Cochrane Library

Cochrane Database of Systematic Reviews

Tea tree oil for Demodex blepharitis (Protocol)

Savla K, Le JT, Pucker AD

Savla K, Le JT, Pucker AD.
Tea tree oil for Demodex blepharitis.
Cochrane Database of Systematic Reviews 2019, Issue 6. Art. No.: CD013333.
DOI: 10.1002/14651858.CD013333.

www.cochranelibrary.com

WILEY

TABLE OF CONTENTS

[Intervention Protocol]

Tea tree oil for Demodex blepharitis

Keyur Savla[1], Jimmy T Le[2], Andrew D Pucker[1]

[1]School of Optometry, University of Alabama at Birmingham, Birmingham, Alabama, USA. [2]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

Contact address: Andrew D Pucker, School of Optometry, University of Alabama at Birmingham, 1716 University Blvd, Birmingham, Alabama, 35294-0010, USA. apucker@uab.edu.

Editorial group: Cochrane Eyes and Vision Group.
Publication status and date: New, published in Issue 6, 2019.

Citation: Savla K, Le JT, Pucker AD. Tea tree oil for Demodex blepharitis. *Cochrane Database of Systematic Reviews* 2019, Issue 6. Art. No.: CD013333. DOI: 10.1002/14651858.CD013333.

ABSTRACT

This is a protocol for a Cochrane Review (Intervention). The objectives are as follows:

To evaluate the effects of tea tree oil on ocular *Demodex* infestation in people with chronic Demodex blepharitis.

BACKGROUND

Description of the condition

Description of the condition

Blepharitis is a chronic inflammation of the eyelids, which may or may not involve the Meibomian glands (Wolffsohn 2017). Blepharitis is characterized by eye itching, burning, dryness, irritation, or watering; the patient may also experience blurry vision or the sensation of heavy eyelids (Amescua 2018; Cheng 2015; Lemp 2009). Demodex blepharitis (or demidocosis) refers to a common form of blepharitis that involves a *Demodex* mite infestation (Cheng 2015; Liu 2010). There are two primary types of *Demodex* mites that are known to inhabit humans (Cheng 2015):

- *Demodex folliculorum* (0.3 to 0.4 mm) is primarily found at the base of the eyelashes and eyelash follicles (anterior blepharitis) (Basta-Juzbasic 2002; Cheng 2015; Liu 2010). They feed on epithelial cells around the hair follicles, which may also cause trichiasis (inward deviation of eyelashes) or madarosis (eyelash loss) (Gao 2007).

- *Demodex brevis* (0.2 to 0.3 mm) tends to inhabit the Meibomian glands (posterior blepharitis) (Basta-Juzbasic 2002; Cheng 2015; Liu 2010). They can block gland orifices, which can prevent meibum expression and induce Meibomian gland dysfunction (English 1981).

Not all patients who present with *Demodex* mites are symptomatic, which suggests that these mites may be considered part of the normal ocular flora (Kemal 2005). *Demodex* mites have been associated with other skin diseases such as papulopustular rosacea, rosacea-like eruptions of the face, and eyelid basal cell carcinomas (Erbagci 2003; Forton 2005).

Epidemiology

Blepharitis is one of the most common ocular disorders. Lemp 2009 reports that eye care providers estimate observing blepharitis in up to 47% of their patients. The prevalence of Demodex blepharitis in particular varies widely in the literature (29% to 100%),

likely due to the lack of standardized procedures for measuring clinically significant infestations (Gao 2005a; Kemal 2005; Roth 1979). Demodex mites, however, are rarely found on the eyelids of those under 16 years old (Coston 1967). Children likely have a lower risk for *Demodex* infestations due to having a lower level of sebaceous gland secretions (Herron 2005). There does not appear to be a sex predilection for *Demodex* infestation (Biernat 2018).

Diagnosis

There are no specific clinical diagnostic tests for diagnosing Demodex blepharitis (AAO 2018). Microscopic evaluation of epilated eyelashes may reveal *Demodex* mites or cylindrical dandruff/collarettes (e.g. debris generated from mites accumulating at the root of eyelashes) (Gao 2005b). Cylindrical dandruff is considered to be a pathognomonic sign for Demodex blepharitis (Gao 2005b). The microscopic evaluation involves epilating two or more random eyelashes from each eyelid; placing the eyelash on the slide; adding a drop of oil to the eyelash slide; applying a coverslip; and viewing the eyelashes with a microscope set to 25x magnification (Coston 1967; Gao 2005b). Detection and counting of *Demodex* eggs, larvae, and adult mites is done to grade the severity of *Demodex* infestation (Liu 2010). The literature reports a range of scales for grading Demodex blepharitis severity. Coston 1967 suggests that six or more *Demodex* mites per 16 lashes is clinically significant, while Biernat 2018 suggests that the presence of one *Demodex* mite, larva, or egg is clinically significant. Others have simply counted the number of *Demodex* mites present and reported them as mites per eyelash (Gao 2005b). Gao 2005b has also proposed a modified method for sampling and counting the *Demodex* mites that entails intentional selection of two lashes with cylindrical dandruff per eyelid, applying them to a coverslip, and counting the mites with a microscope while adding 100% alcohol to the slide to break up the cylindrical dandruff. Gao and colleagues also suggest that if no cylindrical dandruff is observed, mites can still be searched for by collecting lashes as described above, adding saline to the slide instead of 100% alcohol because the cylindrical dandruff will not need to be degraded.

