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Therapeutics

Tea tree oil reduces histamine-induced skin inflammation

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Summary

Background Tea tree oil is the essential oil steam-distilled from *Melaleuca alternifolia*, an Australian native plant. In recent years it has become increasingly popular as an antimicrobial for the treatment of conditions such as tinea pedis and acne.

Objectives To investigate the anti-inflammatory properties of tea tree oil on histamine-induced weal and flare.

Methods Twenty-seven volunteers were injected intradermally in each forearm (study and control assigned on an alternating basis) with histamine diphosphate (5 μg in 50 μL). Flare and weal diameters and double skin thickness were measured every 10 min for 1 h to calculate flare area and weal volume. At 20 min, 25 μL of 100% tea tree oil was applied topically to the study forearm of 21 volunteers. For six volunteers, 25 μL paraffin oil was applied instead of tea tree oil.

Results Application of liquid paraffin had no significant effect on histamine-induced weal and flare. There was also no difference in mean flare area between control arms and those on which tea tree oil was applied. However, mean weal volume significantly decreased after tea tree oil application (10 min after tea tree oil application, $P = 0{\cdot}0004$, Mann–Whitney U-test).

Conclusions This is the first study to show experimentally that tea tree oil can reduce histamine-induced skin inflammation.

Key words: flare, histamine, *Melaleuca alternifolia*, tea tree oil, weal

Tea tree oil (TTO) is the essential oil steam-distilled from *Melaleuca alternifolia*, an Australian native plant. TTO contains over 100 components, the majority being monoterpene and sesquiterpene hydrocarbons and their alcohols. Several *in vitro* studies have investigated TTO's antimicrobial properties and there are now susceptibility data on a wide range of bacteria, yeasts and fungi.[1–4] In recent years, TTO has become popular as a naturally occurring antimicrobial and antiseptic agent.

A recent systematic review of randomized clinical trials with TTO for the treatment of acne and fungal infections concluded that owing to promising findings, TTO deserves to be investigated more closely.[5]

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Some clinical and *in vitro* studies have reported indirect anti-inflammatory responses with topical TTO.[1,4,6–8] We hypothesize that these anti-inflammatory responses may reflect control of the tissue damaging effects of a strong immune response induced by an invading organism. This laboratory has reported regulatory properties of TTO on the activity of human monocytes activated *in vitro*.[9,10] In contrast, TTO could not control superoxide production by human neutrophils *in vitro*.[10] However, monocytes, macrophages and neutrophils may not be part of the immediate hypersensitivity response to allergens or components of an insect bite, a condition that is also treated with TTO. As allergen-induced weal and flare responses are mediated mainly by histamine,[11] in this study we have tested topical TTO on experimentally induced skin inflammation induced by histamine.

Materials and methods

Participants

The control oil, the group treated with liquid paraffin, comprised five females and one male (mean age 37 years, range 23–54). Twenty-one people were tested with TTO (16 females, five males, mean age 35 years, range 23–56). Participants had no severe generalized skin conditions such as eczema or psoriasis, atopy (eczema, hay fever or asthma), or previous skin or systemic sensitivity to TTO and had had no severe allergic reactions in the past. Subjects with a past history of pityriasis versicolor, tinea pedis, minor acne and minor scalp psoriasis were included in the study. The participants were not on systemic immunosuppressant therapy and had not taken oral antihistamines or topical corticosteroids in the preceding 2 weeks. This study was approved by the Clinical Investigation Committee of Flinders Medical Centre, Adelaide, Australia.

Induction of weal and flare

Histamine (50 μL of 100 μg mL^{-1} solution) was injected intradermally into the inner forearm skin (approximately midway along the volar aspect) of both arms and the resulting weal and flare measured at 10 min intervals for 60 min. After 20 min, undiluted TTO (25 μL) or liquid paraffin (25 μL) was applied topically with a pipette to cover the flare and weal on the experimental arm. Study arms (TTO or liquid paraffin) and control arms were assigned in an alternating fashion from subject to subject. In this way each subject acted as his or her own control. Weal and flare diameters (cm) were measured with calipers (Mitutoyo Corp., Tokyo, Japan). Weal skin double thickness (mm) was measured by lightly pinching the skin and measuring with a spring-loaded gauge (Mitutoyo).

