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Topical application of *Terminalia chebula* extract helps croton oil-induced dermatitis in mice

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Abstract

Numerous herbs have been used successfully in treating dermatologic disorders for thousands years and still widely used because it is easy to find, cheap and to avoid the side effects and complications of topical steroids. *Terminalia chebula*, commonly found in India and Southeast Asia, is thought to be effective for treating several diseases including inflammation and reported as an anti-oxidant. *T. chebula* extract was tested on croton oil-induced ear dermatitis. The anti-inflammatory response was evaluated by observing redness, ear thickness and ear plug weight. The analysis showed significantly different thickness at the time of 30 minutes till the end, at 5 hours of experiment. Ear plug weight also showed significant difference between treated and untreated ears. Topical application of *T. chebula* extract on croton oil-induced mouse ear dermatitis can help reducing inflammation and could be a novel topical anti-inflammatory drug candidate.

Introduction

Herbal therapy is becoming increasingly popular among patients and physicians. Numerous herbs have been used successfully in treating dermatologic disorders for thousands years and it is still widely used in Asia including in Thailand (Bedi and Shenefelt, 2002). Dermatitis is the most common skin problem reported every past year and topical corticosteroids are well known as a gold standard treatment in this disease (Habif, 1990). Topical steroids are most commonly prescribed because their highly effective anti-inflammatory property, however, there are many common side effects and complications occurred such as steroid allergy, steroid-induced skin atrophy, addiction and rebound, hypertrichosis, steroid acne, skin hypopigmentation. Furthermore, long term or usage in high doses could lead to the secondary infection from bacteria, virus and fungus, Norwegian scabies, Kaposi's sarcoma, adrenal suppression and much more (Wolverton, 2001). To avoid the side effects and complications from the use of topical steroids, alternative treatments for dermatitis are needed. Consequently, plant remedies seem to be the most convenient solution because of their accessibility and diversity in tropical regions. Thailand is located in tropical rain forest zone above the equator. Therefore, we have a lot of raw materials for research to investigate whether they could be used as new candidate drugs. In addition, Thai local plants are not expensive, variety and easy to find all over Thailand and were found used to cure some diseases. Unfortunately, there is less number of studies in Thai herbal plant. This study attempts to investigate the new local plants if it may provide the good effect to use as anti-inflammatory topical drug. *Terminalia chebula* or Thai local name; ''Sa-mor-thai" (ST) is regarded as a universal panacea in the Ayur-Vedic Medicine and in the Traditional medicine. The dry nut's peel is used to cure cold-related nagging coughs. The bark or peel of the nut is placed in the cheek. Its fruit has many properties, for instance, effect digestive system, anthelmentic, cardiotonic, cough, colds and anti-inflammation. It has been reported that *T. chebula* could protect the rat pheochromatoma cells *in vitro* from ishemic damage and its mechanism showed the inhibition of oxidation and inflammatory effects (Gaire *et al*., 2013). Moreover, there was a report found that *T. chebula* contains terflavin B which is one type of tannin while chebulinic acid is found in the fruits which possibles help reducing inflammation including the inflamed skin (Quanbin *et al.*, 2006). Thus, our experiment aimed to investigate whether the extract of *T. chebula* could be a novel topical anti-inflammatory herbal drug candidate.

Materials and Methods

Experimental animals

Animals used in this study were pathogen-free,

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Twenty - 6 week old male ICR mice were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakorn Pathom, Thailand. All laboratory mice were acclimatized in the laboratory animal room of Anatomy building, Department of Anatomy, Faculty of Medicine, Siriraj Hospital, Mahidol University.

Chemicals

Croton oil; Stock of croton oil was purchased from Sigma, USA. Calypsol; Ketamine (Gedeon Richter Ltd., Budapest Hungary)

Herbal extraction

Terminalia chebula extraction

T. chebula fruits extracts was provided from Dr. Aikkarach Kettawan, Institute of Nutrition, Mahidol University, Salaya, Nakorn Pathom. Briefly, dried fruits were grinded into tiny pieces and dissolved in 95% ethanol and shaken for hours. Evaporation into dry powder was performed. The final concentration of T. chebula used in this experiment was 100 mg/ml. This concentration was selected since it gave the good result in *in vitro* study (personal communication).