Treatment options

Treatment of Demodex blepharitis is geared towards mite eradication (Fromstein 2018). Current treatment approaches include eyelid hygiene, 1% sulfur ointment, 1% mercury oxide ointment, pilocarpine gel, iodized solutions, warm compresses, intense pulsed light, ivermectin, and tea tree oil (Coston 1967; Filho 2011; Fromstein 2018; Liu 2010; Zhang 2018). Of all the treatment options investigated, tea tree oil has been shown to be the most promising option for killing *Demodex* mites (Liu 2010). Tea tree oil therapies may be more effective as they are known to have antibacterial, antifungal, and anti-inflammatory properties (Liu 2010).

Description of the intervention

Tea tree oil is an essential oil derived via distillation from the leaves and terminal branches of a "small paper-barked tree" known as Australian *Melaleuca alternifolia* (Lam 2018; Swords 1978). Tea tree oil contains over 100 different components (Hammer 2006; Swords 1978), although only 15 of the most common components are included in the 2017 International Organization for Standardization (ISO) tea tree oil standard (ISO-4730; see Table 1). Tea tree oil is typically applied topically to the eyelid in the form of a scrub via eyelid wipes or foam when attempting to fight ocular *Demodex* infestations (Cheng 2015), but it should never be taken orally because it is highly toxic if ingested (Hammer 2006). It has been recommended to use tea tree oil treatments for at least two *Demodex* mite life cycles (i.e. approximately six weeks) in order to ensure adequate killing of the parasite (Cheng 2015).

How the intervention might work

Although tea tree oil likely has multiple modes of action and likely contains multiple molecular species with antimicrobial properties, since it has action against bacteria, fungus, and parasites (Cheng 2015; Li 2017; Schelz 2006), terpinen-4-ol is the most active tea tree oil molecular compound against *Demodex* (Tighe 2013). In fact, data from Tighe 2013 suggest that terpinen-4-ol is the only component in the 2004 ISO tea tree oil standard that is able to effectively kill *Demodex* mites at a 1% concentration when diluted in mineral oil. Determining that terpinen-4-ol alone has high antiparasitic properties at a 1% concentration is an important finding because higher concentrations of tea tree oil and oxidation products within tea tree oil have been associated with ocular irritation and allergic reactions, respectively, which are the primary side effects associated with tea tree oil treatment (Hammer 2006). Tighe 2013 also found that "α-terpineol, 1,8-cineole, sabinene, limonene, terpinolene, and α-terpinene" are effective at killing *Demodex* mites when used at a therapeutic concentration (all > 2.5%), and they found that the action of terpinen-4-ol could be enhanced by the inclusion of terpinolene and inhibited by the inclusion of α-terpineol, suggesting that the components within tea tree oil interact in a complex manner.

While tea tree oil's full mechanism of action against *Demodex* mites is unknown, tea tree oil causes the *Demodex* mites to migrate out of the skin, which may make it easier for treatments to take action against them (Liu 2010). Tea tree oil, or more specifically terpinen-4-ol and 1,8-cineole within tea tree oil, may also act against *Demodex* by competitively inhibiting acetylcholinesterases (Lam 2018). *Demodex* mites also have the ability to carry bacteria (internally or externally), which may further promote blepharitis and a *Demodex* infestation-associated immune reaction (Liu 2010), therefore the antibacterial effects (e.g. increased membrane permeability) of tea tree oil may also contribute to its therapeutic effects (Lam 2018). Tea tree oil has also been found to have anti-

inflammatory properties, as it has been shown that terpinen-4-ol is able to suppress monocyte-derived pro-inflammatory proteins (e.g. tumor necrosis factor alpha, interleukin-8) and oxygen-derived reactive species production (superoxide) (Brand 2001; Hart 2000), which may further promote resolution of the condition.

Why it is important to do this review

Recalcitrant multifactorial dry eye commonly occurs in those with ocular *Demodex* infestations (Cheng 2015; Post 1963). While there has been a general systematic review on chronic blepharitis (Lindsley 2012), this review is out of date, and there has yet to be a systematic review focusing on the treatment of ocular *Demodex* infestations with tea tree oil. While the use of tea tree oil for the treatment of ocular *Demodex* infestations is clinically accepted (Cheng 2015), the evidence supporting this practice is not fully formed. This review is therefore important because it will summarize the current understanding of the use of tea tree oil for treating ocular *Demodex* infestations along with the clinical trials that either support or refute the clinical utility of tea tree oil.

OBJECTIVES

To evaluate the effects of tea tree oil on ocular *Demodex* infestation in people with chronic Demodex blepharitis.

METHODS

Criteria for considering studies for this review

Types of studies

We will include randomized controlled trials (RCTs) and quasi-RCTs. We will define quasi-RCTs as trials that have not used randomization to allocate participants to treatment groups but that have attempted to use a non-biased method of treatment assignment such as birth date, Social Security number, or medical record number of a consecutive sample of eligible patients.
We will include trials using a cross-over design if it is possible to determine that the treatment sequence was randomly or quasi-randomly assigned.

