Research Article

Inhibitory effect of Phlai capsules on skin test responses among allergic rhinitis patients: a randomized, three–way crossover study

Pattara Tanticharoenwiwat1,2, Prapasri Kulalert1,2, Thaweephol Dechatiwongse Na Ayudhya3, Sittichai Koontongkaew4,5, Weena Jiratchariyakul3, Rudeed Soawakontha6, Prakongsiri Booncong7, Orapan Poachanukoon1,2,3
1. Center of Excellence for Allergy, Asthma and Pulmonary Disease, Thammasat University Hospital, Pathumthani 12120, Thailand
2. Department of Pediatrics, Faculty of Medicine, Thammasat University, Pathumthani 12120, Thailand
3. Medicinal Herb Research Unit for Asthma, Faculty of Medicine, Thammasat University, Pathumthani 12120, Thailand
4. Faculty of Dentistry, Thammasat University, Pathumthani 12120, Thailand
5. Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand
6. Faculty of Pharmaceutical Sciences, Huachiew Chalermprekiet University, Samut Prakan 10540, Thailand
7. Department for Development of Thai Traditional and Alternative Medicine, Ministry of Public Health, Nonthaburi 11000, Thailand

ABSTRACT

BACKGROUND: Zingiber cassumunar Roxb., commonly known as Phlai in Thai, has been used as a traditional medicine in Thailand for the treatment of various diseases, including inflammation and chronic airway disease.

OBJECTIVE: The purpose of this study was to assess the antihistaminic effect of Phlai on skin testing.

DESIGN, SETTING, PARTICIPANTS AND INTERVENTION: This was a randomized, open-label, three-way crossover study. Twenty allergic rhinitis (AR) patients were enrolled. In randomized sequence, patients received a single dose of Phlai capsules (100 or 200 mg) or loratadine (10 mg) with a washout period of 1 week between each treatment.

MAIN OUTCOME MEASURES: Skin prick testing for histamine and common aeroallergen (house dust mite) were performed before treatment and after 1, 2, 3, 4, 6, 8, 12 and 24 hours of treatment. The main treatment outcomes were the mean wheal and flare responses to the skin prick test after treatment.

RESULTS: Both 100 mg and 200 mg Phlai doses suppressed wheal and flare responses to house dust mite allergen, but only 200 mg of Phlai capsules significantly suppressed wheal and flare responses to histamine. Repeated measures analysis of variance showed that loratadine caused more wheal and flare suppression than Phlai capsules in responses to the histamine skin prick test. However, there were no significant differences among the effects of 100 mg Phlai capsules, 200 mg Phlai capsules and loratadine in suppression of wheal and flare induced by the mite skin prick test. Both doses of Phlai were well-tolerated with no adverse events.
1 Introduction

Zingiber cassumunar Roxb. (Zingiberaceae), commonly known as Phlai in Thai, has been used as a traditional medicine in Thailand for the treatment of various diseases, including inflammation and asthma. Several phenylbutenoids and cyclohexene derivatives have been identified in the rhizomes of Phlai. (E)-4-(3´, 4´-dimethoxyphenyl)but-3-en-1-ol (compound D; Figure 1), isolated from the rhizomes of Z. cassumunar, has been reported to possess a potential anti-inflammatory activity.\[^{1}\]\(^{1}\] Z. cassumunar extract exhibits an anti-allergic effect against antigen-induced β-hexosaminidase, released as a marker of degranulation in rat basophil leukemia cell line (RBL-2H3).\[^{2}\]\(^{2}\] It inhibits the cleavage of promatrix metalloproteinase-9 (MMP-9) by house dust mites and inhibits phorbol 12-myristate 13-acetate-induced MMP-9 gene expression and protein synthesis in human airway epithelial cells.\[^{3}\]\(^{3}\] It also suppresses mucin production, including MUC2 and MUC5AC expression in human airway epithelial cells.\[^{4}\]\(^{4}\] Additionally, compound D has an antispasmodic effect in the guinea pig ileum and tracheal smooth muscle.\[^{5}\] Pulverized rhizome of Phlai is effective in relieving symptoms of asthma in children\[^{6}\]\(^{6}\] and adults.\[^{7}\] A previous study compared the antihistaminic effect of Phlai powder with chlorpheniramine in asthmatic children. It showed that Phlai produced significant inhibition of wheal response to the histamine skin prick test. However, the suppressive effect of chlorpheniramine was greater than that of Phlai. No adverse reaction to Phlai was observed during the study.\[^{8}\] However, the antihistaminic effect of Phlai has not yet been compared to that of second-generation H1-antagonist drugs, which are now recommended for treatment of allergic rhinitis and chronic urticaria because of reduced incidence of adverse effects.\[^{9,10}\] The primary objective of this study was to compare the antihistaminic effect of the standardized Phlai extract with that of loratadine, a commonly used second-generation antihistamine. Secondarily, we tested for dose-dependency in the effects of standardized Phlai extract, using 100 and 200 mg Phlai capsules, equivalent to 4 and 8 mg of compound D.

