Analgesic Therapeutic Effects of *Bombax malabaricum* flowers, *Osmanthus fragrans* and Their Compatibility in Mice

Peng LING[1], Yumei ZHANG[1], Yulin ZHAO[1], Xingwang WANG[1], Aipeng LI[2], Lixin SUN[2], Wenjing YANG[2], Yongshan JIN[3], Suoyi HUANG[3,4]*

1. School of Clinical Medicine, Youjiang Medical University for Nationalities, Baise 533000, China; 2. School of Basic Medical Sciences, Youjiang Medical University for Nationalities, Baise 533000, China; 3. School of Pharmacy, Youjiang Medical University for Nationalities, Baise 533000, China; 4. Key Laboratory of Guangxi Universities on National Medicine in Youjiang River Basin, Baise 533000, China

Abstract [**Objectives**] To investigate the impact of the compatibility of *Bombax malabaricum* flowers and *Osmanthus fragrans* on the analgesic therapeutic effects in mice. [**Methods**] The analgesic effects of *B. malabaricum* flowers and *O. fragrans*, as well as their compatibility at a 1 ∶ 1 ratio, were investigated using the hot plate test and the acetic acid writhing test. Observations were made regarding the reactions of mice, specifically the licking of their forepaws and hindpaws, both prior to and following drug administration. The duration of these reactions was recorded, and the pain threshold of the mice was assessed following drug administration. Additionally, the frequency of writhing responses was documented following the injection of acetic acid into the abdominal cavity of the mice for 20 min. [**Results**] The pain thresholds observed in the positive control group, the *B. malabaricum* flowers group, the *O. fragrans* group, and the compatibility group were significantly elevated compared to those of the blank control group following 14 d of drug administration ($P < 0.05$). This finding indicates that the positive control group, the *B. malabaricum* flowers group, the *O. fragrans* group, and the compatibility group of *B. malabaricum* flowers and *O. fragrans* at a 1 ∶ 1 ratio exhibited analgesic efficacy in mice. Furthermore, the pain thresholds of the *B. malabaricum* flowers and *O. fragrans* groups were significantly lower than that of the compatibility group ($P < 0.05$), suggesting that the compatibility group demonstrated a significantly superior analgesic effect compared to the *B. malabaricum* flowers group in mice. A statistically significant difference was observed in the frequency of writhing responses among the five experimental groups: the normal saline group, the positive control group, the *B. malabaricum* flowers group, the *O. fragrans* group, and the compatibility group ($P = 0.01 < 0.05$). Post hoc analyses revealed that the frequency of writhing responses in the *O. fragrans* group was significantly lower than that observed in both the normal saline group and the compatibility group. Additionally, the frequency of writhing responses in the positive control group was significantly lower than that in the normal saline group. [**Conclusions**] *B. malabaricum* flowers and *O. fragrans*, as well as their compatibility at a 1 ∶ 1 ratio, exhibits analgesic effects, with the analgesic effect being more pronounced in the compatibility group compared to the *B. malabaricum* flowers group or the *O. fragrans* group.

Key words *Bombax malabaricum* flowers, *Osmanthus fragrans*, Compatibility, Analgesic effect

1 Introduction

Pain is defined as an uncomfortable sensory and emotional experience that is associated with actual or potential tissue damage, presenting a significant challenge in clinical diseases[1]. In the context of surgical procedures, pain can induce fear of movement, diminish postoperative mobility in patients, and may even result in complications[2]. Chronic pain is characterized by its prolonged duration and is associated with a range of complications, including dysfunction and diminished immune response, which exacerbate the life and economic stress experienced by numerous patients[3]. While there are various treatment methods available, pharmacological interventions, particularly opioids, present significant limitations, including the potential for addiction and adverse side effects[4–5]. Presently, a major global challenge is the identification of an effective pain management program that minimizes side effects and remains within the financial constraints of patients.