Types of participants

We will include trials that enrolled adult participants aged 18 years or older diagnosed with ocular Demodex blepharitis.

Types of interventions

We will compare treatment of Demodex blepharitis with any form of tea tree oil (any concentration or formulation) to another treatment (e.g. baby shampoo, eyelid scrubs, antimicrobial, anti-inflammatory, antiallergic medications, or a combination of the above mentioned) or no treatment (e.g. no treatment, placebo). We will also include studies comparing different concentrations of tea tree oil to each other.

Types of outcome measures

Primary outcomes

- M**ean change in number of *Demodex mites*** per eyelash from baseline at four to six weeks, measured by any method.
- **Mean change in participant-reported change in symptoms,** including but not limited to irritation, burning, tearing, itching, eyelid sticking, photophobia, and increased frequency of blinking, from baseline; measured using participant symptom reports, questionnaires, interviews, or visual analog scale (VAS) at four to six weeks. Although it is ideal for studies to use validated scales, we will consider all scales used in included studies for inclusion since standardized information is not available. We will conduct sensitivity analyses to examine the impact of any assumptions made in this regard.

For both primary outcomes, when mean change from baseline is not available, we will use mean difference at four to six weeks instead.

Secondary outcomes

- **Mean change in number of *Demodex mites per eyelash*** from baseline at 10 to 12 weeks, measured by any method.
- **Mean change in participant-reported change in symptoms** from baseline at 10 to 12 weeks, measured by any method.
- **Proportion of participants with an improvement in visual acuity (i.e. improvement of two or more lines)**, measured on a visual acuity chart with a LogMAR scale (or equivalent) at 4 to 6 weeks and 10 to 12 weeks. When continuous LogMAR data are available, we will analyze the mean change in visual acuity from baseline.
- **Mean change in number of cylindrical dandruff** by eyelid from baseline at 4 to 6 weeks and 10 to 12 weeks, as measured by the reduction in the number of collarettes compared to baseline.
- **Proportions of participants with Meibomian gland dysfunction** (as defined by study investigators, e.g. meibum quality or expressibility, or both) from baseline at 4 to 6 weeks and 10 to 12 weeks.

- **Proportions of participants experiencing conjunctival injection (redness)** (as defined by study investigators) from baseline at 4 to 6 weeks and 10 to 12 weeks.
- **Adverse events**, as reported by study investigators.

For continuous secondary outcomes, when mean change from baseline is not available, we will use mean difference at a follow-up time point instead. Data from time points greater than four weeks, which are not included in the above time frames, will be analyzed when possible.

Search methods for identification of studies

Electronic searches

The Cochrane Eyes and Vision Information Specialist will search the following electronic databases. There will be no restrictions on language or year of publication.

- Cochrane Central Register of Controlled Trials (CENTRAL) (which contains the Cochrane Eyes and Vision Trials Register) in the Cochrane Library (latest issue) (Appendix 1).
- MEDLINE Ovid (1946 to present) (Appendix 2).
- Embase.com (1980 to present) (Appendix 3).
- PubMed (1948 to present) (Appendix 4).
- Latin American and Caribbean Health Sciences Literature Database (LILACS) (1982 to present) (Appendix 5).
- US National Institutes of Health Ongoing Trials Register ClinicalTrials.gov (www.clinicaltrials.gov) (Appendix 6).
- World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictrp) (Appendix 7).

Searching other resources

We will search the reference lists of included trials for any additional trials not identified by the electronic searches. We will contact experts in the field for information on current, past, or unpublished trials. We will not handsearch any conference proceedings or journals for the purpose of this review.

Data collection and analysis

Selection of studies

After removing duplicates from the search results, two review authors will independently screen titles and abstracts of all records identified by the search using a web-based review management software (Covidence). The review authors will classify each record as either relevant (a vote of 'yes') or not relevant (a vote of 'no') for full-text review. Two review authors will retrieve and then independently review the full texts of all studies identified as relevant during title and abstract screening to determine if the studies meet the inclusion criteria (a vote of 'include') or not (a vote of 'exclude'). We will contact trial authors to clarify any details needed to make a complete assessment of eligibility, and document reasons for exclusion for each study assessed as ineligible after full-text review. Any discrepancies will be resolved by discussion between review authors at each stage of the screening process.

Data extraction and management

Two review authors will independently extract data using a web-based electronic data collection form. We will extract the information as described in Appendix 8, including: study setting, countries where recruitment took place, sample size, study duration and follow-up time, study design, analysis choice, sources of funding, and potential conflicts of interests; characteristics of participants (e.g. inclusion/exclusion criteria), underlying disease conditions, and medical history; interventions (e.g. dose and duration of tea tree oil), comparators, outcomes (e.g. domain, specific measurement, specific metric, method of aggregation, and the time frame); and quantitative results.

We will compare the extracted data and resolve any discrepancies by discussion. One review author will complete data entry into Review Manager 5 (Review Manager 2014), and a second review author will verify the data entered.

Assessment of risk of bias in included studies

Two review authors will independently assess the risk of bias in included trials following the guidance described in Chapter 8 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2017). Specific items for consideration will include: random sequence generation and allocation concealment (selection bias), masking of participants and study personnel (performance bias), masking of outcome assessors who assessed participant-reported changes in symptoms and number of mites (detection bias), missing data and intention-to-treat analysis (attrition bias), selective outcome reporting (reporting bias), and other potential sources of bias.

