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Experimental study on antinociceptive and anti-allergy effects of patchouli oil

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Pogostemon cablin (Blanco) Benth. (Lamiaceae), which is named ‘Guang-Huo-Xiang’ in Chinese, has various therapeutic functions to remove dampness, relieve summer-heat and exterior syndrome, stop vomiting, and stimulate appetite. Patchouli oil is the essential oil of *Pogostemonis herba*. This study is aimed to investigate the antinociceptive and anti-allergy activities of patchouli oil. The antinociceptive activity of patchouli oil (20, 40, and 80 mg/kg) was evaluated using the acetic acid-induced writhing test (NIH mice) and the hot-plate test (NIH mice), and those for anti-allergy activity were tested using Schultz–Dale reaction, the passive cutaneous anaphylaxis (PCA) test, and delayed-type hypersensitivity (DTH) test. All doses of patchouli oil could significantly ($p < 0.05$) extend writhing latent period and moderately decrease writhing frequency. In the hot-plate test, patchouli oil treatment significantly ($p < 0.01$) increased the latency period at all three doses at 30, 60, and 90-minute time intervals compared with the control group. Patchouli oil could remarkably ($p < 0.01$) inhibit the contraction induced by the Schultz–Dale reaction. Moreover, patchouli oil at all doses significantly ($p < 0.01$) inhibited the PCA reaction induced by Sprague–Dawley rat anti-ovalbumin serum and the DTH reaction induced by the 2,4-dinitrochlorobenzene when comparing with those of the control group. The findings suggested that patchouli oil may provide additional evidence for its potential uses as antinociceptive and anti-allergy agents.

Keywords: patchouli oil; antinociceptive activity; anti-allergy activity

Introduction

Pogostemonis Herba is the dried aerial part of *Pogostemon cablin* (Blanco) Benth. (Lamiaceae) (also known as ‘Guang-Huo-Xiang’ in Chinese). This plant is cultivated extensively in China, Indonesia, Malaysia, and Brazil for its essential oil productions (1). Its therapeutic functions in Chinese medicine include removing dampness, relieving summer-heat and exterior syndrome, stopping vomiting, and stimulating appetite (2), and it is traditionally used for treatment of common cold, nausea, diarrhea, headache, and fever (3). Moreover, this herb is a major component herb of many popular traditional Chinese herb product formulations, such as Baoji pill and Huoxiangzhengqi oral liquid products (4, 5). These traditional products are used in the treatment of heat stroke, diarrhea, and vomiting problems. Pharmacological studies showed that the *Pogostemonis Herba* has a variety of activities including anti-inflammatory and antinociceptive (6, 7), anti-emetic (8), immunomodulatory (9), and antimicrobial actions (10).

Patchouli oil was obtained by steam distillation of *Pogostemon cablin* (Blanco) Benth. (Lamiaceae) leaves. It has been extensively used in perfumes, soaps and cosmetic products, as well as in food and alternative medicines (11). Research has showed that patchouli oil consists of over twenty-four sesquiterpenes (12), and the major constituent is patchouli alcohol or pogostone based on the different regions of cultivation and harvesting seasons (13), which suggests that it is a type of chemotype plant. Patchouli oil has many pharmacological activities including anti-inflammatory, antinociceptive and antimicrobial actions (6, 14). Our previous studies demonstrated that the *Pogostemonis Herba* has anti-inflammatory activity *in vitro* and *in vivo* (15, 16), and the effect was due in part to the inhibition of the expression of cyclooxygenases (COXs) for decreasing in prostaglandin (PG) production, which consequently reduces pain and inflammation. Moreover, the prostaglandin E2 (PGE2) can reduce the production of cAMP, which could trigger the release of allergy mediators.

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Based on the findings above, we hypothesized that patchouli oil may potentially have antinociceptive and anti-allergy uses, and this present study is to investigate these activities of patchouli oil.

Experimental

Plant materials

Patchouli oil was purchased from Nanhai Zhongnan Co., Ltd (Lot 090601, Foshan, China).