Flare area was calculated by using the following formula:[12]

$$\text{Flare area } (\text{cm}^2) = \pi/4 \times (D_1 + D_2)^2/2$$

where D_1 = diameter of flare (cm), D_2 = second perpendicular diameter of flare (cm).

Assessment of weal volume was calculated using the following formula:[12]

$$\text{Weal volume}(\mu\text{L}) = \pi/4 \times (d_1 + d_2)^2/2 \times (T_t - T_0)/2$$

where d_1 = diameter of weal (cm), d_2 = second perpendicular diameter of weal (cm), T_t = skinfold thickness at time t (mm), T_0 = skinfold thickness at time 0 (mm).

Subjects were also questioned about level of itch during the experiment and asked to grade pruritus as follows: 0, no itch; 1, mild; 2, moderate and 3, severe. They were also asked if they had previously used TTO products.

Tea tree oil and liquid paraffin

The TTO was provided by Thursday Plantation (Ballina, NSW, Australia) as is commercially available. Gas chromatographic analysis of the TTO used in this study, was done by the Wollongbar Agricultural Institute (Wollongbar, Australia) (Table 1). TTO was kept in 10-mL aliquots (brown glass bottles) to minimize oxidation and discarded after 1 month. Liquid paraffin (BP) was obtained from Orion Laboratories (Welshpool, Western Australia).

Results

All of the subjects tolerated intradermal histamine injection and topical application of TTO or liquid paraffin without any adverse effects. Of the 21 subjects in the TTO study, seven had not used any TTO product before on the skin. The other 14 subjects had each used one or more of a range of products with unknown concentrations of TTO (e.g. cream, deodorant, moisturizer, soap, handwash) as well as 100% pure oil, on limbs or face from 1 week to 1 year preceding the study. Stated uses for the products were for insect bites, cuts, acne or skin irritation.

Table 1. Gas chromatographic analysis of the tea tree oil used

Component	Percentage
Terpinen-4-ol	41·6
γ-Terpinene	21·5
α-Terpinene	10·0
Terpinolene	3·5
α-Terpineol	3·1
α-Pinene	2·4
1,8-Cineole	2·0
p-Cymene	1·8
Aromadendrene	1·1
δ-Cadinene	1·0
Limonene	0·9
Ledene	0·9
Globulol	0·5
Sabinene	0·4
Viridiflorol	0·2

There was no significant difference in itch scores between the control, TTO- and liquid paraffin oil-treated arms. The control oil, liquid paraffin, had no effect on mean flare area over the 60-min period following histamine injection (Fig. 1A). There was also no significant difference in mean flare area between control and TTO arms over the same period following histamine injection (Fig. 1B).