We will assign each item as having 'low risk,' 'high risk,' or, if the information provided is insufficient to make an assessment, 'unclear risk.' We will document reasons for our assessments and resolve any discrepancies through discussion. We will present the overall assessments as the 'Risk of bias' summary figure and graph (Higgins 2017).

Measures of treatment effect

We will treat ordinal outcomes and scales measuring participant-reported symptoms as continuous data or dichotomous data as

appropriate, depending on the length of the scale used and the manner in which the outcomes are reported.

We will report mean differences in change from baseline with 95% confidence intervals (CIs) for continuous outcomes (i.e. in number of *Demodex* mites, participant-reported change in symptoms, visual acuity, number of collarettes, Meibomian gland dysfunction, and conjunctival infections) and risk ratios (RRs) with 95% CIs for any dichotomous outcomes (i.e. proportion of participants reporting change in symptoms and proportion of participants with improvements in visual acuity). If any trials compared eyes within individuals (e.g. one eye was randomized to the treatment while the other was randomized to no treatment), then we will note whether or not the study investigators included statistical methods accounting for the correlation between eyes belonging to the same individual.

Unit of analysis issues

When the unit of analysis is one study eye per individual participant, accounting for non-independence of eyes is not necessary. When both eyes from the same individual are randomized, we will use the estimates accounting for the correlation. We recognize that the unit of analysis is the eyelash for some outcomes, and the individual participant for others, and will therefore exercise caution when extracting the data and summarize any unit of analysis issues that we encounter.

Dealing with missing data

We will address missing study data for the outcomes of interest or any unclear information by writing to study investigators. We will wait two weeks for investigators to reply before considering multiple imputation or other imputation approaches for missing data. In the event that the quality of the available data prevents any meaningful analysis, we will omit the study from the analyses and note this decision in the Discussion.

Assessment of heterogeneity

We will evaluate clinical and methodological heterogeneity by examining participant characteristics, types or dosing of tea tree oil, and outcomes by carefully reviewing the available data and taking into consideration potential risk of bias. We will assess statistical heterogeneity by assessing forest plots and examining the I^2 statistic (Deeks 2017). The I^2 statistic describes the proportion of total variation across trials that is due to heterogeneity rather than chance (Higgins 2017). We will consider an I^2 greater than 70% as the cut-off point to identify the presence of considerable heterogeneity (Higgins 2017). We will give consideration to the consistency of the effect estimates. For example, if we find that all effect estimates are in the same direction, we will report a meta-analysis even though there might have been considerable statistical heterogeneity.

Assessment of reporting biases

We will examine selective outcome reporting as part of the 'Risk of bias' assessment, by comparing the outcomes reported in the included studies and the outcomes listed in study registration or study protocols (where available). We will not examine funnel plots of intervention effect estimates for evidence of asymmetry.

Data synthesis

We will follow Chapter 9 of the *Cochrane Handbook for Systematic Reviews of Interventions* for data analysis (Deeks 2017). In the absence of considerable clinical and methodological heterogeneity, we will use a random-effects model to compute a quantitative synthesis. If the number of studies included in the quantitative synthesis is less than three with no evidence of substantial statistical heterogeneity, we will use a fixed-effect meta-analysis. We will provide a descriptive, qualitative synthesis of included trials and their results.

Subgroup analysis and investigation of heterogeneity

We will consider one subgroup analysis: severity (mild versus moderate) of *Demodex* infestation. The effects of tea tree oil may vary based on severity of infestation. If sufficient data are available, we will also conduct subgroup analyses based on types of comparators, for example no treatment, placebo, or other non-tea tree oil treatments (such as baby shampoo, eyelid scrubs, antimicrobial, anti-inflammatory, antiallergic medications, or a combination of the above-mentioned comparators).

Sensitivity analysis

We will conduct two sensitivity analyses to determine the effect of excluding studies at high risk of bias for incomplete outcome data (i.e. the amount or distribution of missing outcomes differs between treatment groups) (Higgins 2017); and the effect of excluding studies that are quasi-randomized trials. We will also conduct additional sensitivity analyses to determine the impact of any post hoc decisions made during the review process.

Summary of findings

We will prepare a 'Summary of findings' table for each available outcome. We will include the following outcomes in the 'Summary of findings' table.

- **Mean change in number of *Demodex mites per eyelash*** from baseline at 4 to 6 weeks or 10 to 12 weeks, measured by any method.
- **Mean change in participant-reported change in symptoms,** including but not limited to irritation, burning, tearing, itching, eyelid sticking, photophobia, and increased frequency of blinking, from baseline; measured using participant

symptom reports, questionnaires, interviews, or visual analog scale (VAS) at 4 to 6 weeks or 10 to 12 weeks.

- **Proportion of participants with an improvement in visual acuity (i.e. improvement of two or more lines)**, measured on a visual acuity chart with a LogMAR scale (or equivalent) at 4 to 6 weeks or 10 to 12 weeks.
- **Mean change in number of cylindrical dandruff** by eyelid from baseline at 4 to 6 weeks or 10 to 12 weeks, as measured by the reduction in the number of collarettes compared to baseline.
- **Proportions of participants with Meibomian gland dysfunction** (as defined by study investigators, e.g. meibum quality or expressibility, or both) from baseline at 4 to 6 weeks or 10 to 12 weeks.
- **Proportions of participants experiencing conjunctival injection (redness)** (as defined by study investigators) from baseline at 4 to 6 weeks or 10 to 12 weeks.
- **Adverse events**, as reported by study investigators.