Animals

National Institutes of Health (NIH) mice (*Mus musculus*, weight: 18–22 g) of six weeks old *in vivo* samples, Sprague–Dawley (SD) rats (*Rattus norvegicus*, weight: 150–200 g) of four months old samples and guinea pigs (*Cavia porcellus*, weight: 200–250g), of either sex, were all obtained from Medical Experimental Center of Guangzhou University of Chinese Medicine. All animals were raised under natural conditions (temperature: 23–25°C; 12-hour/12-hour light–dark cycle). The animals were received in humane care conditions for the use of laboratory animals, as published by the US National Institution of Health (NIH Publication, revised in 1985). The experimental procedures were approved by our institutional animal research ethics committee (approval number: SCKX (YUE) 2008-0020) with reference to the European Community guidelines for the use of experimental animals.

Reagents

Indomethacin reagent was purchased from Huanan Pharmacy Group Co., Ltd. (Dongguan, China). Tramadol hydrochloride solution was from Neptunus Co., Ltd (Shenzhen, China). Naloxone hydrochloride solution was from Qinghai Pharmacy Group Co., Ltd. (Xining, China). Cyproheptadine hydrochloride solution was from Changzhou Siyao Pharmacy Group Co., Ltd. (Changzhou, China). Evans blue was from National Pharmacy Group Chemical Reagent Co., Ltd (Shanghai, China). Ovalbumin was from Sigma (Shanghai, China). 2,4-Dinitrochlorobenzene (DNCB) was from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). C₇–C₃₀ straight-chain hydrocarbons were from Shanghai ANPEL Scientific Instrument Co., Ltd (Shanghai, China). Other solvents such as acetic acid, *n*-hexane, acetone and ethanol were purchased from Tianjin Damao Chemical Reagent Works (Tianjin, China).

Gas chromatography–mass spectrometry analysis

The chemical composition of patchouli oil was analyzed by a gas chromatography with mass spectrometry detector (GC–MS). It was performed with an Agilent 6890-5975 model gas chromatograph–mass

spectrometer (Agilent Corporation, Santa Clara, CA, USA). The essential oil samples were diluted with *n*-hexane at a ratio of 1:6 prior to injection. The column used was a non-polar column of HP-5MS with a dimensions of 30 m × 0.25 mm ID × 0.25 µm; the carrier gas used was helium at a flow rate of 1 mL/minute split 1:60; and, the injector temperature was 250°C. The temperature was programmed from 150°C (isothermal oven programme for 23 minutes), and then it was raised to 230°C at 8°C/minute. Thereafter, the conditions were held for 2 minutes. The mass spectrometer measurement was scanned from 50 to 400 *m/z*. The ionization source temperature was 280°C. The injection volume was 1 µL. Individual components were identified by NIST database matching. Retention indices (RIs) of constituents were determined using standard C₇–C₃₀ straight-chain hydrocarbons. The reference RI database is the Database of Insect Pheromones and Semiochemicals.

Quantitative analysis of patchouli oil using gas chromatography–flame ionization detector

Quantitative analysis of patchouli oil was performed by a flame ionization gas chromatography (FID), using Varian-3900 equipment (Varian Corporation, Palo Alto, CA, USA). Samples were diluted with *n*-hexane (10 mg/mL) prior to injection. The column used was a ZB-WAX, 30 m × 0.32 mm ID × 0.25 µm film thickness (Phenomenex Corporation, Torrance, CA, USA); the carrier gas used was helium at a flow rate of 1 mL/minute; the inlet mode was split 1:20; and, the injector temperature was 250°C. The temperature was programmed from 150°C (isothermal oven programme for 23 minutes), and then it was raised to 230°C at 8°C/minute. Thereafter, the conditions were held for 2 minutes. The ionization source temperature was 280°C. The injection volume was 1 µL. Quantification of patchouli oil was estimated by an internal standard method. The internal standard was octadecane solution (1.5 mg/mL).

