Efficacy and safety of topical Trikatu preparation in, relieving mosquito bite reactions: A randomized controlled trial

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KEYWORDS
Trikatu preparation; Mosquito bite; Papule size reduction; Mosquito bite symptoms; Eucalyptus oil

Summary
Introduction: Trikatu is composed of dried fruits of Piper nigrum L and Piper retrofractum Vahl, and dried rhizomes of Zingiber officinale R. Although this preparation has been used to relieve pruritus, pain, and inflammation for a long time, there is no clinical evidence to confirm its efficacy and safety. Therefore, we performed a double-blind, within-person-randomized controlled study of 30 healthy volunteers to determine efficacy and safety of topical Trikatu on mosquito bite reactions.

Methods: All subjects were bitten by Aedes aegypti laboratory mosquitoes on their forearms and they were randomly assigned arms to apply either Trikatu or reference product on the mosquito bite papule. The main outcome was the difference of papule size reduction at 30 min, measured by a caliper, between the Trikatu and reference arms. Pruritis, redness, pain, and patient satisfaction were assessed at 15, 30, 60, 180, and 360 min as secondary outcomes.
Introduction

Trikatu is one of the popular Thai traditional preparations comprising of dried fruits of black pepper (Piper nigrum Linn., family Piperaceae), long pepper (Piper retrofractum Vahl., family Piperaceae) and dried rhizomes of ginger (Zingiber officinale Roscoe., family Zingiberaceae) in various ratio. The various formulas of Trikatu have been used to adjust patient’s element during rainy season for the treatment of illness due to fire, wind, and water, respectively.1,2 Trikatu is also used as Ayurvedic in India for a wide range of diseases and symptoms such as cold, asthma, pruritis, pain, inflammation.3,4 Dried rhizomes of ginger are composed of various substances such as essential oils, 6-gingerol, 6-shogaol, 6-gingessulfonic acid, gingerglycolipids A, B and C.5-8 Ethanol extract of ginger has antioxidant, anti-edema, antipyretic, analgesic and anti-inflammatory effects.8-13 Dried fruits of black pepper are composed of piperine, chavicine, pipermine, piperdine, and volatile oils.14-16 Ethanol extract and volatile oils of pepper have anti-inflammatory, and anti-edema effects.17-21 Dried fruits of long pepper are composed of piperine, piperonialine, piperundecalidine, and dehydropipperonaline.15,22,23 Its ethanol extract and volatile oils have anti-inflammatory, and can decrease abdominal pain and peptic ulcer.14-26 The main active ingredients of Trikatu are piperine from black and long pepper dried fruits, and gingerol from ginger dried rhizomes.5,27 Although the Trikatu capsule (500 mg) is now available commercially in India and other preparations of Trikatu have been used in India and Thailand for a long time in various conditions,1,2,4,5 the majority of research was on pharmacology and toxicology without any studies demonstrating clinical evidence of such preparation.2,5,28-31

Mosquito bites frequently cause skin symptoms including pruritis, redness, and pain. These symptoms are mediated by antiasiva IgE antibodies and histamine reaction leading to induce inflammatory process.32,33 Current therapeutic options for treating mosquito bite associated symptoms are oral antihistamines and topical steroids and antihistamine. Moreover, OTC products containing menthol, eucalyptus have been used for relieving insect bite reactions.34-37 It was postulated that the Trikatu product exerting anti-inflammatory effects may have efficacy for controlling symptoms of mosquito bites. This study was conducted to determine the efficacy and safety of this product on mosquito bite reactions.

Methods

A double blinded, within person-randomized, controlled trial was conducted at Clinical Research Unit, Department of Medical Sciences, Thailand to compare the effects of a topical Trikatu product with a reference product. The study was conducted in compliance with the principles of the Good Clinical Practice, in accordance with Declaration of Helsinki and approved by the Institutional Review Boards of Naresuan University. Thirty subjects participated to the study and all of them provided informed consent before study participation. This study was supported by the National Research Council of Thailand (NRCT) which had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Results: There were no significant differences between treatment and reference arms on any outcome at any time of measurement.

Conclusion: Trikatu did not show additional effects for relieving mosquito bite reaction as compared with the reference product containing camphor, menthol, and eucalyptus. For further study, it is very important to consider a proper selection of subjects, comparator product, and concentration of extract when Trikatu preparation is investigated.

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Mosquito exposure and measurement of bite reactions

Mosquito-bite reaction was induced by *Aedes aegypti* laboratory mosquitoes. One mosquito in a cage was allowed to bite on the right or left forearm for 5 min. The reactions including papule size, intensity of pruritis, erythema, and edema were measured after biting for 30 min as baseline. The papule size was measured (two perpendicular diameters in mm) 3 times per each arm by a physician using digital caliper, then areas of papules were calculated and average of them were reported. For irregularly shaped papules, the two perpendicular diameters were measured and the area was calculated based on its multiplication. Symptoms of mosquito bite (pruritis, erythema and edema) and other adverse reactions (edema, burning and pain) were evaluated by a physician and subjects using visual analogue scale (VAS: range from 0 to 100 mm) and rating score (range from 0 to 3), respectively, as previous described.35–37 Effects of Trikatu and reference products were measured at 15, 30, 60, 180, 360 min after the applications. Physician measuring outcomes was trained as general physicians, had been practicing medicine more than 10 years, and had attended continuing good clinical practice of randomized controlled trial training programs.