We will assess the certainty of the evidence using the GRADE approach with GRADEpro GDT software (GRADEpro GDT 2015).

ACKNOWLEDGEMENTS

Cochrane Eyes and Vision (CEV) will create and execute the electronic search strategies. We are grateful to the following peer reviewers for their time and comments on the protocol: Jennifer Harthan (Illinois College of Optometry) and Justin Kwan (Professional Eye Care Center).

REFERENCES

Additional references

AAO 2018
American Academy of Ophthalmology. Blepharitis - preferred practice pattern. www.aao.org/preferred-practice-pattern/blepharitis-ppp-2018 (accessed prior to 17 April 2019).

Amescua 2018
Amescua G, Akpek EK, Farid M, Garcia-Ferrer FJ, Lin A, Rhee MK, et al. Blepharitis Preferred Practice Pattern. *Ophthalmology* 2019;**26**(1):P56–93.

Basta-Juzbasic 2002
Basta-Juzbasic A, Subic JS, Ljubojevic S. Demodex folliculorum in development of dermatitis rosaceiformis steroidica and rosacea-related diseases. *Clinics in Dermatology* 2002;**20**(2):135–40.

Biernat 2018
Biernat MM, Rusiecka-Ziolkowska J, Piatkowska E, Helemejko I, Biernat P, Gosciniak G. Occurrence of Demodex species in patients with blepharitis and in healthy individuals: a 10-year observational study. *Japanese Journal of Ophthalmology* 2018;**62**(6):628–33.

Brand 2001
Brand C, Ferrante A, Prager RH, Riley TV, Carson CF, Finlay-Jones JJ, et al. The water-soluble components of the essential oil of Melaleuca alternifolia (tea tree oil) suppress the production of superoxide by human monocytes, but not neutrophils, activated in vitro. *Inflammation Research* 2001; **50**(4):213–9.

Cheng 2015
Cheng AM, Sheha H, Tseng SC. Recent advances on ocular Demodex infestation. *Current Opinion in Ophthalmology* 2015;**26**(4):295–300.

Coston 1967
Coston TO. Demodex folliculorum blepharitis. *Transactions of the American Ophthalmological Society* 1967;**65**:361–92.

Covidence [Computer program]
Veritas Health Innovation. Covidence. Version accessed prior to 9 April 2019. Melbourne: Veritas Health Innovation.

Deeks 2017
Deeks JJ, Higgins JPT, Altman DG (editors) on behalf of the Cochrane Statistical Methods Group. Chapter 9: Analysing data and undertaking meta-analyses. In: Higgins JPT, Churchill R, Chandler J, Cumpston MS (editors). Cochrane Handbook for Systematic Reviews of Interventions version 5.2.0 (updated June 2017). The Cochrane Collaboration, 2017. Available from www.training.cochrane.org/handbook.

English 1981
English FP, Nutting WB. Demodicosis of ophthalmic concern. *American Journal of Ophthalmology* 1981;**91**(3): 362–72.

Erbagci 2003
Erbagci Z, Erbagci I, Erkilic S. High incidence of demodicidosis in eyelid basal cell carcinomas. *International Journal of Dermatology* 2003;**42**(7):567–71.

Filho 2011
Filho PA, Hazarbassanov RM, Grisolia AB, Pazos HB, Kaiserman I, Gomes JA. The efficacy of oral ivermectin for the treatment of chronic blepharitis in patients tested positive for Demodex spp. *British Journal of Ophthalmology* 2011;**95**(6):893–95.

Forton 2005
Forton F, Germaux MA, Brasseur T, De Liever A, Laporte M, Mathys C, et al. Demodicosis and rosacea: epidemiology and significance in daily dermatologic practice. *Journal of the American Academy of Dermatology* 2005;**52**(1):74–87.

Fromstein 2018
Fromstein SR, Harthan JS, Patel J, Opitz DL. Demodex blepharitis: clinical perspectives. *Clinical Optometry* 2018; **10**:57–63.

Gao 2005a
Gao YY, Di Pascuale MA, Li W, Baradaran-Rafii A, Elizondo A, Kuo CL, et al. In vitro and in vivo killing of ocular Demodex by tea tree oil. *British Journal of Ophthalmology* 2005;**89**(11):1468–73.

Gao 2005b
Gao YY, Di Pascuale MA, Li W, Liu DT, Baradaran-Rafii A, Elizondo A, et al. High prevalence of Demodex in eyelashes with cylindrical dandruff. *Investigative Ophthalmology and Visual Science* 2005;**46**(9):3089–94.

Gao 2007
Gao YY, Di Pascuale MA, Elizondo A, Tseng SC. Clinical treatment of ocular demodecosis by lid scrub with tea tree oil. *Cornea* 2007;**26**(2):136–43.

GRADEpro GDT 2015 [Computer program]
McMaster University (developed by Evidence Prime Inc). GRADEpro GDT. Version accessed prior to 9 April 2019. Hamilton (ON): McMaster University (developed by Evidence Prime Inc), 2015.