Acetic acid-induced writhing test

The writhing test in mice was carried out by using the method of the previous study with slight modification (17). Mice of either sex were divided into five groups consisting of ten mice (*n*=10), fasted overnight with a free access to tap water. Positive drug indomethacin (20 mg/kg, p.o.) and patchouli oil dissolved in soybean oil (20, 40 and 80 mg/kg, p.o.) were given for four days (q.d.). The control group was given equal amounts of soybean oil as a vehicle during this period. Sixty minutes after the last administration, writhing was induced by injecting 0.2 mL of 0.6% acetic acid intraperitoneally. The writhing was monitored for 10 minutes, and the onset time of writhing for each

mouse was recorded (reaction time). Percentage inhibition of writhing was calculated as the following formula:

$$\text{Inhibition of writhing (\%)} = \frac{\text{Control Mean} - \text{Test Mean}}{\text{Control Mean}} \times 100$$

where Control Mean=mean number of writhes in the control group; Test Mean=mean number of writhes in the treated group.

Hot-plate test

The hot-plate test employed in this study was as previously described (18) with slight modifications made. Mice of either sex were fasted overnight with a free access to tap water. A hot-plate apparatus (YLS-6B, China) was used for determining the antinociceptive effect of patchouli oil. In this experiment, mice were placed individually on a hot plate maintained at $55 \pm 0.5^\circ\text{C}$ before administration. The time for each mouse to lick its paws or jump was recorded as the pretreatment reaction time. Mice showed a pretreatment reaction time >30 seconds or <5 seconds were not used in the subsequent test. A cutoff time of 45 seconds was imposed to avoid tissue damage. Positive drug tramadol (10 mg/kg, i.p.) and patchouli oil (20, 40 and 80 mg/kg, p.o.) were given for four days (q.d.). The control group was given equal amounts of soybean oil as a vehicle during this period. To examine the possible connection of endogenous opioids to antinociceptive activity, patchouli oil (80 mg/kg) and tramadol (10 mg/kg) were investigated in groups of mice pretreated with naloxone (5 mg/kg, i.p.). Reaction time was again measured in mice 30, 60 and 90 minutes after the last administration. The percentage increase in pain threshold was calculated by using the following formula:

$$\text{Increase in Pain threshold (\%)} = \frac{\text{Test Mean} - \text{Control Mean}}{\text{Control Mean}} \times 100$$

where Control Mean=mean time in the control group; Test Mean=mean time in the treated group.

The Schultz–Dale reaction of the guinea pig ileum

Guinea pigs (200–250 g) of either sex were sensitized to ovalbumin as previously described (19). Guinea pigs were subjected to intramuscular injection of 0.8 mL of Krebs–Henseleit buffer solution containing of 40 mg ovalbumin, introduced into the rear legs. Three weeks later, they were sacrificed by a blow on the head. Some pieces of ileum (1 cm) were divided into five groups of

ten each, removed and suspended in an organ bath containing Krebs–Henseleit buffer solution (118 mM NaCl; 4.7 mM KCl; 2.5 mM CaCl_2 ; 1.2 mM KH_2PO_4 ; 1.2 mM $\text{MgSO}_4 \cdot 2\text{H}_2\text{O}$; 25 mM NaHCO_3 ; 11 mM glucose; pH 7.4) at 37°C and gassed with 5% CO_2 in oxygen. Contractions of the intestinal segments were detected by a tension transducer, and the dates were recorded. The tissues were allowed to stabilize for 15 minutes. A 0.1 mL of Krebs–Henseleit solution containing 5 mg/mL ovalbumin was then given until reproducible contractions were obtained. This concentration of ovalbumin gave a submaximal contraction of the guinea pig intestine. Positive drug cyproheptadine of hydrochloride (0.00045 mg/mL) and patchouli oil (0.01, 0.02 and 0.04 mg/mL) were given 5 minutes before challenge with ovalbumin; the control group was given equal amounts of soybean oil as a vehicle. The antigen (OA)-induced contractile responses were evaluated by the maximum tension of each challenge.

The passive cutaneous allergy skin test in rats

The passive cutaneous anaphylaxis (PCA) test was performed as described previously with minor modifications (20). Briefly, ten male SD rats (weight: 150–200 g) received a total of 2 mL (by means of four 0.25-mL portions) of saline; 20 mg ovalbumin was injected into four feet intramuscularly and 0.1 mL 4% $\text{Al}(\text{OH})_3$ was injected intracutaneously. Two weeks later, the blood was obtained by decapitation. The serum was obtained by centrifugation. Thereafter, the serum was stored at -4°C .