Bombax malabaricum, a member of the genus *Bombax* within the family Bombacaceae, is primarily distributed across regions such as Guangdong, Guangxi, and Yunnan in China. This species is characterized by its flat and pungent properties. The roots, flowers, and leaves of *B. malabaricum* are known for their therapeutic effects, including the alleviation of heat and dampness, promotion of blood circulation, and reduction of swelling[6]. Recent studies have highlighted its rich composition of various chemical constituents, which have led to its extensive application in anti-inflammatory and antibacterial treatments. Additionally, *B. malabaricum* is a key ingredient in the Five Flowers Tea. Research on the anti-inflammatory and analgesic properties of *B. malabaricum* has been conducted in China. *Osmanthus fragrans*, a member of the genus *Osmanthus* within the family Oleaceae, is extensively cultivated in the southern regions of China. This species is characterized by its golden-yellow flowers and is recognized for its dual role in both medicinal and culinary applications. Historical records indicate that it was utilized in ancient times for the treatment of arthralgia and myalgia. Contemporary research has demonstrated that *O. fragrans* possesses antioxidant properties and enhances immune function[7]. Furthermore, studies suggest that the analgesic and anti-inflammatory effects of extracts derived from its dried flowers may surpass those of aspirin[8–9]. However, research focusing specifically on the dried flowers of this plant remains relatively limited. This study was conducted to investigate the analgesic therapeutic effects of *B. malabaricum* flowers and *O. fragrans*, as well as their compatibility at a 1 ∶ 1 ratio, based on findings from previous research.

Received: March 10, 2025 Accepted: September 6, 2025

Supported by Undergraduate Innovation and Entrepreneurship Training Program of Guangxi Zhuang Autonomous Region (S202310599060).

* Corresponding author. Suoyi HUANG, doctoral degree, professor.

The hot plate stimulation method was employed to assess the antinociceptive properties of the drugs by measuring the pain threshold in mice. Additionally, acetic acid was utilized to induce a writhing response in the abdominal cavity of the mice, thereby facilitating the screening of analgesic drugs[10]. The primary objective of this research is to explore a novel and effective approach to pain management, while also exploring the potential applications of *B. malabaricum* flowers, *O. fragrans*, and their compatibility in the field of analgesia.

2 Materials and methods

2.1 Test subjects A total of 68 SPF-grade, healthy adult male mice, aged 6 weeks and weighing 30 – 45 g, were procured from Changsha Tianqin Animal Technology Co., Ltd., production license No.: SCXK(X)2022-0011. The mice were maintained in an SPF-grade environment with a temperature of 22 ℃ and a humidity level of 50%. They were provided with feed and water ad libitum, and were subjected to a light/dark cycle with alternating illumination for 12 h.

2.2 Drugs *B. malabaricum* flowers (specification: 500 g) and *O. fragrans* (specification: 500 g) were procured from Youjiang District Zhengyang Pharmacy in Baise City, Guangxi. Aspirin (specification: 250 g) was manufactured by Beijing Solarbio Science & Technology Co., Ltd. Sodium chloride injection (specification: 4.5 g) was produced by Cisen Pharmaceutical Co., Ltd., while glacial acetic acid (specification: 500 mL) was produced by Xilong Chemical Co., Ltd.

2.3 Main consumables and equipment The consumables and equipment utilized in the study comprised the following: a RE-3000A rotary evaporator (Shanghai Yarong Biochemical Instrument Factory), a TWCL magnetic stirrer (Shanghai Biaohe Instrument Co., Ltd.), a ZN-10002 electronic balance (Hangzhou Youheng Weighing Equipments Co., Ltd.), a TH-UP-10 laboratory specific ultra pure water purifier (Shanghai Hetai Instrument Co., Ltd.), a pulverizer (Yongkang Jiupin Industry & Trade Co., Ltd.), clean grade laboratory mice maintenance feed (Beijing Keao Xieli Feed Co., Ltd.), and an RB-200 intelligent hot plate apparatus (Chengdu Taimeng Software Co., Ltd.).

