prescriptions. Perhaps the essential oil recipe can not directly work to protect against stroke, but the supporting role is worth us to study.

Inflammation is a basic reaction to irritation, infection or other injury¹¹. It is an essential step in the mechanisms of ischemic stroke and other forms of ischemic brain injury^{12, 13}. Some pre-clinical stroke studies showed that inhibition of

*Correspondence to: Xueyan Fu, School of Pharmacy, Ningxia Medical University, Yinchuan 750004, China, Ningxia Engineering and Technology Research Center for Modernization of Hui Medicine, Yinchuan 750004, China

E-mail: xueyanfu2661@163.com

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inflammatory responses reduced brain injury and improved neurological outcome. Clinical studies suggested that systemic inflammation could influence the susceptibility of patients to stroke and subsequent prognosis^{12, 13}. Therefore test of the anti-inflammatory and antinociceptive effect of the essential oil recipe (named OR) consisting of white pepper, long pepper, cinnamon, saffron and myrrh may help us to understand the traditional theory.

White pepper (*Piper nigrum* L.) and long pepper (*Piper longum* L.) that from the family Piperaceae, are common used as spices all over the world. They have been used for the treatment of vomiting, diarrhea and digestion in traditional Chinese medicine¹⁴. Piperine, a main component of Piper species, is alkaloid with a long history of medical use in a variety of inflammatory disorders like rheumatoid arthritis¹⁵. The extensive presence of the alkaloids and amides (e.g. piperidine, piperine and piperlonguminine) on long pepper, has been attributed with the treatment of tuberculosis, sleeping disorders, menstrual pain, muscular pain, and certain form of arthritis¹⁶. Piperlonguminine showed various biological properties including anti-hyperlipidemic, anti-platelet and anti-melanogenesis activities¹⁷⁻¹⁹. Meanwhile it's useful as a therapy for vascular inflammatory diseases in both cell and animal models¹⁶.

Cinnamon has been used for cuisine, also as a stomachic and carminative for gastrointestinal complaints as well as other ailments such as common cold and cardiovascular disease^{20, 21}. It is worth that essential oil from indigenous cinnamon (one of the indigenous tree species in Taiwan) twigs and leaves, and water extracts from cinnamon bark have been shown activities against various types of tumor cell lines and inflammation in LPS-induced models^{20, 22-25}.

Crocus sativus L. is a flowering plant in the Iridaceae family and is commonly known as saffron. The important constituents of stigmas of *C. sativus* are carotenoids (e.g. crocetin, crocins, α -carotene), monoterpene aldehydes (e.g. picrocrocin and safranal), monoterpenoids (e.g. crocusatines), isophorones and flavonoids. Various pharmacological activities of saffron have been extensively studied, including anticonvulsive, anti-Alzheimer's, anti-ischemic, hypolipidemic effects. Saffron also offers protective effects against cardiovascular disease, diabetes, Parkinson's disease, depression, tumor, apoptosis, atherosclerosis and other conditions²⁶. The water and ethanolic extracts of saffron's flower had anti-nociceptive and anti-inflammatory activities by writhing test, xylene induced ear edema in mice and formalin induced edema in the rat paw. Those works support its traditional use as an anti-edematogenic remedy^{17, 27}.

Myrrh belongs to *Commiphora* genus in the family Burseraceae, which is an aromatic gum resin that exists in its stem and is used worldwide. The constituents include volatile oil (2-8%), resin (23-40%), gum (40-60%) and bitter principles (10-25%)²⁸. It was shown that myrrh oil exhibit-

ed cytotoxic and anti-inflammatory activities in human cells²⁹. At the same time 85% ethanol extract and its sub-fractions of myrrh were verified to possess anti-inflammatory activity by animal experiments²⁸.

Review of past researches, the anti-inflammatory activity of some components (e.g. piperine and piperlonguminine) and aqueous and ethanolic extracts of some herbs have been suggested in several different experiments. But those evidences are insufficient to explain the theory about essential oils. The aim of the present study was to evaluate anti-inflammatory and analgesic effects of the OR in rodent models of inflammation and pain. Additionally, the chemical composition of OR were reported.