Fifty male SD rats were divided into five groups consisting of ten mice ($n=10$). Positive drug cyproheptadine hydrochloride (20 mg/kg, p.o.) and patchouli oil (20, 40 and 80 mg/kg, p.o.) were given for 14 days (q.d.). The control group was given equal amounts of soybean oil as a vehicle during this period. At the last administration, 24 hours later, the dorsal hair of the rat was removed and 0.4 mL of 10-fold diluted antiserum was injected intracutaneously. At 48 hours, PCA was elicited by intravenously injecting 1.0 mL of saline containing 10 mg Evans blue and 10 mg ovalbumin. The rats were sacrificed 30 minutes after induction of the cutaneous reaction, and the skin at the reaction site was excised. The skin specimen was dissolved in 5 mL of saline and acetone (7:3) mixture solutions for 48 hours. Thereafter, the absorbance of dye extracted in the supernatant was measured at 610 nm with a spectrophotometer (UNICO7200). The leaked dye amount was calculated by subtracting the dye content of the untreated site from that of the antiserum-injected site.

2, 4-Dinitrochlorobenzene induced delayed-type hypersensitivity in mice

Mice of either sex were divided into five groups consisted of ten mice ($n=10$); mouse fur on the abdomen was shaved. Positive drugs of cyproheptadine hydrochloride (40 mg/kg, p.o.) and patchouli oil (20, 40 and 80 mg/kg, p.o.) were given for nine days (q.d.). The control group was given equal amounts of soybean oil as a vehicle during this period. At the last administration 24 hours later, 1 mL 5% DNCB solution was smeared on the shaved skin. At seven days, 1 mL 1% DNCB solution was smeared on the dorsal surface of the right ear of mice. Then 24 hours later, mice were sacrificed; the anti-allergy activity of patchouli oil was investigated by measuring increments between the right and the left ear biopsies of the same mouse.

Statistical analysis

All results are expressed as mean \pm standard errors of mean (SEM). The statistical significances within a parameter were evaluated by using analysis of variance (ANOVA) followed by Tukey's test. Values were considered significantly different at $p<0.05$.

Results

Composition of patchouli oil

Table 1. showed the composition of patchouli oil with their mass spectra matching similarity and RI. Patchouli oil is composed mainly of terpenoids, of which the monoterpenes and sesquiterpenes were accounted for more than 90% (peak area percentage) total in the oil. Quantitative assay by GC-FID showed that the major constituent was patchouli alcohol (26.5%) of the oils.

Table 1. Composition of patchouli oil.

Peak no.	Composition	RI database	RI	% in oil	Matching similarity (%)
1	β -Patchoulene	1378	1374	7.0	96
2	β -Elemene	1389	1388	1.0	94
3	cis-Thujopsene	1428	1400	0.9	77
4	Isocaryophyllene	1413	1412	6.3	94
5	β -Gurjunene	1432	1433	3.3	74
6	α -Patchoulene	1456	1447	8.6	88
7	Δ -Patchoulene	1452	1450	0.7	96
8	β -Caryophyllene	1467	1471	1.0	92
9	cis- β -Guaiane	1489	1482	0.7	72
10	γ -Gurjunene	1473	1493	3.2	43
11	β -Selinene	1485	1501	0.4	79
12	β -guaiane	1500	1510	14.2	85
13	Elemol	1547	1546	3.0	88
14	Globulol	1576	1568	0.8	85
15	trans-Longipinocarveol	1639	1574	0.6	35
16	Caryophyllene oxide	1606	1619	0.8	92
17	Patchouli alcohol	1659	1647	26.5	98
18	Bulnesol	1666	1656	0.7	86
19	Aristolone	1756	1752	0.6	90

Note: The reference retention index (RI) database is the Database of Insect Pheromones and Semiochemicals.