2 Materials and methods

2.1 Materials

Each Phlai capsule contains 25 mg of standardized Phlai extract, which is equivalent to 1 mg of compound D, and the following inactive ingredients: dibasic calcium phosphate, microcrystalline cellulose, corn starch, sodium starch glycolate and polyvinyl pyrrolidone K30. Phlai capsules were produced by the Quality Herbal Product Project, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand.

Twenty volunteers, 18–50 years old, with a clinical history of allergic rhinitis and a positive skin test to house dust mite (Dermatophagoides pteronyssinus) were eligible for the study. Exclusion criteria involved the presence of uncontrolled asthma, upper respiratory tract infection, history of severe reaction to skin prick tests, immunotherapy treatment and other internal organ disorders. Drugs known to alter the skin prick test response were not to be used prior to and during the study to avoid suppressant effects on skin test; thus any antihistamine drugs were withheld for 7 d prior to and during the study, topical and systemic steroids were withheld for 2 weeks prior to and during the study.\[^{11,12}\] Blinded co-investigators conducted the informed consent interviews, and all participants signed a written informed consent to participate into the study. The study was approved by the human ethics committee of our hospital (MTU-EC-PE-1-012/59) and registered with http://www.clinicaltrials.in.th (TCTR20160510001).

CONCLUSION: Both 100 mg (compound D 4 mg) and 200 mg (compound D 8 mg) Phlai capsules, when taken as a single therapeutic dose, inhibited skin reactivity to histamine and mite skin prick tests in AR patients.

TRIAL REGISTRATION: Thai clinical trial registry (TCTR20160510001).

Keywords: Zingiber cassumunar; Phlai; antihistamine; skin test; rhinitis, allergic; loratadine

2.2 Study design

This study was conducted at the outpatient clinic of Thammasat University Hospital, Thailand. A prospective, randomized, open-label, three-way crossover trial design was used. After the initial enrollment visit, each volunteer was assigned to one of six randomized groups which would receive either a single dose of 4 Phlai capsules (100 mg standardized Phlai extract), 8 Phlai capsules (200 mg standardized Phlai extract) or one tablet of 10 mg loratadine. A hold period of approximately 7 d was placed between each treatment to minimize carryover effects from repeated treatments (Figure 2). In each treatment period, subjects underwent skin testing with histamine (histamine 1 mg/mL, ALK-ABELLÓ, Madrid, Spain) and common aeroallergen (D. pteronyssinus, ALK-ABELLÓ, Madrid, Spain) skin prick tests before received the drug at 7:30–8:00 a.m. (time 0). The tests were subsequently administered 1, 2, 3, 4, 6, 8, 12 and 24 hours after the administration. All skin tests were administered and read by the same investigator.

2.3 Assessment of antihistaminic activity

Antihistamine activity was evaluated at each time point by suppression of wheal and flare areas. Fifteen minutes after each skin prick test, the wheal and flare areas induced by the test were traced on the forearm with a ballpoint pen and transferred to a sheet of paper using transparent tape. Wheal and flare areas were each assessed by measuring the largest diameter and its perpendicular diameter to quantify the area of skin response (INSIZE Digital Vernier Caliper®). The wheal and flare responses were determined by calculating the mean of these two values.

2.4 Adverse effects

Participants were asked to report any unusual symptoms occurring after the drug intake such as sedation, dizziness, dry mouth or headache.

2.5 Statistical analysis

Results are expressed as the mean ± standard error of mean (SEM). Percentage inhibition of the wheal and flare was calculated according to the following equation:

\[
\text{Percentage inhibition} = \left( \frac{W(F)_{\text{mean baseline}} - W(F)_{\text{mean posttreatment}}}{W(F)_{\text{mean baseline}}} \right) \times 100
\]

Statistical analysis was performed using repeated measures analysis of variance. Student’s t test for paired data was used to test differences between baseline and posttreatment results when data were normally distributed. The Wilcoxon signed-rank test was used for analysis of nonnormally distributed data. P value < 0.05 was taken to indicate statistical significance. The analyses were conducted in SPSS (Version 13.0 Chicago, USA SPSS Inc).

3 Results

3.1 Demographic data

The study included 20 allergic rhinitis patients (14 women and 6 men). According to allergic rhinitis and its impact on asthma definition, there were 12 patients with mild intermittent allergic rhinitis, 5 patients with mild persistent allergic rhinitis and 3 patients with severe persistent allergic rhinitis. The mean age of the patients was (34.3 ± 2.5) years.