2 EXPERIMENTAL

2.1 Plant material

White pepper (*Piper nigrum* L.), long pepper (*Piper longum* L.), cinnamon (*Cinnamomum cassia* Presl.), saffron (*Crocus sativus* L.) and myrrh (*Commiphora myrrha* Engl.) were purchased from Anhui Taiyuan Chinese Herbal Pharmacology Co., Ltd. (Voucher No.: SCHM 121215). All of them were classified by Professor Chang-Cai Bai at Pharmacognosy Department, College of Pharmacy, Ningxia Medical University.

2.2 OR extraction and analysis

The white pepper, long pepper, cinnamon, saffron and myrrh were mixed at the ratio of 1:2:2:2:2 (w/w) and smashed as coarse dust. Then 1000 g of mixture was extracted with supercritical CO₂ extraction instrument at 50°C under 30 MPa for 2 h. Percentage content of OR was calculated on basis of the plant dry weight, resulting in the oil yield of 5.82%.

The main constituents were analyzed by GC/MS. The analytical GC was carried out on a Shimadzu QP2010 plus gas chromatograph with a Rxi-5Sil MS (5% phenyl, 95% dimethyl polysiloxane, 30 m $\times$ 0.25 mm; 0.25 μ m film thickness) fused-silica capillary column with helium at 1.33 mL/min as carrier gas temperature setup: injector at 250°C. The oven temperature program was from 100°C to 150°C, at 5°C/min for 5 min, then increased to 240°C at a rate of 5°C/min where it remained for 15 min. The mass spectra were taken at 70 eV from 35 to 500 Da.

Peak identifications were assigned on the basis of a comparison of their retention indices relative to an n-alkane homologous series obtained by co-injecting the oil sample with a linear hydrocarbon mixture. Individual components were identified by comparing the mass spectra with those of authentic compounds previously analyzed and stored in the database from the National Institute of Standards and Technology (NIST). The interpretation of RI values was assisted by data published literatures³⁰⁻³⁹.

2.3 Drugs and chemicals

The following drugs and chemicals were used: acetic acid (no. 130325) and dimethylbenzene (no. 120503) was purchased from Damao Chemical Company (Tianjin, China). Indomethacin was obtained from Shanxi Yunpeng Pharmaceutical Co., Ltd. (no. A130602). Evans blue was purchased from Sigma Co., Ltd. (no. E2129). Custom Alkanes Solution was purchased from SangHai Anpel Scientific Instrument Co., Ltd. (no. CDGG-115320-05). The OR was dissolved in sesame oil just before use.

2.4 Animals

ICR mice, at 6-8 weeks of age (or 18-22 g) were purchased from the Medicine Experiment Animal Center of Ningxia Medicine University. The animals, left for two days for acclimatization to animal room conditions, were maintained on standard pellet diet and water ad libitum. The food was withdrawn on the day before the experiment, but animals were allowed free access to water. All animals were cared for in compliance with the Guide for the Care and Use of Laboratory Animals (1996). The experimental procedures were approved by our institutional animal research ethics committee (approval number: SYXK(NING)2011-0001).

2.5 Anti-inflammatory activity

2.5.1 Dimethylbenzene (DMB)-induced ear vasodilatation assay

The weight of oil recipe is 54 g and the oil yield is 5.82%. The common human daily dose of OR is $(54 \times 5.82\%) \text{ g}/75 \text{ kg}$ in body weight. According to the formula: $d_{\text{mice}} = d_{\text{human}} \times (10 \text{ to } 12)$, the common dose of OR for mice should be 419 to 503 mg/kg/day. In the present study we selected 1000, 500, 250, 62.5 mg/kg/day as high, middle, low and very low dosages for mice, respectively. The test was carried out according to the previously described method⁽⁴⁰⁾. Mice with half males and half females were randomly divided in 6 groups ($n = 8$ per group). Sesame oil (control group), indomethacin (10 mg/kg, positive group), and OR (1000, 500, 250, 62.5 mg/kg) were administered via oral gavage. 60 min after gavage of test samples, the mice were topical applied 30 μl DMB to both inner and outer surface of right ear. Mice were sacrificed by cervical dislocation 30 min later then the ear biopsies of both ears were obtained with a punch (a diameter of 8 mm) and weighed. Weight-increase-rate of the right ear over the left one indicated the vasodilatation.