Acetic acid-induced writhing test

All doses of patchouli oil could significantly ($p<0.05$) extend the latent time while simultaneously reduce the number of mouse abdominal constrictions when compared with the control group. In details, inhibition of 20, 40, and 80 mg/kg groups were 12.25%, 20.41%, and 29.59%, respectively. Moreover, the highest dose (80 mg/kg) inhibited the writhing significantly ($p<0.05$) when compared with the control group. (Table 2.).

Hot-plate test

The mean thresholds at different time intervals after treatment in different groups were shown in Table 3. A significant ($p<0.01$) increase in reaction time for all doses at 30, 60, and 90 minutes after treatment was noted. Moreover, more than 40% protection was achieved at 60 and 90 minutes. Tramadol also caused significant antinociception. In combination studies using naloxone, an opioid receptor antagonist, the analgesic activity of tramadol was diminished by naloxone. Moreover, naloxone antagonized patchouli oil (80 mg/kg) antinociception at the 30, 60, and 90 minutes (Table 4).

The Schultz-Dale reaction of the guinea pig ileum

The control group showed a strong contraction after being challenged by the previously sensitization with ovalbumin. The positive drug cyproheptadine hydrochloride (0.00045 mg/mL) had a significant inhibiting effect when compared with the control group. Patchouli oil treatments were significantly inhibited the contraction at $p<0.01$ compared with the control (Figure 1).

Table 2. Inhibitory effect of patchouli oil on the acetic acid-induced writhing in mice.

Group	Number of writhes per 10 minutes	Reaction time (seconds)	Percentage inhibition (%)
Control	29.4±2.0	234.4±36.8	
Indomethacin (20 mg/kg)	1.9±0.3*	790.2±33.0**	93.537
Patchouli oil (20 mg/kg)	25.8±2.1	354.0±33.7*	12.245
Patchouli oil (40 mg/kg)	23.4±2.0	511.1±39.0**	20.408
Patchouli oil (80 mg/kg)	20.7±1.9*	567.9±37.3**	29.592

Note: Each value represents mean±SEM ($n=10$).

* $p<0.05$, ** $p<0.01$ indicates significant different compared with the control group.

Table 3. Effect of patchouli oil on hot-plate test in mice.

Group	Hot-plate latency (s)			
	Before treatment	30 minutes	60 minutes	90 minutes
Control	10.2±1.0	10.7±0.7	10.3±1.0	9.3±0.6
Tramadol (10 mg/kg)	10.8±0.8	21.3±1.5**	20.0±1.5**	18.0±1.6**
Patchouli oil (20 mg/kg)	10.0±1.1	15.1±1.1=	15.7±1.1**	17.2±1.0**
Patchouli oil (40 mg/kg)	10.8±1.0	14.5±0.9**	14.7±1.1**	15.4±1.3**
Patchouli oil (80 mg/kg)	11.1±1.1	16.2±1.3**	15.1±1.1**	15.3±1.0**
Tramadol (10 mg/kg)+naloxone (5 mg/kg)	10.1±1.1	16.8±1.6**	14.7±1.2**	12.6±0.7**
Patchouli oil (80 mg/kg)+naloxone (5 mg/kg)	10.2±0.7	12.0±0.9	11.8±0.9	11.1±0.9*

Note: Each value represents mean±SEM ($n=10$).

* $p<0.05$, ** $p<0.01$ indicates significant different compared with the control group.

Table 4. Effect of patchouli oil on hot-plate test in mice.

Group	% protection against hot-plate stimuli			
	Before treatment	30 minutes	60 minutes	90 minutes
Control	0.0	0.0	0.0	0.0
Tramadol (10 mg/kg)	5.9	99.0	94.2	93.5
Patchouli oil (20 mg/kg)	-2.0	41.1	54.2	84.9
Patchouli oil (40 mg/kg)	5.9	35.5	42.7	65.6
Patchouli oil (80 mg/kg)	8.9	51.4	46.6	64.5
Tramadol (10 mg/kg)+naloxone (5 mg/kg)	-0.1	57.0	42.7	35.5
Patchouli oil (80 mg/kg)+naloxone (5 mg/kg)	0	12.1	14.6	19.4

Note: Each value represents mean±SEM ($n=10$).

* $p<0.05$, ** $p<0.01$ indicates significant different compared with the control group.