3.2 Histamine-induced skin test

Mean wheal and flare responses to the histamine skin prick test and percentage inhibition of this test by Phlai and loratadine are shown in Figures 3 and 4, respectively. The 100 mg Phlai capsules appeared to suppress the histamine-induced wheal size slightly from (4.02 ± 0.20) mm to (3.66 ± 0.18) mm at \( t = 6 \) h, but this was not significant (\( P = 0.165 \)). At \( t = 6 \) h, the same dose significantly suppressed the histamine-induced flare from (16.96 ± 1.85) mm to (11.90 ± 1.93) mm (\( P = 0.013 \)).

The 200 mg Phlai capsules significantly inhibited the histamine-induced wheal between 6 and 12 h and the histamine-induced flare between 4 and 8 h when compared to baseline. The peak inhibition effects of the 200 mg Phlai capsules occurred 6 h postdose, when the wheal size was reduced from (4.58 ± 0.20) mm to (3.73 ± 0.15) mm (\( P = 0.002 \)) and the flare size was reduced from (16.96 ± 1.85) mm to (11.90 ± 1.93) mm (\( P = 0.013 \)).
reduced from (16.15 ± 1.07) to (12.04 ± 1.85) mm \((P = 0.024)\).

Loratadine produced significant inhibition of the histamine-induced wheal from 2 to 12 h. The peak effects were observed at 3 h posttreatment and the wheal size was reduced from (4.83 ± 0.24) mm to (3.23 ± 0.20) mm \((P < 0.001)\). Loratadine produced significant inhibition of the histamine-induced flare from 2 h until the end of observation, with a peak effect at 8 h, when the flare size was reduced from (18.54 ± 1.37) mm to (3.19 ± 1.33) mm \((P < 0.001)\).

Loratadine suppressed the wheal and flare responses to the histamine skin test more than \textit{Phlai} capsules throughout the observation period, but this suppression was not significant at all time points. Loratadine suppressed the wheal response significantly more than 100 mg \textit{Phlai} capsules from 2 to 8 h and 200 mg \textit{Phlai} capsules from 2 to 4 h. Loratadine suppressed the flare response significantly more than 100 mg \textit{Phlai} from 4 to 8 h and 200 mg \textit{Phlai} from 4 h until the end of the observation period.

When comparing \textit{Phlai} 100 mg and 200 mg capsules, it was observed that although the 200 mg capsules had higher activity on wheal and flare responses than the 100 mg capsules, the differences were not statistically significant at any time point.

### 3.3 Mite-induced skin test

Mean wheal and flare responses to the mite skin prick test and percent inhibition of wheal and flare size by treatment with \textit{Phlai} and loratadine are shown in Figures 5 and 6.

Treatment with 100 mg \textit{Phlai} capsules significantly inhibited both the mite allergen-induced wheal and flare responses at 3 h postadministration. The wheal size decreased from (6.34 ± 0.79) mm to (5.15 ± 0.52) mm \((P = 0.023)\), and the flare size decreased from (22.10 ± 3.05) mm to (17.75 ± 2.65) mm \((P = 0.013)\).

The 200 mg \textit{Phlai} capsules produced significant suppression from 4 h to 8 h with a peak effect at 4 h. The mean wheal diameter decreased from (5.98 ± 0.65) mm to (4.98 ± 0.66) mm \((P = 0.025)\). The peak effect of 200 mg \textit{Phlai} capsules occurred at 4 h posttreatment, inhibiting the mite-induced flare response from (19.71 ± 2.30) mm to (16.69 ± 2.38) mm \((P = 0.022)\).

Loratadine significantly inhibited the mite-induced wheal response from 2 to 8 h, with a peak effect at 4 h, when wheal size decreased from (6.03 ± 0.77) mm to (4.00 ± 0.45) mm \((P < 0.001)\). Loratadine inhibited the mite-induced flare response at 1 h through the end of observation (24 h) with a peak effect at 6 h, with flare diameter decreasing from (20.06 ± 2.57) mm to 12.64 ± 2.44 mm \((P = 0.001)\).
were no significant differences in suppression of the mite-induced wheal and flare responses among the three treatment groups at any time point during the experiment.

Both doses of the *Phlai* capsules and loratadine 10 mg were well-tolerated by participants, and no adverse events were reported.

### 4 Discussion

In this study we compared the effectiveness of *Phlai* capsules at the dosage of 100 mg (4 mg of compound D), 200 mg (8 mg of compound D) and that of loratadine 10 mg in inhibiting skin test responses to histamine and aeroallergen (mite). We found that histamine-induced wheal and flare responses were inhibited significantly by 200 mg *Phlai* capsules but to a significantly greater degree by loratadine. Both doses of *Phlai* capsules (200 mg and 100 mg of *Phlai* capsules) significantly inhibited mite allergen-induced wheal and flare responses. However, the dose-dependent inhibitory effects of *Phlai* capsules were observed in histamine-induced wheal and flare responses.