2.4 Grouping and drug administration of experimental animals A total of 65 healthy mice of SPF grade were selected, acclimatized, and fed for one week. Their weights were recorded every 2 d throughout the experiment. The mice were randomly assigned to groups, with each group consisting of 13 mice. Mice were labeled using picric acid and categorized into the following groups: blank control group, positive control group, *B. malabaricum* flowers group, *O. fragrans* group, and a compatibility group consisting of *B. malabaricum* flowers and *O. fragrans* at a 1 : 1 ratio. During the experiment, mice in the traditional Chinese medicine group were administered the corresponding extracts of traditional Chinese medicine via gavage at a dosage of 20 g/kg based on their body weight. Mice in the blank control group received an equivalent volume of normal saline, while those in the positive control group were treated with an aspirin solution at a dosage of 20 g/kg once daily for 14 d. The animal experiments were conducted in accordance with the ethical standards governing animal research.

2.5 Extraction of traditional Chinese medicines *B. malabaricum* flowers and *O. fragrans* were dried, weighed, and subsequently ground into a fine powder using a pulverizer. The powdered *B. malabaricum* flowers were then mixed with 15 times their volume of distilled water and subjected to condensation reflux evaporation extraction in a water bath maintained at 60 ℃ for 2 h. Following this, the mixture was filtered through filter paper, and an additional 10 times the volume of distilled water was added to continue the condensation reflux evaporation extraction for an additional 1.5 h, resulting in a total of three extraction cycles. The filtrates from all three extractions were combined, filtered again, and then evaporated and concentrated to yield an infiltration paste. Upon cooling, distilled water was added to achieve a final mass concentration of 1 g/mL. The solution was stored in the refrigerator at a temperature of 4 ℃ for future use. The preparation method and final concentration of *O. fragrans* were identical to those of *B. malabaricum* flowers.

2.6 Measurement of experimental indicators According to the method in the literature[11], a hot plate apparatus was employed to initially identify qualified mice exhibiting pain thresholds ranging from 5 to 30 sec. Ultimately, 40 mice were selected and subsequently randomized into five equal groups for the experimental procedure.

Hot plate test: the pain threshold of mice was measured 1 h following the conclusion of the final gavage. The temperature of the hot plate apparatus was maintained at (55 ± 0.1) ℃, and the mice were subsequently placed on the hot plate. The onset of pain was indicated by any of the following behaviors: licking of the forepaws and hindpaws, withdrawal of the hind paw, or jumping. The duration from the moment the mice were placed on the hot plate to the occurrence of any of these behaviors was recorded, thereby determining the pain threshold value for each mouse. The rate of increase in pain threshold was calculated at the conclusion of the measurement period.

Rate of increase in pain threshold (%) = (Mean pain threshold of control group – Mean pain threshold of administered group)/Mean pain threshold of control group × 100%.

Writhing method: 30 min after the last gavage, mice were injected intraperitoneally with 20 mL/kg of a 0.5% acetic acid solution and observed for 20 min. If the mice exhibited abdominal concavity, extension of the trunk and limbs, and elevation of the buttocks, these behaviors were classified as a writhing response. The frequency of writhing responses in the mice was then recorded.

2.7 Statistical methods Data that conformed to a normal distribution were presented as ($\bar{x} \pm s$). For the analysis of data across multiple groups, one-way ANOVA was employed, along with subsequent multiple comparisons between groups. Conversely, data that did not meet the criteria for normal distribution were analyzed using k independent samples in non-parametric tests, with the aid of SPSS 27.0 software.

3 Results and analysis

3.1 Comparison of body weight of mice in each group As illustrated in Table 1, 1 d prior to administration, the body weight of the *O. fragrans* group and the compatibility group was significantly lower than that of the positive control group ($P<0.05$). The remaining body weight values did not exhibit statistical significance.

Table 1 Comparison of body weight of mice in each group ($\bar{x} \pm s$, $n=8$, g)

Group	1 d prior to administration	7 d post administration	14 d post administration
Blank control	35.11 ±2.63	35.22 ±2.07	34.23 ±3.32
Positive control	33.18 ±2.40	34.24 ±2.05	34.91 ±2.07
Bombax malabaricum flowers	34.95 ±1.23	35.08 ±1.50	35.20 ±1.76
Osmanthus fragrans	36.00 ±2.05#	35.29 ±2.11	35.15 ±2.24
Compatibility	35.36 ±1.29#	33.08 ±3.36	34.14 ±2.38

NOTE Compared to the positive control group, #$P<0.05$.