Dermatitis induced by croton oil

Mice were randomly divided into 4 groups (5 mice per each group) as normal (N), control (C), Placebo (P), and ST extract (ST). All mice were weighed and all ears were measured the thickness by using a digital vernier caliper. N-mice were left in cages until the experiment end without any disturbance. In the other three groups, the skin inflammation or irritant contact dermatitis was induced to the inner surface of both ears of mice by topical application of 50 µl of 35 µg/µl of croton oil dissolved in acetone per each ear. The C-mice were left untreated both ears whereas the P-mice were treated by 50 µl of acetone on the right ear only. For the ST-mice, 50 µl of the ST extract at the concentration of 100 mg/ml, respectively, were applied topically only on the right ear at 15 minutes after the application of croton oil solution while the left ear remained untreated. Thirty minutes after croton oil induced dermatitis performed, the redness was observed, the thickness of both ears of all group of mice were measured and photo taken, then repeated the observation and measurement every 1 hour till the end of the experiment. After 6 hours, all mice were sacrificed by intra-peritoneal injection of overdose Calypsol®. A 6mm in diameter of ear plug was removed from all the treated and the untreated ears. Inflammation was measured quantifying the edematous response be the weight difference and the difference of thickness between the two plugs.

Evaluation of the skin inflammatory response

The redness and swelling of the ear produced by congestion of the capillaries in the skin after the inflammatory process occurred within few minutes by croton oil application. Thus, the edema response of the skin was expressed as an increase in ear thickness due to the acute inflammatory process. It has been established that inflammation induced by croton oil is related to the activation of PLA2, which releases arachidonic acid from the cell membrane and is metabolized to prostaglandins and leukotrienes (Paula, 2003). The release of inflammatory mediators response for the clinical signs of inflammation. Vasodilation and its resulting increased blood flow causing the redness and increased heat. Increased permeability of the blood vessels results in an exudation of plasma proteins and fluid into the tissue, which manifests itself as swelling or edema. Ear thickness was measured before the croton oil application (Cabrini *et al.*, 2011), after the dermatitis was induced (15 minutes after application), 15 minutes after applied placebo or ST extract and every hour after treatments using a digital vernier caliper (Insize). The vernier caliper was applied near the tip of the ears just distal to the cartilaginous ridges and the numbers were recorded. To minimized technique variations, a single investigator performed the measurements throughout each experiment. At the end of the experiment, all ear plugs were biopsied 6 mm in diameter and weighted by digital scale to evaluate the edematous response.

Statistical analysis

All data were represented as tables. The difference of among groups was evaluated by using One-way ANOVA whereas the difference between ears was evaluated by using Student t-test. All values were expresses as mean and standard deviation (SD). Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

In the present study, after all mice were randomized, we found that the body weight of every groups of mice were 35.21 g. in average, which have no significant difference between all groups. The thickness of both ears of all groups measured at the starting was varied from 0.17 to 0.22 mm., averaging 0.19825 mm. in right ears and 0.19875 mm. in left ears and also found no significant difference between groups (Table 1).

Ear thickness

As above-mentioned, we measured the ear

Table 1. Weight and ear thickness of all groups of mice (n = 5; mean ± SD)

Group of mice	Weight (gram)	Thickness of right ear (mm)	Thickness of left ear (mm)
Normal	36.12±2.87	0.194±1.82	0.190±0.71
Control	34.28±1.60	0.196±1.34	0.202±1.3
Placebo	32.92±1.08	0.202±1.30	0.206±1.14
ST	36.34±2.79	0.192±0.84	0.198±0.84