The primary outcome was the difference of papule size reduction, measured by a digital caliper, after application Trikatu and reference products for 30 min. The digital caliper (range; 0–300 mm, resolution; 0.01 mm) has been used to determine the efficacy of drugs for relieving mosquito bite symptoms in previous studies.35–37 Secondary outcomes were adverse reactions and other mosquito bite associated clinical symptoms including pruritis, erythema, and edema which were measured at all measurement times by physician and subject using VAS.

In addition, subject’s satisfactions were assessed as secondary outcome at the end of study in various aspects including overall efficacy, efficacy for reducing papule size, erythema, edema and pruritis, product cost, continuous use, and recommendation each product in the future.

Statistical analysis and sample size

The sample size required in this study was 30 participants. The sample size calculation using within-person study designed was based on a pilot study of 5 participants. The sample size was calculated with type 1 error of 0.05% and 80% power to detect 37.94% difference in size reduction of papule (SD; 37.9) after application Trikatu product for 30 min. This calculation has taken into account the possible withdrawal rate of 10%.

All analyses used the intention-to-treat approach. Descriptive statistics were used to describe characteristics of enrolled subjects. Paired simple t-test was used to compare size reduction of papule between treatment and reference arms. McNemar’s test was used to compare the differences of binary outcomes between treatment and reference arms at any time of assessment.

Results

Thirty-seven subjects were screened for eligibility but seven of them did not meet inclusion criteria. As a result, 30 subjects were enrolled and included into the intent-to-treat analysis (ITT analysis). There was no lost follow-up or drop out during study period (Fig. 1). Thirty subjects with average age of 33 years (SD; 6.95) were included in the study. Sixty-three percents of them were female and 70% of them were employee. Both treatment and reference arms had similar baseline characteristics (Table 1).

At 30 min after application of each product, papule size was not changed in both arms. However, reduction of papule size in both arms was seen after application of each product for 1 h and this reduction was statistically significant different from baseline at 3 h after product application (p-value < 0.05). However, there was no statistically significant difference in percentage of papule size between the treatment and reference arms at all times of reduction measurement (Table 2). In addition, there were no statistically significant differences in erythema, edema, and pruritis symptoms between treatment and reference arms at any time of assessment (data not shown). Subject’s satisfactions in various aspects such as overall efficacy, efficacy for reducing papule size and relieving erythema, edema, and pruritis symptoms of Trikatu product were similar to those of reference product (data not shown). In terms of safety, there was no loss to follow up or drop out due to adverse effects and no need for additional drug to treat symptoms of mosquito bites during study period. No signs and symptoms of allergic reaction related to study preparation at any time of assessment were reported.
Discussion

Our finding indicates that the efficacy of topical Trikatu preparation in reducing symptoms of mosquito bites is similar to that of reference preparation. However, both preparations can reduce papule size and relieve erythema intensity, edema and pruritis symptoms from mosquito bite reaction.

The absence of difference in efficacy between Trikatu preparation and reference preparation can be explained by several reasons. First, the reference product contains eucalyptus which is good anti-inflammatory agent. In addition, camphor and menthol have cooling effect that can reduce pruritis symptom of mosquito bites. This might lead to a small room for Trikatu product to exert its anti-inflammatory effect. Second, the level of inflammatory reaction induced by mosquito bites might be so low that it does not require the anti-inflammatory effects obtained from the combination of Trikatu and eucalyptus. Other conditions with higher level of inflammatory reaction such as insect stings (bees, wasps etc.) may increase chance of Trikatu product to exert its full benefits. Third, the amount of Trikatu in the preparation might be too low.

Current evidence has suggested that all ingredients in Trikatu preparation were quite safe. Therefore, there was no need for performing irritation test before conducting a clinical study. In addition, other ingredients in Trikatu product had been used in several current topical products in the market and safety data of each agent indicated minimal irritation effect. In this study, we found no adverse events detected at all times of assessment, thereby supporting the safety and tolerability of topical use Trikatu product.

The strength of our research work is that we performed and reported this clinical trial with high standard adhering to CONSORT statement. The sample size was calculated based on the pilot study and all analyses were performed using intention to treat approach. We believe that our good adherence to the protocol ensure the high quality and internal validity of our findings. In addition, we chose

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reference product that was similar in appearance and odor to treatment product to blind both physicians and subjects. However, the inflammatory and cooling effects of some ingredients in reference product result in reduction of sensitivity to detect the specific effects of Trikatu in treatment product.

A key lesson can be drawn from this research work. Selection of the right comparator and subjects to be investigated is the most crucial process. At early stage of product development, choosing a placebo (without active ingredient) will increase a chance of a product of interest to exert its potential benefits. Including subjects with high inflammatory reaction such as those induced by insect stings (bees, wasps etc.) may increase chance of Trikatu product to exert its full benefits. This should be taken into account for consideration during the design and conduct of research work investigating of herbal medicine products.

Conclusion

Trikatu preparation containing Trikatu extract, camphor, menthol, and eucalyptus did not provide additional anti-inflammatory effect compared to the reference product containing camphor, menthol, and eucalyptus.

Conflict of Interest statement

There is no conflict of interest in this study.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ctim.2013.08.014.

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