A previous study found that chlorpheniramine (10 mg) had greater activity than *Phlai* powder (500 mg) in inhibition of the wheal response to histamine.\[^{8}\] By assessing histamine-induced skin prick test responses, Simons et al.\[^{13}\] observed that loratadine significantly suppressed the size of the histamine-induced wheal from 2 to 12 h, with a maximal effect at 5 h. Our results are in accordance with these findings.

In contrast to suppression of histamine-induced responses, we found that both therapeutic dose of *Phlai* and loratadine suppressed mite-induced wheal and flare responses, and we found no significant difference between *Phlai* and loratadine in the degree of this suppression.

The main cause of difference between the effectiveness of *Phlai* and loratadine might be related to pharmacological properties of the agents, such as the distribution volume in human body. If a drug has a high plasma distribution volume, it will have a weak effect.\[^{14}\] Loratadine is 97% to 99% plasma protein bound and has a large volume distribution (119 L/kg). Following oral administration of loratadine 40 mg, a peak concentration of 24.3 to 30.5 µg/L occurs between 1 and 1.5 h. Distribution half-life (t1/2α) values in both single (20 and 40 mg) and multiple doses of loratadine (40 mg) range from 0.9 to 1 h. Plasma elimination half-life (t1/2β) values range from 7.8 to 11 h.\[^{15}\]

However, there is incomplete information available for the pharmacokinetics of compound D. Khemawoot et al.\[^{16}\] examined the pharmacokinetic profiles of compound D in male Wistar rats. After giving the standardized extract at 25 mg/kg by oral administration, the maximum concentration of compound D was 18.44 µg/L after 0.14 h. Compound D demonstrated good tissue distribution to most organs at 1 and 4 h after administration.

From until now, we have no data about human pharmacokinetic study of this herbal medicine. Moreover, the duration of action for antihistamine effects was unknown. For these reasons, the timing for the assessment of skin reaction of *Phlai* in this study may not be enough. Long lasting effects on skin test reactivity should be done in future study.
The efficacy of Phlai (compound D) for suppressing allergen-induced responses in allergic rhinitis patients is still not clear. However, our data suggest that Phlai should be considered for further examination in the context of nasal allergen challenge studies.

The EAACI/ARIA guidelines note that histamine-induced wheal and flare studies do not predict the clinical efficacy of different antihistamines in allergic rhinitis.[9] Therefore, a study comparing the clinical effectiveness and safety of Phlai capsules in the setting of nasal allergen challenge is a necessary next step.

The crossover design was chosen in this study to minimize variability by ensuring within subject treatment comparisons, and a one-week washout interval between treatment periods was designed to minimize any carryover effects. The antihistamine activity of Phlai was evaluated using the skin prick test with a common aeroallergen and histamine; this is the accepted method for objectively assessing the cutaneous histamine antagonistic activity.[13]

5 Conclusion

Our pharmacodynamic study showed good antihistamine activity in both 100 mg Phlai capsules (4 mg of compound D) and 200 mg Phlai capsules (8 mg of compound D) when used as a single therapeutic dose to inhibit reactivity to skin prick tests in allergic rhinitis patients. The inhibitory effect of Phlai capsules to the histamine skin prick test was found to be less potent than that of loratadine. There was no significant difference between Phlai capsules and loratadine in the suppression of wheal and flare induced by mite skin test.

6 Acknowledgements

We thank Mrs. Patchara Boonya-anuchit for excellent assistant. This study was supported in part, by research grants from Thai Traditional Medical Knowledge Fund, Ministry of Public Health, and Thammasat University research fund, Thailand (Fund number 3/2556).

7 Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES


Journal of Integrative Medicine (JIM) is an international, peer-reviewed, PubMed-indexed journal, publishing papers on all aspects of integrative medicine, such as acupuncture and traditional Chinese medicine, Ayurvedic medicine, herbal medicine, homeopathy, nutrition, chiropractic, mind-body medicine, Taichi, Qigong, meditation, and any other modalities of complementary and alternative medicine (CAM). Article types include reviews, systematic reviews and meta-analyses, randomized controlled and pragmatic trials, translational and patient-centered effectiveness outcome studies, case series and reports, clinical trial protocols, preclinical and basic science studies, papers on methodology and CAM history or education, editorials, global views, commentaries, short communications, book reviews, conference proceedings, and letters to the editor.

- No submission and page charges
- Quick decision and online first publication

For information on manuscript preparation and submission, please visit JIM website. Send your postal address by e-mail to jcim@163.com, we will send you a complimentary print issue upon receipt.