The passive cutaneous allergy skin test

The effect of patchouli oil on PCA-reaction induced in the back skin of rats is presented in Figure 2. Cutaneous injection of anti-ovalbumin serum induced prominent enhancement of vascular permeability exhibited by dye elusion into the back skin in the control rats. The enhancement of vascular permeability was significantly ($p<0.01$) suppressed by oral administrations of patchouli oil (20, 40, 80 mg/kg).

2,4-Dinitrochlorobenzene induced delayed-type hypersensitivity skin test in mice

Ear vasodilatation was induced by DNCB application in the sensitized mice ears. An increase of ear weight was seen in all animals throughout the observation

period. Both of patchouli oil and positive drug cyproheptadine hydrochloride had a significant inhibiting effect when comparing with the control group. All doses significantly ($p<0.01$) decreased the vasodilatation (Figure 3.) compared with the control.

Discussion

Antinociceptive activity

The results of the present study demonstrated that oral administration of patchouli oil dosage in mice exerted significant antinociception against the chemicals (acetic acid) and thermal stimuli of nociception. The acetic acid-induced abdominal writhing test is a typical model commonly used to assess the inflammatory pain with

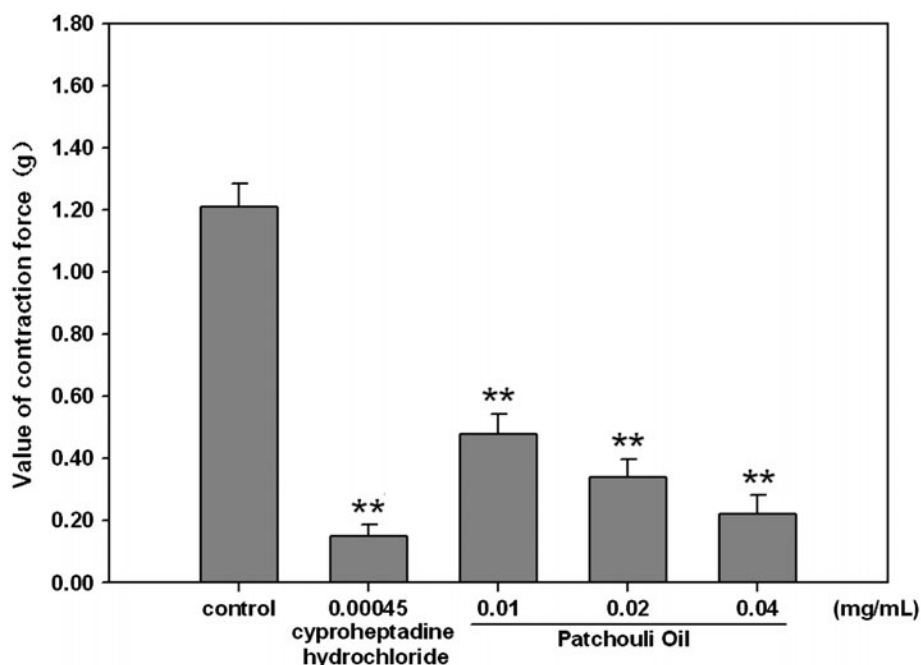


Figure 1. Effects of administration of patchouli oil on ovalbumin-induced contraction of the guinea pig ileum. Each value represents mean \pm SEM ($n=10$). * $p<0.05$, ** $p<0.01$ indicates significant different compared with the control group.