Hammer 2006
Hammer KA, Carson CF, Riley TV, Nielsen JB. A review of the toxicity of Melaleuca alternifolia (tea tree) oil. *Food and Chemical Toxicology* 2006;**44**(5):616–25.

Hart 2000
Hart PH, Brand C, Carson CF, Riley TV, Prager RH, Finlay-Jones JJ. Terpinen-4-ol, the main component of the essential oil of Melaleuca alternifolia (tea tree oil), suppresses inflammatory mediator production by activated human monocytes. *Inflammation Research* 2000;**49**(11):619–26.

Herron 2005
Herron MD, O'Reilly MA, Vanderhooft SL. Refractory Demodex folliculitis in five children with acute lymphoblastic leukemia. *Pediatric Dermatology* 2005;**22**(5): 407–11.

Higgins 2017
Higgins JPT, Altman DG, Sterne JAC (editors). Chapter 8: Assessing risk of bias in included studies. In: Higgins JPT, Churchill R, Chandler J, Cumpston MS (editors). Cochrane Handbook for Systematic Reviews of Interventions version 5.2.0 (updated June 2017). The Cochrane Collaboration, 2017. Available from www.training.cochrane.org/handbook.

Kemal 2005
Kemal M, Sumer Z, Toker MI, Erdogan H, Topalkara A, Akbulut M. The Prevalence of Demodex folliculorum in blepharitis patients and the normal population. *Ophthalmic Epidemiology* 2005;**12**(4):287–90.

Lam 2018
Lam NS, Long XX, Griffin RC, Chen MK, Doery JC. Can the tea tree oil (Australian native plant: Melaleuca alternifolia Cheel) be an alternative treatment for human demodicosis on skin?. *Parasitology* 2018;**145**(12):1510–20.

Lemp 2009
Lemp MA, Nichols KK. Blepharitis in the United States 2009: a survey-based perspective on prevalence and treatment. *Ocular Surface* 2009;**7**(2):S1–14.

Li 2017
Li Y, Shao X, Xu J, Wei Y, Xu F, Wang H. Tea tree oil exhibits antifungal activity against Botrytis cinerea by affecting mitochondria. *Food Chemistry* 2017;**234**:62–7. DOI: 10.1016/j.foodchem.2017.04.172

Lindsley 2012
Lindsley K, Matsumura S, Hatef E, Akpek EK. Interventions for chronic blepharitis. *Cochrane Database of Systematic Reviews* 2012, Issue 5. DOI: 10.1002/14651858.CD005556.pub2

Liu 2010
Liu J, Sheha H, Tseng SC. Pathogenic role of Demodex mites in blepharitis. *Current Opinion in Allergy and Clinical Immunology* 2010;**10**(5):505–10.

Post 1963
Post CF, Juhlin E. Demodex folliculorum and blepharitis. *Archives of Dermatology* 1963;**88**(3):298–302.

Review Manager 2014 [Computer program]
Nordic Cochrane Centre, The Cochrane Collaboration. Review Manager 5 (RevMan 5). Version 5.3. Copenhagen: Nordic Cochrane Centre, The Cochrane Collaboration, 2014.

Roth 1979
Roth AM. Demodex folliculorum in hair follicles of eyelid skin. *Annals of Ophthalmology* 1979;**11**(1):37–40.

Schelz 2006
Schelz Z, Molnar J, Hohmann J. Antimicrobial and antiplasmid activities of essential oils. *Fitoterapia* 2006;**77** (4):279–85.

Swords 1978
Swords G, Hunter GL. Composition of Australian tea tree oil (Melaleuca alternifolia). *Journal of Agricultural and Food Chemisty* 1978;**26**(3):734–7.

Tighe 2013
Tighe S, Gao YY, Tseng SC. Terpinen-4-ol is the most active ingredient of tea tree oil to kill demodex mites. *Translational Vision Science and Technology* 2013;**2**(7):2.

Wolffsohn 2017
Wolffsohn JS, Arita R, Chalmers R, Djalilian A, Dogru M, Dumbleton K, et al. TFOS DEWS II diagnostic methodology report. *Ocular Surface* 2017;**15**(3):539–74.

Zhang 2018
Zhang X, Song N, Gong L. Therapeutic effect of intense pulsed light on ocular demodicosis. *Current Eye Research* 2019;**44**(3):250–6.

* *Indicates the major publication for the study*

ADDITIONAL TABLES

Table 1. Components of International Organization for Standardization Tea Tree Standard

Molecule	Minimum concentration	Maximum concentration
Terpinen-4-ol R isomer ratio S isomer ratio	35.00 67.00 29.00	48.00 71.00 33.00
γ-terpinene	14.00	28.00
α-terpinene	6.00	12.00
1,8-cineole	< 0.01	10.00
p-cymene	0.50	8.00
Terpinolene	1.50	5.00
α-terpineol	2.00	5.00
α-pinene	1.00	4.00
Sabinene	< 0.01	3.50
Aromadendrene	0.20	3.00
Ledene	0.10	3.00
δ-cadinene	0.20	3.00
Limonene	0.50	1.50
Globulol	< 0.01	1.00
Viridiflorol	< 0.01	1.00