2.5.2 Acetic acid-induced vascular permeability

The Acetic acid-induced increased vascular permeability in mice was carried out using the reported technique^(40, 41). Briefly, grouping and administration were the same as mentioned in Section 2.5.1. 60 min after via gavage, 10 ml/kg body weight of 2% Evans blue in normal saline solution to the tail vein then applied 20 ml/kg body weight of 0.6%

(v/v) acetic acid immediately (i.p.). 20 min after the administration of acetic acid, the mice were killed by cervical dislocation. The peritoneal cavity was rinsed by 10 ml of saline. The washing solutions were collected in centrifuge tube and centrifuged at 1000 r/min for 5 min. The supernatant was measured at 590 nm by spectrometry and the absorbance of Evans blue in the exudates under 590 nm represented the capillary permeability.

2.6 Antinociceptive activity

2.6.1 Acetic acid-induced writhing response

Mice were used according to the method previously reported^(42, 43). The doses and the routes of administration are provided in Section 2.5.1. 60 min after oral gavage, the response to an intraperitoneal injection of acetic acid solution, manifesting as a contraction of the abdominal muscles stretching of hind limbs, were counted cumulatively after 5 min of stimulus over a period of 20 min. Nociception was induced by intraperitoneal injection of 0.6% acetic acid solution at the dose of 20 ml/kg body weight.

2.6.2 Hot plate test

This test was performed in order to measure the latency of the response^(42, 43). A glass cylinder was placed on a hot-plate with adjustable temperature. The temperature of the hot-plate was then regulated to $55 \pm 1^\circ\text{C}$. Female mice with baseline latencies of more than 20 s were eliminated from the study and the cut-off time of 40 s was fixed to avoid damage to the paws. Each mouse was placed in the glass on the hot-plate in order to obtain the animal's response to heat-induced nociceptive pain stimulus (linking of the hind paws, jumping or shaking). The reaction times obtained at 0, 30, 60, 90 and 120 min prior to via gavage of vehicle and OR.

2.7 Statistical analysis

The results were analyzed using a statistical program SPSS Statistics, version 17.0. One-way ANOVA followed by Dunnett's test or Dunnett's T3 test were used for determining the statistically significant differences between the values of various experimental groups. Data are expressed as means $\pm$ SD and $p < 0.05$ and $p < 0.01$ were considered statistically significant.

3 RESULTS

The chemical analysis of the oil sample used in the present investigation identified 37 compounds, representing 74.42% of the total oil content, β -selinene (7.38%), aromadendrene (5.30%), β -elemene (5.22%), cis-piperitol (5.21%), cis- β -guaiene (4.67%), ylangene (3.70%), 3-heptadecene (3.55%), δ -cadinene (3%), β -cadinene (2.87%) were found to be the major constituents of the oil (Table 1).

Table 1 Chemical composition and retention indices of OR.

<i>Compound</i>	^a <i>Rt</i>	^b <i>RI</i> ^{calc}	^c <i>RI</i> ^{lit}	^d %
γ-Terpinene	3.095	1052	1057	0.43
Terpinolene	3.140	1088	1088	0.51
Linalool	3.199	1100	1096	1.94
Benzennepropanal	4.185	1165	—	0.49
Terpinen-4-ol	4.345	1176	1178	0.58
cis-Piperitol	4.567	1190	1193	5.21
α-Terpineol	4.747	1201	1188	2.49
cis-Cinnamaldehyde	5.146	1223	1219	1.29
Thymol	6.332	1288	1289	1.10
Tetradecane	6.534	—	—	1.22
δ-Elementene	7.337	1340	1339	1.73
Ylangene	8.220	1384	1375	3.70
β-Elementene	8.447	1395	1398	5.22
β-Caryophyllene	8.790	1411	1419	1.72
α-trans-Bergamotene	8.900	1417	—	0.60
Aromadendrene	9.158	1429	1436	5.30
Elixene	9.235	1432	1431	0.48
β-copaene	9.300	1435	1436	1.40
β-Farnesene	9.638	1451	1459	1.38
β-selinene	10.518	1492	1487	7.38
cis-β-Guaiene	10.743	1502	1491	4.67
β-himachalene	10.913	1508	1510	2.93
α-Murolene	11.083	1514	1500	0.68
β-Cadinene	11.215	1519	1518	2.87
δ-Cadinene	11.288	1521	1525	3.00
Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	11.559	1531	—	0.63
(E)-γ-Bisabolene	11.711	1536	1535	1.19
α-calacorene	11.788	1539	1537	0.58
Elemol	11.951	1544	1545	0.52
Caryophyllene oxide	13.024	1582	1582	1.39
Aromadendrene epoxide	14.574	1627	1627	1.35
γ-Murolol	15.118	1641	1642	1.39
α-Eudesmol	15.617	1655	1655	0.73
1-Nonadecene	16.528	1679	—	2.75
3-heptadecene	16.842	1687	—	3.55
nonadecanol	22.178	1875	—	0.70
(±)-Cembrene	22.830	1901	—	1.83
Total identified				74.42