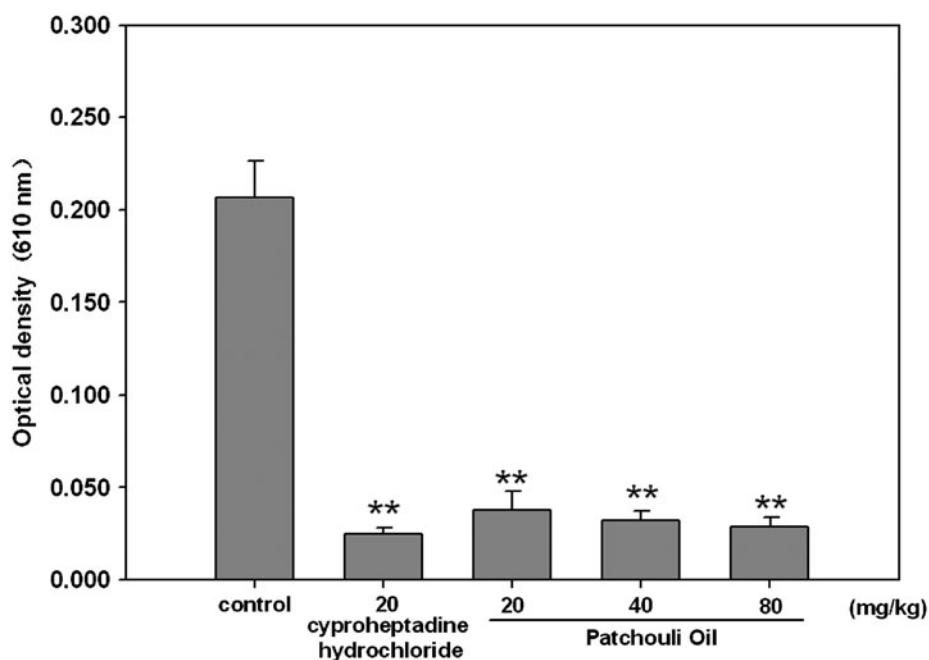


Figure 2. Effects of administration of patchouli oil on the passive cutaneous allergy reaction. Each value represents mean \pm SEM ($n=10$). * $p<0.05$, ** $p<0.01$ indicates significant different compared with the control group.

high sensitivity, but low specificity (21). Intraperitoneal injection of acetic acid produced pains through activation of chemosensitive nociceptors or irritation of the visceral surface, which lead to the liberation of histamine, bradykinin, PGs and serotonin (22). A

positive result of the writhing test indicated that the antinociceptive activity of patchouli oil can be due in part to inhibition of the release of inflammatory mediators or indirect blockade of peripheral COXs activity (23). The writhing test has the advantage of allowing

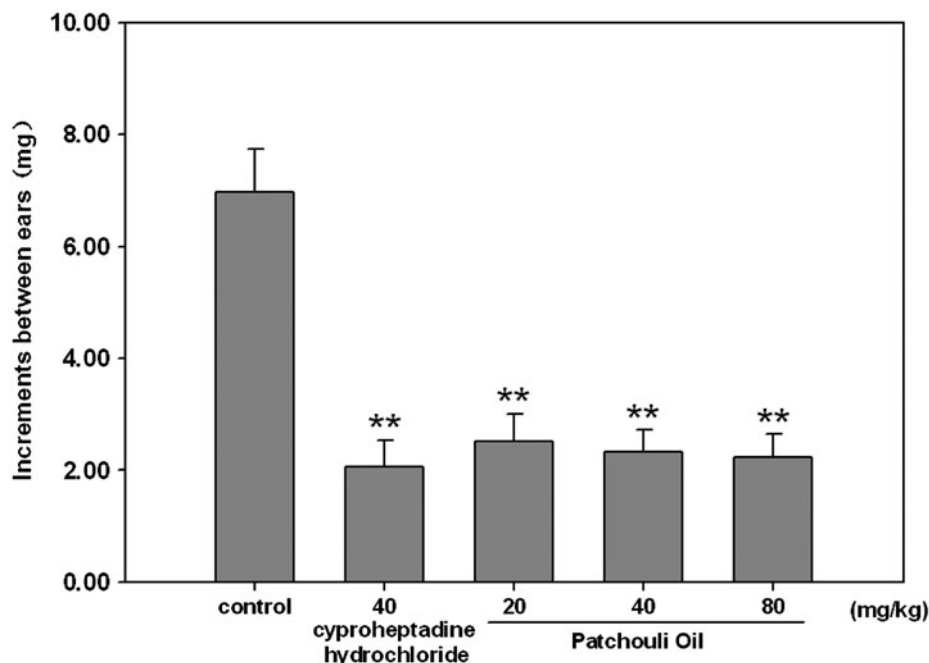


Figure 3. Effects of administration of patchouli oil on delayed-type hypersensitivity in mice. Each value represents mean \pm SEM ($n=10$). * $p<0.05$, ** $p<0.01$ indicates significant different compared with the control group.