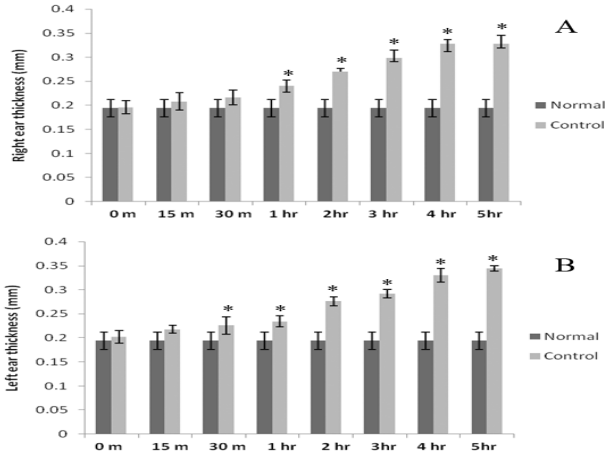

Figure 1. Thickness of right (A) and left (B) in normal and in control groups

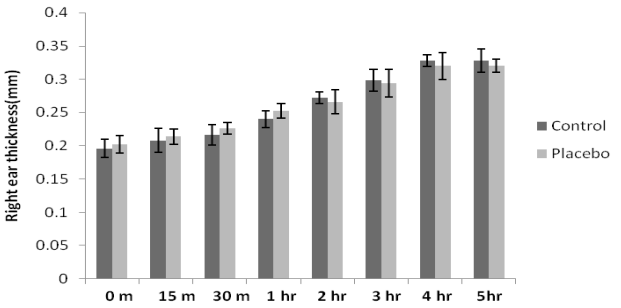

Figure 2. Thickness of right ear in control and placebo groups

thickness to evaluate the swelling of ear tissue. Compared N-mice with C-mice, we found that the thickness of both ears between these two groups were different. The ears of control group, which were applied by croton oil, were gradually thicker significantly at longer period of time. This could be concluded that the croton oil induced dermatitis was performed correctly. (Figures 1A and B). Observing C-mice and P-mice, there showed no significant difference between the right ears of both group which might be summarized that there was no external or environmental factors affected swelling of ears and had no experimenter's bias (Figure 2)

Ears of ST-mice, compared the right ear with the left ear, showed significantly different thickness at the time of 30 minutes till the end, at 5 hours of experiment (Figure 3). It might be assumed that the extract of ST seems to have a topical anti-inflammatory effect in the skin inflammation. However, compared the

Table 2. Time interval comparison of right ear thickness of ST mice comparing to 0 min (mean ± SD)

Thickness	0 min	15 min	30 min	1 hour	2 hours	3 hours	4 hours	5 hours
(mm)	0.192± 0.84	0.214± 0.89	0.226± 0.89*	0.21± 1.22*	0.218± 1.30*	0.232± 1.92*	0.24± 1.58*	0.25± 1.58*

*significantly difference at $p < 0.05$

Table 3. Ear Plug weight of ST-treated mice (gram; mean ± SD)

Group of mice	Right (g)	Left (g)
ST treatment	0.013±0.006*	0.018±0.004

*significantly difference at $p < 0.05$

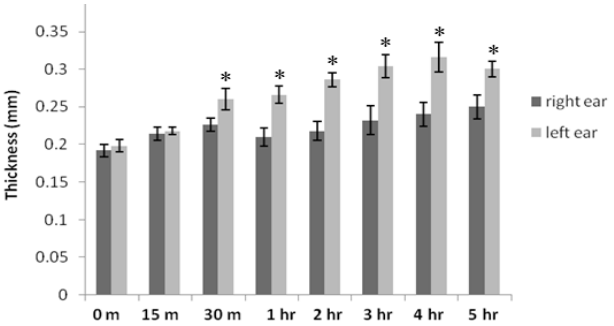

Figure 3. Thickness of both ears in ST treated group

right ears of ST group at the time before experiment (0 min) with their right ears at the time during the experiment, we found the ear thickness was different significantly which reflected that the inflammatory process still went on and ST extract at prepared concentration might not effective enough to cure the inflamed in ears to recover as normal. (Table 2). It has been confirmed that arachidonic acid metabolites act as mediators of the inflammatory response via COX and lipoxygenase activity. Therefore, these have been a target for the development of therapeutic agents. T.chebura was studied and found that the extract of this fruit could inhibit COX-1, COX-2 and 5-LOX. Therefore, the down regulation of NFkB was observed (Shairaslee *et al*., 2012). For the topical application of *T. chebura* extract on mouse's ears could decrease ear swelling resulted in ear thickness reduction. Further investigation in immunology and histology should be done to find out more evidence of topical anti-inflammatory property of ST extract.

Ear plug weight

In the croton oil ear test, the inflammatory response is usually quantified by measuring the ear plug weight (EPW). The 6 mm in diameter biopsied ear tissue were weighted at the end and showed significant difference between N-mice and C-mice in both ears, no significant difference between the right ear of C-mice and P-mice which was correlated with the results in ear thickness evaluation we have discussed. Compare right with left ears of ST-mice, it presented the EPW was different significantly

(Table 3). This could be concluded that at the end of experiment, the inflammation, swelling response was probably inhibited by the application of ST extract and might be resumed as nearly normal ear.

Conclusion

Base on all of results, it can be suggested that *T. chebula* has topical anti-inflammatory property which helps curing the dermatitis in mice. Further studies of the anti-inflammatory activity would be necessary to determine its possible mechanism of action.

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