(ISO-4730: 2017; Reproduced from Lam 2018**)**

APPENDICES

Appendix 1. CENTRAL search strategy

#1 MeSH descriptor: [Blepharitis] explode all trees
#2 blephariti*
#3 demodicosis or demodicidosis or demodecosis or "cylindrical dandruff"
#4 demodex or "d. folliculorum" or "d. brevis"
#5 blepharoconjunctivitis
#6 ocular near (rosacea or mites)
#7 MeSH descriptor: [Meibomian Glands] explode all trees
#8 meibomian near gland*
#9 ocular near gland*
#10 (eye* or ocular) near inflamm*
#11 (eye* or ocular) near infect*
#12 eye* near seborrheic
#13 eye* near staphylococcal
#14 {OR #1-#13}
#15 MeSH descriptor: [Tee Tree Oil] explode all trees
#16 "Tea tree" OR TTO OR Melaleuca
#17 Cliradex
#18 ISO4730 OR "ISO 4730" OR "4-Terpineol" OR "terpinene-4-ol" or terpin* OR "T4O"
#19 {OR #15-#18}
#20 #14 AND #19

Appendix 2. MEDLINE Ovid search strategy

1. exp BLEPHARITIS/
2. blephariti*.tw.
3. (demodicosis or demodicidosis or demodecosis or "cylindrical dandruff").tw.
4. (demodex or "d. folliculorum" or "d. brevis").tw.
5. blepharoconjunctivitis.tw.
6. (ocular adj3 (rosacea or mites)).tw.
7. exp Meibomian Glands/
8. (meibomian adj3 gland*).tw.
9. (ocular adj3 gland*).tw.
10. ((eye* or ocular) adj3 inflamm*).tw.
11. ((eye* or ocular) adj3 infect*).tw.
12. (eye* adj3 seborrheic).tw.
13. (eye* adj3 staphylococcal).tw.
14. or/1-13
15. exp "Tea Tree Oil"/
16. ("Tea tree" or TTO or Melaleuca).tw.
17. Cliradex.tw.
18. (ISO4730 or "ISO 4730" or terpin* or "T4O").tw.
19. or/15-18
20. 14 and 19

Appendix 3. Embase.com search strategy

#1 'blepharitis'/exp
#2 blephariti*:ti,ab,kw
#3 demodicosis:ti,ab,kw OR demodicidosis:ti,ab,kw OR demodecosis:ti,ab,kw OR 'cylindrical dandruff':ti,ab,kw
#4 demodex:ti,ab,kw OR 'd. folliculorum':ti,ab,kw OR 'd. brevis':ti,ab,kw
#5 blepharoconjunctivitis.:ti,ab,kw
#6 (ocular NEAR/3 (rosacea OR mites)):ab,ti,kw
#7 'meibomian gland'/exp
#8 (meibomian NEAR/3 gland*):ab,ti,kw
#9 (ocular NEAR/3 gland*):ti,ab,kw
#10 ((eye* OR ocular) NEAR/3 inflamm*):ti,ab,kw
#11 ((eye* OR ocular) NEAR/3 infect*):ti,ab,kw
#12 (eye* NEAR/3 seborrheic):ti,ab,kw
#13 (eye* NEAR/3 staphylococcal):ti,ab,kw
#14 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13
#15 'tea tree oil'/exp
#16 'tea tree':ti,ab,kw,tn OR tto:ti,ab,kw,tn OR melaleuca:ti,ab,kw,tn
#17 cliradex:ti,ab,kw,tn
#18 iso4730:ti,ab,kw,tn OR 'iso 4730':ti,ab,kw,tn OR terpin*:ti,ab,kw,tn OR 't4o':ti,ab,kw,tn
#19 #15 OR #16 OR #17 OR #18
#20 #14 AND #19

Appendix 4. PubMed search strategy

#1 blephariti*[tw]
#2 (demodicosis[tw] OR demodicidosis[tw] OR demodecosis[tw] OR "cylindrical dandruff"[tw])
#3 (demodex[tw] OR "d. folliculorum"[tw] OR "d. brevis"[tw])
#4 Blepharoconjunctivitis[tw]
#5 (ocular[tw] AND (rosacea[tw] OR mites[tw]))
#6 (Meibomian[tw] AND gland*[tw])
#7 (ocular[tw] AND gland*[tw])
#8 ((eye*[tw] OR ocular[tw]) AND inflamm*[tw])
#9 ((eye*[tw] OR ocular[tw]) AND infect*[tw])
#10 (eye*[tw] AND seborrheic[tw])
#11 (eye*[tw] AND staphylococcal[tw])
#12 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11
#13 ("Tea tree"[tw] OR TTO[tw] OR Melaleuca[tw])
#14 Cliradex[tw]
#15 (ISO4730[tw] OR "ISO 4730"[tw] OR terpin*[tw] OR "T4O"[tw])
#16 #13 OR #14 OR #15
#17 #12 AND #16
#18 Medline[sb]
#19 #17 NOT #18