^a Rt – retention time (min) from a linear temperature program.^b RI^{calc} – retention index calculated for each compound.^c RI^{lit} – retention index obtain from published literatures³⁰⁻³⁹.^d % – relative abundances from the peak area integration.

3.1 Anti-inflammatory effects on DMB-induced ear vasodilatation

Treatment with OR (62.5-1000 mg/kg, p.o.) 1h before applied DMB in mice ears significantly (Fig. 1; $p < 0.01$) inhibited the edema formation when compared to the control group. Although no dose-dependent manner was observed, OR at all doses could remarkably decrease the vasodilatation with inhibition rate over 50%. Moreover OR at the dose of 1000 mg/kg, which showed significant (Fig. 1; $p < 0.01$) inhibition on ear vasodilatation when compared to Indomethacin (10 mg/kg, p.o.) with inhibition rate of 91.13%. The inhibitory percentage of Indomethacin was only

Fig. 1 Effects of oral Pretreatment with OR (62.5-1000 mg/kg) or indomethacin (10 mg/kg) on DMB-induced ear vasodilatation. (A) The result of ear edema represents mean $\pm$ S.D. ($n = 8$). $*p < 0.05$, $**p < 0.01$ when compared to the control group; $\#p < 0.05$, $\#\#p < 0.01$ when compared with Indomethacin. (B) Inhibition of ear vasodilatation (%) is presented as the ratio of the mean increase of treatment group (%) on that of control group (%).

48.92%.

3.2 Anti-inflammatory effects on acetic acid-induced vascular permeability enhancement

Figure 2 showed acid-induced vascular permeability enhancement were significantly ($p < 0.01$, compared to control group) inhibited in mice pretreated orally with OR (62.5-1000 mg/kg, p.o.) with an inhibition of 66.6%, 52.73%, 51.33% and 91.33%, respectively. There is no dose-activity dependence relationship of OR. Absorbances

Fig. 2 Effects of oral Pretreatment with OR (62.5-1000 mg/kg) or indomethacin (10 mg/kg) on the capillary permeability under acetic acid challenge. (A) The absorbance of Evans Blue in the leakage under 590 nm represented indicated the capillary permeability. Data represents mean $\pm$ S.D. ($n = 8$). $*p < 0.05$, $**p < 0.01$ when compared to the control group; $\#p < 0.05$, $\#\#p < 0.01$ when compared with Indomethacin. (B) Inhibition of leakage (%) is presented as the ratio of the mean absorbance of treatment group (%) on that of control group (%).

Fig. 3 Effects of oral Pretreatment with OR (62.5-1000 mg/kg, grey columns) or indomethacin (10 mg/kg, black column) on acetic acid-induced writhing in mice. (A) Each value represents mean $\pm$ S.D. ($n = 8$). * $p < 0.05$, ** $p < 0.01$ when compared to the control group; # $p < 0.05$, ## $p < 0.01$ when compared with Indomethacin. (B) Inhibition of writhes (%) was presented as the ratio of the mean increase of treatment group (%) on that of control group (%).

of 1000mg/kg group was 0.39 ± 0.15 , significantly ($p < 0.01$) different with that of indomethacin (0.84 ± 0.15).