3.2 Comparison of pain thresholds caused by hot plates As presented in Table 2, the pain thresholds of the positive control group, the *B. malabaricum* flowers group, the *O. fragrans* group, and the compatibility group were significantly elevated compared to those of the blank control group at 14 d post administration ($P<0.05$). This finding indicates that the positive control group, the *B. malabaricum* flowers group, the *O. fragrans* group, and the compatibility group, which consisted of *B. malabaricum* flowers and *O. fragrans* at a 1∶1 ratio, all exhibited analgesic effects in mice. The pain thresholds observed in the *B. malabaricum* flower group and the *O. fragrans* group were significantly lower than those recorded in the positive control group ($P<0.05$). This finding suggests that the analgesic effect of aspirin on mice was markedly superior to that of the *B. malabaricum* flowers and *O. fragrans* groups. Furthermore, the pain thresholds of the *B. malabaricum* flower group were also significantly lower than those of the compatibility group ($P<0.05$), indicating that the analgesic effect of the compatibility group was significantly more effective than that of the *O. fragrans* group. The data presented in the table further illustrate that the analgesic effect of the compatibility group was weaker in comparison to the aspirin positive control group.

Table 2 Comparison of pain thresholds caused by hot plates ($\bar{x} \pm s$, $n=8$)

Group	Pain threshold//sec	Rate of increase in pain threshold//%
Blank control	7.79 ±1.59	–
Positive control	15.47 ±3.81$^{1)}$	98.59
Bombax malabaricum flowers	11.09 ±3.70$^{1)2)3)}$	42.30
Osmanthus fragrans	11.87 ±2.69$^{1)2)}$	52.37
Compatibility	14.55 ±3.82$^{1)}$	86.78

NOTE Compared to the blank control group, $^{1)}P<0.05$; compared to the positive control group, $^{2)}P<0.05$; compared to the compatibility group, $^{3)}P<0.05$.

3.3 Differential analysis of the frequency of writhing responses As presented in Table 3, the median frequency of writhing responses was observed to be 58 in the normal saline group, 36 in the positive control group, 41.5 in the *B. malabaricum* flowers group, 30 in the *O. fragrans* group, and 54.5 in the compatibility group. The statistical analysis yielded $H=13.305$, $P=0.01<0.05$, indicating a significant difference in the frequency of writhing responses among the groups. Further multiple comparisons revealed that the frequency of writhing responses in the *O. fragrans* group was significantly lower than that in both the normal saline group and the compatibility group. Additionally, the frequency of writhing responses in the positive control group was significantly lower than that in the normal saline group.

Table 3 Differential analysis of the frequency of writhing responses ($n=8$)

Group	Frequency of writhing responses	*H*	*P*	Multiple comparison
Blank control	58.00 (41.50, 61.50) a	13.305	0.01	$d<a$, $d<b$, $c<a$
Positive control	36.00 (28.00, 39.75) c			
Bombax malabaricum flowers	41.50 (36.50, 42.75)			
Osmanthus fragrans	30.00 (27.50, 41.50) d			
Compatibility	54.50 (25.75, 58.25) b			

NOTE Different lowercase letters in the same column indicate a statistically significant difference ($P<0.01$).

4 Discussion

Pain is recognized as the fifth vital sign of life[12]. Opioids serve as the primary treatment modality for moderate to severe pain[13]. However, nonsteroidal anti-inflammatory drugs such as indomethacin, ibuprofen, and diclofenac sodium, while effective as analgesics, are also linked to adverse gastrointestinal effects[14]. These side effects can lead to considerable discomfort for patients during pain management and may exacerbate the burden on the healthcare system. Consequently, the development of new, potent, and cost-effective novel anti-inflammatory and analgesic medications should prioritize the assessment of adverse drug reactions, as these reactions are critical for patients experiencing side effects from medications. In light of the current medication status, this study investigated the compatibility of *B. malabaricum* flowers and *O. fragrans* at a 1∶1 ratio for analgesic treatment. The objective was to determine whether this compatibility could yield a synergistic effect, thereby enhancing the efficacy of analgesic medications for patients and establishing a reliable foundation for their clinical application.

