

# Anti-demodectic effects of okra eyelid patch in *Demodex* blepharitis compared with tea tree oil

WENTING LIU<sup>1,2</sup> and LAN GONG<sup>1,2</sup>

<sup>1</sup>Department of Ophthalmology and Vision Science, The Eye, Ear, Nose and Throat Hospital of Fudan University;

<sup>2</sup>NHC Key Laboratory of Myopia, Laboratory of Myopia, Chinese Academy of Medical Sciences, Fudan University, Shanghai 200031, P.R. China

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**Abstract.** *Demodex* infection gradually develops to *Demodex* blepharitis, which is characterized as chronic inflammation of the eyelid and meibomian gland (MG) and ultimately leads to MG dysfunction. In the present prospective study, the anti-demodectic effects of an okra eyelid patch in patients *Demodex* blepharitis were investigated. A total of 52 patients with *Demodex* blepharitis with ocular discomfort were recruited. Patients were randomized to receive either an okra eyelid patch treatment (treatment group, n=27) or tea tree oil (TTO) eye care patch treatment (control group, n=25) for three months. The *Demodex* count, the ocular surface disease index (OSDI) score, MG expressibility (MGE) and meibum quality, Schirmer I test (SIT), tear break-up time (TBUT) and corneal fluorescein staining (CFS) were determined prior to treatment and after 1 and 3 months of treatment. Changes in the parameters were compared between the treatment group and control group after 1 and 3 months of treatment. The average survival time in the okra group was 115.25±11.87 min, which was significantly lower compared with the average ST of 378.75±37.94 min in the blank group (P<0.01). After 3 months of okra eyelid patch treatment, the *Demodex* count was significantly reduced from 10.15±4.53 to 1.30±1.41 (P<0.01) and the OSDI score of the patients was reduced by 16.84±10.17 (P<0.01). There was no significant difference in the *Demodex* count (P=0.716) and OSDI (P=0.873) between the treatment and control groups. The

rate of complete *Demodex* eradication in the treatment group (11/27, 40.74%) was slightly lower than that in the control group (12/25, 48%), but there was no significant difference between the two groups ( $\chi^2=0.277$ , P=0.598). Regarding the other ocular parameters, no significant difference was observed in the TBUT, meibum quality and MGE between the two groups (P<0.05). TTO group has a significantly improvement compared with Okra group in terms of SIT (P=0.035) and CFS (P=0.023). In conclusion, okra eyelid patch treatment is able to significantly eradicate ocular *Demodex* as well as markedly alleviate ocular symptoms. Due to causing less irritation than TTO, the okra eyelid patch may be more suitable for sensitive patients with *Demodex* blepharitis, such as the elderly and children. The study was registered as a clinical trial in the Chinese Clinical Trial Registry (ChiCTR) in November 2018 (registration no. ChiCTR-1,800,019,466).

## Introduction

*Demodex* (class Arachnida, superorder Acariformes), a microscopic and elongated mite, is one of the most common ectoparasites in humans and may be present on the face and most other parts of the body (1,2). The facial T-zone (forehead, cheeks and nose), meibomian glands (MGs) and follicles of eyelashes are the most common habitats of *Demodex*. Two parasitic species have been detected in humans, namely *Demodex folliculorum* and *Demodex brevis*. Eyelash follicles are the habitat of *Demodex folliculorum*, while *Demodex brevis* resides in the MGs and the sebaceous glands of the eyelids (3-5). *Demodex* infection gradually develops to *Demodex* blepharitis, which is characterized as chronic inflammation of the eyelid and MG and ultimately leads to MG dysfunction (MGD). MGD is the major cause of evaporated dry eye disease, characterized as tear film instability and chronic ocular surface inflammation (6,7). Tea tree oil (TTO) may forcefully eradicate *Demodex* mites and alleviate ocular surface inflammation associated with *Demodex* blepharitis (8). Hence, TTO treatment is currently accepted as the most effective therapy for *Demodex* blepharitis. Although TTO has a certain therapeutic effect on *Demodex* blepharitis, the pungent odor of TTO may cause mild to moderate ocular irritation and discomfort at higher concentrations or with prolonged periods of exposure, especially to the elderly and children (6).