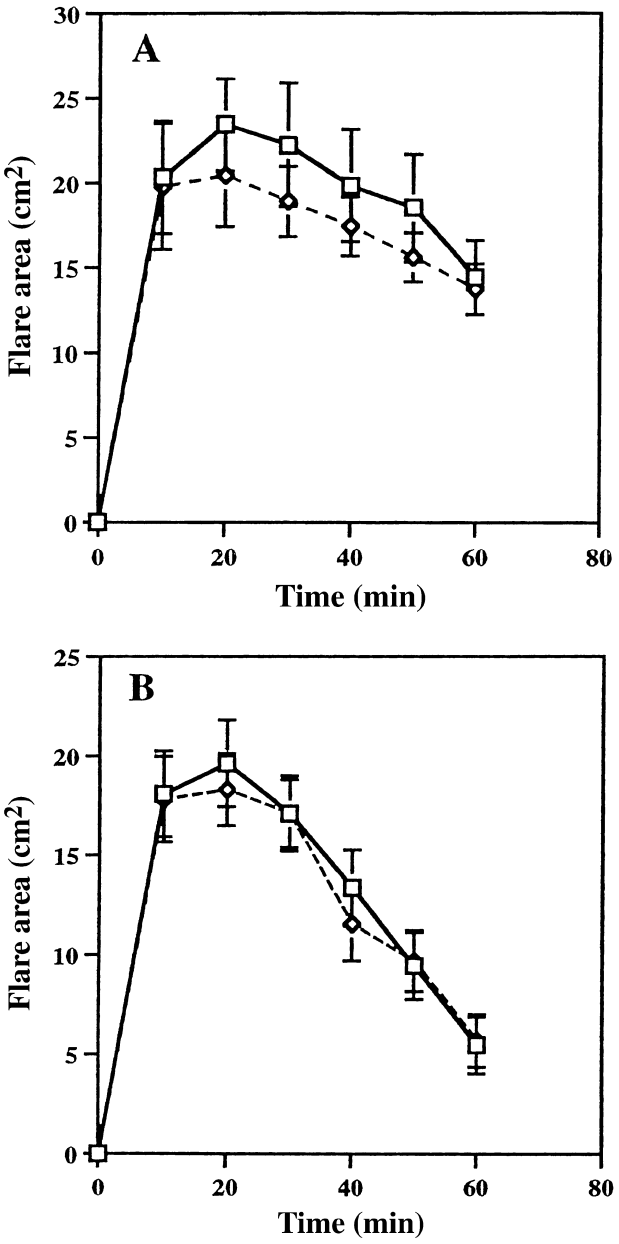

Figure 1. The effect of (A) liquid paraffin, and (B) tea tree oil (TTO) on histamine-induced flare. The mean flare area (cm^2) for the control (solid line) and study arms (broken line) with increasing time after histamine injection is shown. In (A) liquid paraffin, and in (B) TTO, was applied 20 min after histamine administration. The mean ± SEM is shown for (A) six volunteers ± SEM, and (B) 21 volunteers.

The mean weal size 20 min after histamine injection was 2·05 μL, range 0·26–10·90 (Table 2). As there was considerable interindividual, as well as intraindividual variability in the size of the histamine-induced weal, the results were normalized as a percentage of the weal volume at 20 min. For the six volunteers treated with liquid paraffin 20 min after histamine injection, there was no significant difference in the weal between the control and study arms (Fig. 2A). It is notable that the weal volume continued to increase after application of liquid paraffin. In contrast, the mean weal volume showed a marked decrease following TTO application 20 min after histamine injection (Fig. 2B). At 30 min (10 min after TTO application), the weal had increased in size in only six of the 21 arms treated with TTO. This contrasted with an increased weal in 17 of the 21 untreated control arms. At 30 min (10 min after TTO application), the mean weal volume on the TTO arm was 92% of that seen at 20 min. It decreased to 83%, 62% and 43% at 40, 50 and 60 min, respectively. At 30 min, the mean weal volume on the control arm was 163% of that seen at 20 min, changing to 175%, 130% and 113% at 40, 50 and 60 min, respectively (Fig. 2B). At 30 min, the percentage weal volume of the TTO-treated arms was statistically significantly lower than that of the control arms ($P = 0{\cdot}0004$, Mann–Whitney *U*-test). At 60 min, the percentage weal volume of the TTO-treated arms was also statistically significantly lower than that of the control arms ($P = 0{\cdot}017$).

Discussion

In this study, a weal and flare reaction of significant size was induced by histamine injection in the inner forearms of 27 volunteers. There was considerable interindividual and intraindividual variability in the response to histamine. The intraindividual variability between a patient's arms was surprising as the control and liquid paraffin- and TTO-treated arms were used alternatively. For this reason, the weal results were normalized to that measured at 20 min, i.e. the weal measured immediately before application of liquid paraffin or TTO. TTO significantly reduced the developing oedema to histamine while the weal in the liquid paraffin-treated and control arms continued to develop. Twenty minutes was chosen as the time for application of TTO or the control oil as we hypothesized that this was similar to the timing of medication after an insect

Table 2. Weal volumes (μL) 20, 40 and 60 min after histamine injection for 27 volunteers treated after 20 min with liquid paraffin (LP) or tea tree oil (TTO)