This test aimed to formulate a mixture of *B. malabaricum* flowers and *O. fragrans* at a 1∶1 ratio, based on previous studies. The analgesic effects of *B. malabaricum* flowers and *O. fragrans*, as well as their compatibility, were assessed in mice using the hot plate test and the acetic acid writhing test. The results of the hot plate test indicate that the analgesic effect of the compatibility group comprising *B. malabaricum* flowers and *O. fragrans* at

(To page 36)

[14] TAN W, ZHOU W, LUO HS, *et al.* The inhibitory effect of melatonin on colonic motility disorders induced by water avoidance stress in rats [J]. European Review for Medical and Pharmacological Sciences, 2013, 17(22): 3060–3067.
[15] HU Y, ZHENG YL, MEI QX, *et al.* Effects of Tongxie Yaofang formula ultrafine granular powder in viscera hypersensitive of rats with chronic water avoidance stress[J]. Traditional Chinese Drug Research and Clinical Pharmacology, 2021, 32(3): 322–331. (in Chinese).
[16] HU Y, ZHENG YL, MEI QX, *et al.* HPLC determination of four components in Tongxie Yaofang and its broken wall decoction pieces[J]. Journal of Chinese Medicinal Materials, 2019, 42(7): 1601–1604. (in Chinese).
[17] HU Y, ZHENG YL, MEI QX, *et al.* Simultaneous determination of seven active components in Tongxie Yaofang formula and ultrafine granular powder by ultra-performance liquid chromatography[J]. Traditional Chinese Drug Research & Clinical Pharmacology, 2017, 28(6): 786–791. (in Chinese).
[18] COLOMIER E, ALGERA J, MELCHIOR C. Pharmacological therapies and their clinical targets in irritable bowel syndrome with diarrhea[J]. Frontiers in Pharmacology, 2021, 11: 629026.
[19] LI ZL, XU EP, WANG XG. Research progress on the mechanism of Tongxie Yaofang in regulating IBS-D visceral hypersensitivity[J]. Journal of Liaoning University of Traditional Chinese Medicine, 2024, 26(6): 120–125. (in Chinese).
[20] PROGATZKY F, SHAPIRO M, CHNG SH, *et al.* Regulation of intestinal immunity and tissue repair by enteric glia[J]. Nature, 2021, 599(7883): 125–130.
[21] LI Y, LI YR, JIN Y, *et al.* Involvement of enteric glial cells in colonic motility in a rat model of irritable bowel syndrome with predominant diarrhea[J]. Journal of Chemical Neuroanatomy, 2023, 128: 102235.
[22] LONG X, LI M, LI LX, *et al.* Butyrate promotes visceral hypersensitivity in an IBS-like model via enteric glial cell-derived nerve growth factor [J]. Neurogastroenterology & Motility, 2018, 30(4): e13227.
[23] WANG KK, YANG YL, ZHOU YN, *et al.* Efficacies of Tong-Xie-Yao-Fang disassembled recipes on brain-gut peptideina rat model of diarrhea-predominant irritable bowel syndrome[J]. Chinese General Practice, 2023, 26(5): 569–575. (in Chinese).
[24] WANG P, DU C, CHEN FX, *et al.* BDNF contributes to IBS-like colonic hypersensitivity via activating the enteroglia-nerve unit[J]. Scientific Reports, 2016, 6: 20320.
[25] LIU S. Neurotrophic factors in enteric physiology and pathophysiology [J]. Neurogastroenterology & Motility, 2018, 30(10): e13446.
[26] ZHAO Y. Mechanism of regulating BDNF/TrkB signaling pathway by electroacupuncture to inhibit EGCs overactivation and relieve visceral hypersensitivity in IBS-D rats[D]. Chengdu: Chengdu University of Traditional Chinese Medicine, 2022. (in Chinese).