3.3 Anti-nociceptive activity on acetic acid-induced writhes

In the acetic acid-induced writhing mice (shown in Fig. 3), OR (62.5-1000 mg/kg, po.) evoked a dose-dependent inhibition, the inhibitory percentage of 95.93%, 93.12%, 75.82%, 42.50%, respectively. All doses of OR inhibited the frequency induced abdominal constrictions by acetic acid when compared to the control group, the results were statistically significant ($p < 0.01$ and $p < 0.05$). In addition, treatment with OR (1000 and 500 mg/kg) showed antinociceptive effect in comparison with the reference drug ($p < 0.01$).

3.4 Anti-nociceptive activity on hot plate test

In the hot plate test, the results presented in Table 2 showed that no significant antinociceptive effect at all analyzed periods, although we observed from 30 min to 120 min.

4 DISCUSSION

TCHM insist that the aroma from fragrant drugs is an important part of Hui anti-stroke prescriptions. In order to prevent volatilization of these aromas, lots of prescriptions were grind as powders, and then swallowed down by hydromel. In addition, some fragrant medicines were used in aroma therapy to cure stroke. Although aromas were endowed with divine status, their effects have never been confirmed by scientific research. With reading and studying mass of classic literatures, our laboratory summed up large traditional Hui prescriptions. The result found that 80% of the medicines were aromatic drugs and frequency of white pepper, long pepper, saffron and myrrh were 56.4%, 42.6%, 42.6% and 35.6%, respectively. Notably, the percentage of white pepper, long pepper and saffron simultaneously appeared in the same prescription was 21.78%^{9,10}. White pepper, long pepper and cinnamon have a long history of use in TCHM and are believed to releasing the

Table 2 Effects of oral Pretreatment with OR (250-1000 mg/kg) on hot-plate test in mice.

Treatment	Dose (mg/kg)	Reaction time (s)				
		0 min	30 min	60 min	90 min	120 min
control	—	15.88 $\pm$ 2.42	20.00 $\pm$ 3.12	12.63 $\pm$ 3.42	17.63 $\pm$ 3.74	14.25 $\pm$ 7.29
	1000	14.63 $\pm$ 3.20	17.63 $\pm$ 3.82	14.13 $\pm$ 6.60	13.38 $\pm$ 4.47	13.88 $\pm$ 6.90
OR	500	15.50 $\pm$ 3.12	19.00 $\pm$ 3.07	13.25 $\pm$ 3.11	12.13 $\pm$ 3.76	11.38 $\pm$ 3.40
	250	16.00 $\pm$ 3.02	18.50 $\pm$ 3.93	16.00 $\pm$ 2.67	14.25 $\pm$ 3.66	15.00 $\pm$ 4.90

Note: Data represents mean $\pm$ S.D. ($n = 8$). * $p < 0.05$ when compared to the control group by ANOVA followed by Dunnett's test.

Yang and awaking the brain. Saffron and myrrh have been demonstrated to activating circulation to remove blood stasis. To some degree, these herbs can reflect effects of the whole prescription. That these five drugs have been studied integrally in this report is very meaning.

It is well-known that the aroma of aromatic drugs mainly comes from the essential oil. The essential oil is traditionally obtained by steam distillation. This process can induce hydrolysis, thermal degradation and water solubilization of some fragrance components⁴⁴⁾. What's more, drug residues were wet and broken, not suitable for later processing. Extracts obtained by solvents contain residues that pollute the essential oil to which they are added. These disadvantages can be averted by using the supercritical fluid extraction technology (SFE)⁴⁴⁾. So, the OR recipe was obtained by SFE in this study.