Subject	Time (min)	Control arm (μL)	Study arm (μL)	Subject	Time (min)	Control arm (μL)	Study arm (μL)
LP1	20	8·04	10·9	LP4	20	1·13	2·33
	40	5·45	10·34		40	2·70	2·94
	60	5·31	9·08		60	1·25	1·25
LP2	20	4·32	4·18	LP5	20	1·81	0·93
	40	2·84	5·31		40	1·50	0·67
	60	1·94	4·01		60	1·50	0·08
LP3	20	3·68	4·38	LP6	20	0·67	2·68
	40	2·68	1·42		40	1·35	3·85
	60	2·19	0·53		60	1·11	2·70
TTO1	20	1·58	1·70	TTO12	20	2·39	2·65
	40	1·58	0·78		40	2·76	2·86
	60	1·13	0·73		60	1·47	2·76
TTO2	20	2·15	3·28	TTO13	20	0·66	0·66
	40	3·72	3·31		40	2·65	1·43
	60	2·04	2·80		60	2·31	0·00
TTO3	20	0·26	0·69	TTO14	20	0·61	1·70
	40	1·96	0·76		40	1·99	1·47
	60	1·25	0·90		60	1·35	1·36
TTO4	20	2·12	5·94	TTO15	20	1·33	2·33
	40	1·85	2·86		40	1·43	2·33
	60	0·53	1·58		60	1·56	0·99
TTO5	20	0·53	1·41	TTO16	20	0·43	0·57
	40	0·43	1·41		40	0·48	0·31
	60	0·25	0·00		60	0·09	0·06
TTO6	20	1·45	1·10	TTO17	20	1·38	1·99
	40	1·72	0·62		40	2·65	1·46
	60	0·52	0·98		60	1·33	0·21
TTO7	20	2·60	2·26	TTO18	20	1·72	1·13
	40	1·69	2·15		40	0·90	0·52
	60	1·70	0·23		60	0·00	0·00
TTO8	20	0·35	2·38	TTO19	20	1·28	1·23
	40	0·40	2·86		40	1·84	0·95
	60	0·24	1·85		60	0·39	0·00
TTO9	20	1·33	0·66	TTO20	20	2·00	1·23
	40	1·69	0·14		40	2·48	0·88
	60	1·72	0·12		60	1·99	0·83
TTO10	20	2·08	1·43	TTO21	20	1·28	0·95
	40	2·07	1·77		40	2·58	0·08
	60	0·80	0·94		60	2·28	0·08
TTO11	20	0·54	2·26				
	40	1·36	1·30				
	60	0·95	0·90				

bite or allergen exposure. In this study, we used liquid paraffin as a control oil. Furthermore, it was unable to reduce histamine-induced weal and flare in human skin. No oil could be considered the ideal control oil for TTO. Although less volatile, liquid paraffin was considered the best example of an immunologically inert oil.

Histamine-induced inflammation of the skin is manifest by initial reddening of the skin, plasma extravasation and the development of a weal (tissue oedema) and a flare (wider spread erythema). This reaction is frequently accompanied by pruritus (itch). Local release of vasoactive substances from sensory nerves, the vascular endothelium and infiltrating blood cells mediate these changes in microvascular perfusion and permeability; TTO may be affecting any one of these mechanisms of oedema formation. Histamine-induced inflammation is most often associated with immediate hypersensitivity reactions, with histamine released from mast cell granules.[13]

This laboratory has shown that the water-soluble components of TTO, especially terpinen-4-ol, which constitutes 40% of TTO, can suppress inflammatory mediator production by activated human monocytes.[9] The production of lipopolysaccharide-induced tumour necrosis factor α, often considered the most influential