Appendix 5. LILACS search strategy

(MH:C11.338.133$ OR blephariti$ OR blefariti$ OR blefarit$ OR demodicosis OR demodicidosis OR demodecosis OR "cylindrical dandruff" OR demodex OR "d. folliculorum" OR "d. brevis" OR blepharoconjunctivitis OR (ocular rosacea) OR (ocular mites) OR (meibomian gland$) OR (Glándulas Tarsale$) OR (Glândulas Tarsai$) OR MH:A09.371.337.614$ OR MH:A10.336.827.600$ OR (ocular gland$) OR (eye$ inflamm$) OR (ocular inflamm$) OR (eye$ infect$) OR (ocular infect$) OR (eye$ seborrheic) OR (eye$ staphylococcal)) AND (MH:D10.627.675.775$ OR MH:D10.627.700.940$ OR MH:D20.215.784.750.940$ OR "tea tree" OR TTO OR Melaleuca OR "Árbol de Té" OR Cliradex OR ISO4730 OR "ISO 4730" OR terpin$ OR "T4O")

Appendix 6. ClinicalTrials.gov search strategy

(blepharitis OR demodicosis OR demodicidosis OR demodecosis OR "cylindrical dandruff" OR demodex OR "d. folliculorum" OR "d. brevis" OR blepharoconjunctivitis OR meibomian gland OR ocular gland OR ocular rosacea OR ocular mites OR eye inflammation OR eye infection OR ocular inflammation OR ocular infection) AND ("Tea tree" OR TTO OR Melaleuca OR Cliradex OR ISO4730 OR "ISO 4730" OR terpinen OR terpinene OR terpinolene OR "T4O")

Appendix 7. WHO ICTRP search strategy

Blepharitis AND tea tree OR demodex AND tea tree OR blepharoconjunctivitis AND tea tree OR meibomian gland AND tea tree OR eye infection AND tea tree OR Blepharitis AND MELALEUCA OR demodex AND MELALEUCA OR blepharoconjunctivitis AND MELALEUCA OR meibomian gland AND MELALEUCA OR eye infection AND MELALEUCA OR Blepharitis AND Cliradex OR demodex AND Cliradex OR blepharoconjunctivitis AND Cliradex OR meibomian gland AND Cliradex OR eye infection AND Cliradex OR Blepharitis AND terpinen OR demodex AND terpinen OR blepharoconjunctivitis AND terpinen OR meibomian gland AND terpinen OR eye infection AND terpinen

Appendix 8. Data on study characteristics

Mandatory items		**Optional items**
Methods		
Study design	· **Parallel-group RCT** *i.e. people randomized to treatment* · **Within-person RCT** *i.e. eyes randomized to treatment* · **Cluster-RCT** *i.e. communities randomized to treatment* · **Cross-over RCT** · Other, specify	Exclusions after randomization Losses to follow up Number randomized/analyzed How were missing data handled? *e.g., available case analysis, imputation methods* Reported power calculation (Y/N*), if yes, sample size and power* Unusual study design/issues
Eyes *or* Unit of randomisation/ unit of analysis	· **One eye included in study**, *specify how eye selected* · **Two eyes included in study, both eyes received same treatment**, *briefly specify how* analyzed*(best/worst/average/both and adjusted for within-person correlation/both and not adjusted for within-person correlation) and specify if mixture one eye and two*	

(*Continued*)

	eye · **Two eyes included in study, eyes received different treatments,** *specify if correct pair-matched analysis done*	
Participants		
Country	*This information should be collected for total study population recruited into the study. If these data are reported for the people who were followed up only, please indicate.*	Setting Ethnic group Equivalence of baseline characteristics (Y/
Total number of participants		
Number (%) of men and women		
Average age and age range		
Inclusion criteria		
Exclusion criteria		
Interventions		
Intervention (n =) Comparator (n =) ***See MECIR 65 and 70***	· Number of people randomized to this group · Drug (or intervention) name · Dose · Frequency · Route of administration	
Outcomes		
Primary and secondary outcomes *as defined in study reports* ***See MECIR R70***	List outcomes Adverse events reported (Y/N) Length of follow-up and intervals at which outcomes assessed	Planned/actual length of follow-up

CONTRIBUTIONS OF AUTHORS

- Conception and design of protocol (ADP, JTL).
- Drafting the protocol or commenting on it critically for intellectual content (KS, ADP, JTL).
- Final approval of the document to be published (KS, ADP, JTL).

DECLARATIONS OF INTEREST

KS, JTL, and ADP have no financial interests in the subject matter of this systematic review. This Cochrane Review was prepared while JTL was a methodologist at the Johns Hopkins Bloomberg School of Public Health. The opinions expressed in this article are the author's own and do not reflect the view of the National Institutes of Health, the Department of Health and Human Services, or the United States government.

SOURCES OF SUPPORT

Internal sources

- No sources of support supplied

External sources

- National Eye Institute (NEI), National Institutes of Health (NIH), USA.

Cochrane Eyes and Vision US Project (and JL during his time with the Project) is supported by cooperative agreement UG1EY020522, NEI, NIH

- National Institute for Health Research (NIHR), UK.
- Richard Wormald, Co-ordinating Editor for Cochrane Eyes and Vision (CEV) acknowledges financial support for his CEV research sessions from the Department of Health through the award made by the National Institute for Health Research to Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology for a Specialist Biomedical Research Centre for Ophthalmology.
- This protocol was supported by the NIHR, via Cochrane Infrastructure funding to the CEV UK editorial base.

The views and opinions expressed therein are those of the authors and do not necessarily reflect those of the Systematic Reviews Programme, NIHR, NHS or the Department of Health.