(From page 31)

a 1 : 1 ratio was significantly superior to that observed with the individual application of traditional Chinese medicine. However, this effect was found to be less potent when compared to the positive control group. Additionally, the acetic acid writhing test demonstrated that the frequency of writhing responses in the *O. fragrans* group was significantly lower than that in both the normal saline group and the compatibility group. The experimental results indicate that *B. malabaricum* flowers and *O. fragrans*, as well as their compatibility, exhibits synergistic effects, with the analgesic effect of this compatibility significantly differing from that observed with the individual application of each medicine. This study primarily explored the effects of *B. malabaricum* flowers and *O. fragrans*, as well as their compatibility, on analgesic outcomes in mice, but the underlying mechanisms of action warrant further investigation.

References

[1] PEI JH, HAN CY, ZHAO C, *et al.* Application progress of mindfulness therapy in the treatment of chronic pain patients[J]. Chinese Journal of Pain Medicine, 2020, 26(4): 291–296. (in Chinese).
[2] LUO GR, LUO QY, LI LJ, *et al.* Current status and influencing factors of kinesiophobia in cardiac surgery patients[J]. Journal of Youjiang Medical University for Nationalities, 2025, 47(1): 186–192. (in Chinese).
[3] LI Q, HUANG Y, YAN WJ, *et al.* Impact of chronic pain during pregnancy on growth and development of offspring in mice[J]. Chinese Journal of Pain Medicine, 2020, 26(3): 180–184. (in Chinese).
[4] NAMIGAR T, SERAP K, ESRA AT, *et al.* The correlation among the Ramsay sedation scale, Richmond agitation sedation scale and Riker sedation agitation scale during midazolam-remifentanil sedation[J]. Brazilian Journal of Anesthesiology, 2017, 67(4): 347–354.
[5] FARMER AD, HOLT CB, DOWNES TJ, *et al.* Pathophysiology, diagnosis, and management of opioid-induced constipation[J]. Lancet Gastroenterology & Hepatology, 2018, 3(3): 203–212.
[6] TANG AC, YU Y, HUANG M, *et al.* Advances in studies on chemical constituents and pharmacological effects of *Bombax malabaricum* flowers [J]. Chinese Journal of Ethnomedicine and Ethnopharmacy, 2020, 29(23): 74–79. (in Chinese).
[7] LIU G, WU PK, JIANG XM, *et al.* Bacteriostasis and antioxidant effects of *Osmanthus fragrans* fruit peel ethanol extract[J]. Journal of Sichuan Normal University (Natural Science), 2018, 41(5): 672–676. (in Chinese).
[8] YIN W, YU Y, MA QL, *et al.* Study on chemical constituents and antitumor activities of leaves of *Osmanthus fragrans*[J]. Journal of Tropical and Subtropical Botany, 2018, 26(2): 178–184. (in Chinese).
[9] PANG YM, LU HM, WANG B, *et al.* Study on the acute toxicity and the effect of analgesic and anti-inflammatory by the extract of dry flowers of golden *Osmanthus fragrans*[J]. Journal of Guangxi Agriculture, 2022, 37(4): 51–54. (in Chinese).
[10] LIU HL, LI YL, XUE YZ. Effects of different temperatures and acetic acid concentrations on writhing model in mice[J]. Journal of Baotou Medical College, 2006, 22(2): 137–138. (in Chinese).
[11] ZHAO SB, CHEN XY, YU HL, *et al.* Experimental study on analgesic effect of Lianqiao(Forsythia), Yejuhua(Wild Chrysanthemum) and their compatibility[J]. Chinese Journal of Traditional Medical Science and Technology, 2020, 27(4): 547–549. (in Chinese).
[12] SHE SZ. Innovations lead the clinical application research of artificial intelligence patient controlled analgesia[J]. Guangdong Medical Journal, 2023, 44(3): 265–270. (in Chinese).
[13] PAN FT, HUANG Z, ZHANG CY, *et al.* Research progress on the influence of OPRM1 A118G gene polymorphism on opioid drugs in pain treatment[J]. Journal of Youjiang Medical University for Nationalities, 2019, 41(4): 449–451. (in Chinese).
[14] OHLAN R, SUCHETA, NANDAS, *et al.* Analgesic prodrugs for combating their side-effects: Rational approach[J]. Current Drug Delivery, 2017, 14(1): 16–26.