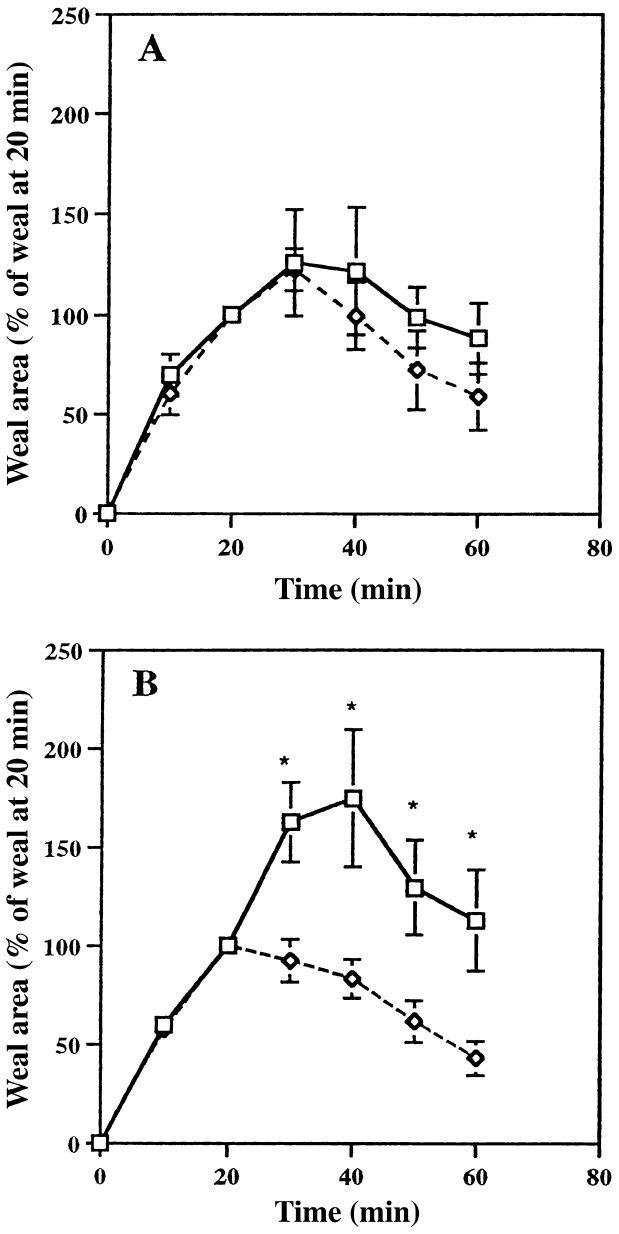

Figure 2. The effect of (A) liquid paraffin, and (B) tea tree oil (TTO), on histamine-induced weal. The weal volume of the control and study arms 20 min after histamine injection (and time of application of liquid paraffin or tea tree oil) was calculated as 100%. The mean percentage change in weal volume for the control (solid line) and study arms (broken line) with increasing time after histamine injection is shown. The mean ± SEM is shown for (A) six volunteers, and (B) 21 volunteers. An asterisk indicates a significant difference between control and TTO-treated arms.

inflammatory cytokine,[14] as well as interleukins 1β, 8 and 10, and prostaglandin E_2, was suppressed. Also, the water-soluble components of TTO can suppress the production of superoxide by human monocytes, but not neutrophils, activated *in vitro*.[10] TTO may enable neutrophils to be fully active in an acute inflammatory response and eliminate foreign antigens, while suppressing monocyte production of superoxide and inflammatory mediators, thereby preventing oxidative damage and the activation of other cells that is seen in more chronic inflammatory states.

Antimicrobial susceptibility studies have found TTO effective *in vitro*.[1,4,7,8] TTO has also been trialled as a pediculocide with 100% mortality of adult head lice.[15] However, few clinical studies have tested TTO's antimicrobial or anti-inflammatory effects *in vivo*. In one study,[7] TTO cream improved the symptoms of tinea pedis (i.e. scaling, inflammation, itch, burning) compared with placebo, with excellent skin tolerance. Interestingly there was no statistically significant difference in fungal clearance between the two groups. In another study,[6] 5% TTO in a water-based gel was an effective topical treatment for acne vulgaris, although less effective than a 5% benzoyl peroxide water-based lotion because of its slower onset of action. The TTO formulation was better tolerated on facial skin, with less skin scaling, dryness and pruritus than with benzoyl peroxide.

In recent years there has been increasing interest in 'natural' medicine products with a special demand for Australian TTO. This study shows that undiluted TTO applied to histamine-induced inflammation can reduce mean weal volume. This is the first study to the best of our knowledge that shows TTO can reduce experimentally induced inflammation in human skin and may give some credence to anecdotal reports that TTO can reduce the hypersensitivity responses to insect allergens. The mechanism by which the active ingredients of TTO regulate weal formation (or fluid resorption) is as yet unknown.

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