Inflammation always started acute inflammation that is characterized by vasodilatation, which occurs first at the arteriole level progressing to the capillary level, and brings about a net increase in the amount of blood present causing the redness, heat and increased capillary permeability of inflammation, is the prime response of inflammatory mediators releasing under the on set of inflammation⁴⁰⁾. So, we tested the impact of OR on the DMB-induced ear edema. Results showed that OR (1000, 500, 250, 62.5 mg/kg for mice) could significantly decrease ear edema by reducing the weight of the edematous ears. From Fig. 1 we could see that the leakage inhibition rate of OR was over 50%. More surprising, the highest dose of OR showed an inhibition of 91%. The acetic acid-induced vascular permeability also used in study of acute inflammation, is known to be an excellent acute inflammatory model in which fluid extravasation. At oral doses of 1000 mg/kg showed an inhibition of 70.89% in vascular permeability assay. The result that OR (1000 mg/kg, p.o.) has significant effect than indomethacin is similar than ear edema test. These results implied that OR owned a vasodilatation and capillary permeability inhibition effect in the first stage of acute inflammation response by decreasing the inflammatory mediator release.

Inflammation can result from chemical stimuli, tissue damage, or autoimmune processes. In each case, stimulation of the immune system causes about the release of inflammatory mediators. These mediators in turn generate numerous effects, including sustained activation and sensitization of both primary nociceptors and higher order neurons involved in the transmission of nociceptive input⁴⁵⁾. In this present paper, the analgesic effects of OR was assayed using the chemically and thermally induced nociceptive pain model in mice. The writhing test induced by acetic acid in mice was commonly considered as classical peripheral inflammatory pain animal model and widely used as a screening for evaluation of analgesic drugs. The peripheral analgesic effect may be triggered by the libera-

tion a variety of inflammatory mediators such bradykinin, substance P, and prostaglandins, especially cyclo-oxygenases, lipoxygenases, as well as some cytokines such as IL-1 β , TNF- α and IL-8^{28, 43)}. The obtained results (Fig. 3) confirmed that OR at four doses have a peripheral analgesic action. It is important to note that the doses of OR tested in this study (500, 1000 mg/kg) significantly inhibited the number of writhes in comparison with the reference drug (indomethacin). The hot plate test has been found suitable to evaluate central antinociceptive effect because of several advantages, particularly the sensitivity to antinociceptives and limited tissue damage⁴⁶⁾. Despite OR displayed a significant antinociception response on the acetic acid induced pain, all test samples showed no significant effects in hot-plate model, suggesting a non-participation on thermal stimulation associated with central neurotransmission in what the heat activates nociceptors.

The research obtains significant results, but still exists some problems worth us to think and improve. Firstly, 1 hour of administration time was selected in our study, it is not fully consider the peak plasma drug concentration and half-life of elimination, although this experimental condition referenced lots of references about anti-inflammatory activity of essential oils. In our further research, we are going to monitor concentrations of the main compounds in rat plasma. Those data will lay the solid foundation to predict the best time to administer drugs for increasing therapeutic effect of the OR extracts. Secondly, there is non-dose dependent relationship on the dose of 250 and 62.5 mg/kg in anti-inflammatory tests. This phenomenon could be related to the potential bioavailability of the OR extracts. Because of the main active compounds of OR extracts are not clear, so there are no good standards to study the potential bioavailability of OR. We hope those problems can be well understood by other researchers.

Chemical compositions of the OR were analyzed by GC/MS and identified the presence of 37 compounds, representing 74.42% of total oil. These chemicals such as β -selinene (7.38%), aromadendrene (5.30%), β -elemene (5.22%), cis-piperitol (5.21%), cis- β -guaiane (4.67%), ylangene (3.70%), δ -cadinene (3%), β -cadinene (2.87%) are present in high amounts. The activities of these components rarely been reported in the past, although a large number of reports revealed essential oil of numerous herbs enriched in these chemicals have antioxidant, anti-inflammatory, anticancer, and antimicrobial activities⁴⁷⁻⁵⁵⁾. It was noteworthy that β -elemene could pass through the blood-brain barrier and treated cerebral malignancy⁵⁶⁾. Together with the previous studies, we can confirm that the anti-inflammatory and antinociceptive effects of OR could be attributed to the single or synergic action of these main components in the oil.

Through all the results in our studies, we can deduce that the essential oil of traditional Hui prescription may

play a role in the treatment of stroke. Lots of main chemical compositions of OR such as β -elemene would help resist the inflammation of stroke. Therefore it is worth to further our exploration of the ability of OR to pass through the blood-brain barrier and the effect of some components on ischemic stroke.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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