evidence to be obtained for effects produced by weak antinociception. However, some other substances without antinociceptive action including muscle relaxants and sedatives can produce similar effects (24). Thus, a positive result with this test does not necessarily mean there is antinociceptive activity. In the reported study on antinociceptive activity of patchouli oil (6), only the writhing test was used to determine the antinociceptive effect. Therefore, in the present study, the hot-plate test was employed to clarify the mechanisms involved. The hot plate test is a selective model for studying the central but not for peripheral antinociceptive activity of the extracts or compounds (25). This method is widely used to screen potential substances that inhibit pain of central origin (24). The results showed that patchouli oil treatment significantly increased the latency period at all three doses at 30, 60, and 90 minutes of time intervals compared with the control group ($p<0.01$). However, the antinociceptive effects of patchouli oil (80 mg/kg) and tramadol were blocked by naloxone which indicates that the antinociceptive effect of patchouli oil was mediated via activation of opioid receptors. Herein, the present findings from both antinociception mice models convinced us that patchouli oil would be a promising essential oil for both the peripheral and central antinociception.

Pogostemon is a large genus from the family Lamiaceae. Although there is little literature regarding the antinociceptive activity of genus *Pogostemon*, many studies have shown that many essential oils extracted

from plants among the family Lamiaceae have antinociceptive properties (26–29). Chemically, the essential oils are composed mainly of terpenoids and phenylpropanoids, including polyketides and a few alkaloids. The terpenoids from essential oils are often monoterpenes and sesquiterpenes, which account for over 90% of the oils (30). Moreover, many literatures reported that thirty-five terpenoids have antinociceptive activity (30). Thus, a major sesquiterpenoid of patchouli alcohol in this essential oil may be one of the bioactive constituents for antinociceptive activity.

Anti-allergy activity

The animal models employed in this part of the study are the most commonly used models in recent pharmacological studies for hypersensitivity. Hypersensitivity reactions involved can be classified into two types: type I and type IV. Type I hypersensitivity is known as immediate or anaphylactic hypersensitivity type, which is mediated by IgE (31). The Schultz–Dale reaction and PCA test are the typical models of type I hypersensitivity. The mechanism of this reaction involves the introduction of antigen, which can produce an antibody (IgE antibody in particular) response. However, IgE binds very specifically to receptors on the surface of mast cells and basophils. The interaction of the antigen with cell-bound IgE causes cell degranulation and triggers the release of chemical mediators that cause smooth muscle contraction, vasodilation, increased vascular permeability and the other allergic symptoms.

The results of the Schultz–Dale reaction and PCA test indicated that patchouli oil may suppress the reaction by reducing the allergen-specific immunoglobulin E level. The DNCB-induced delayed-type hypersensitivity (DTH) skin test in mice is an *in vivo* experimental animal model of type IV hypersensitivity. According to our previous studies, patchouli alcohol has anti-inflammatory activity *in vivo* (16), and this effect was due in part to the inhibition of the expression of COXs for the decreasing in prostaglandin (PG) production, which consequently reduced pain and inflammation. Moreover, the prostaglandin E₂ (PGE₂) can reduce the production of cyclic adenosine monophosphate (cAMP), which could trigger the release of allergy mediators. Patchouli alcohol may be a potential bioactive constituent in patchouli oil.

Our results demonstrated that patchouli oil inhibited the type IV hypersensitivity in a dose-dependent manner. Type IV hypersensitivity is mediated by T lymphocytes (32). Patchouli oil may inhibit the type IV hypersensitivity via the affection of T lymphocytes; however, a concrete mechanism is still unclear. Most of the clinical allergic reactions are more than a single type, as obtained from our study, which displayed the inhibition activity of patchouli oil against type I and type IV hypersensitivity. These studies will be continued for hypersensitivity type II and III in the future, considering patchouli oil may have good prospects as an anti-allergy application.

Conclusions

Based on the results of the present study, it can be concluded that patchouli oil has potentially antinociceptive and anti-allergy activity.

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