**Correspondence to:** Professor Lan Gong, Department of Ophthalmology and Vision Science, The Eye, Ear, Nose and Throat Hospital of Fudan University, 83 Fenyang Road, Shanghai 200031, P.R. China  
E-mail: 13501798683@139.com

**Abbreviations:** CFS, corneal fluorescein staining; CD, cylindrical dandruff; MGE, meibomian gland expressibility; OSDI, ocular surface disease index; SIT, Schirmer I test; TTO, tea tree oil; TBUT, tear break-up time

**Key words:** okra, *Demodex* blepharitis, ocular surface, anti-demodectic

*Abelmoschus esculentus* L., also known as okra, is a well-known tropical vegetable that is widely grown worldwide. In addition to serving as a food source, okra may serve as a traditional medicine to cure numerous diseases, such as dysentery and diarrhea (9). Due to abundant bioactive compounds, such as polysaccharides, flavonoids, polyphenols, caffeine and pectin in okra, the antibacterial, anti-inflammatory and immune regulatory effects of okra have been gradually proven (10-12). Okra has been applied widely in the cosmetics industry, where the safety and efficacy of okra have been gradually confirmed (13). Okra has been reported to exert anti-oxidative and anti-inflammatory effects by suppressing the Akt-mediated NF- $\kappa$ B pathway in a murine BV2 microglial cell line (14). Akt-mediated NF- $\kappa$ B pathway has been demonstrated to serve an important role in dry eye and blepharitis (15,16). Due to the curative and medicinal effects of okra, it could be speculated that okra may confer therapeutic effects in patients with blepharitis and dry eye.

The present study was designed to determine the potential anti-demodectic and therapeutic effects of an okra eyelid patch on *Demodex* blepharitis *in vivo* and *in vitro*.

## Materials and methods

**Subjects.** The present study was performed in accordance with the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the Eye, Ear, Nose and Throat (EENT) Hospital of Fudan University. The study was registered as a clinical trial in the Chinese Clinical Trial Registry (ChiCTR) in November 2018 (registration no. ChiCTR1800019466). A total of 52 patients who experienced *Demodex* blepharitis were recruited from the EENT hospital between December 2018 and May 2019. *Demodex* blepharitis was confirmed by the total number of *Demodex* on epilated lashes under light microscopic examination. In brief, the procedure was as follows: A total of two lashes with cylindrical dandruff (CD) each from the upper and lower eyelids were epilated and mounted on glass slides; one drop of cedar oil was dripped on the bottom of each eyelash to dissolve the CD and to allow the embedded *Demodex* to migrate out, which occurs almost immediately; the total number of *Demodex* mites was counted by the same inspector (WL) under a light microscope (CX23; Olympus Corp.). If the number of *Demodex* mites was  $\geq 3$ , the patient was considered as *Demodex*-positive (17,18). Furthermore, 'absolute *Demodex* eradication' was defined as complete *Demodex* eradication with the *Demodex* count reduced to zero after treatment (19,20).

The inclusion criteria for the present study were *Demodex* blepharitis patients with *Demodex*-positive eyelashes aged 18-70 years who voluntarily participated in the experiment. As patients with certain ocular diseases (recurrent herpes simplex, ocular impairment associated with immune diseases) or receiving physiotherapy for blepharitis (intense pulsed light, baby shampoo, LipiFlow® and other *Demodex* treatments) in the last 6 months may have confounded the results, they were excluded from the study. Patients were also excluded if they had a related ocular surgery, including cataract surgery, trichiasis surgery or refractive surgery in the past 3 months. After the procedure and potential consequences of the treatments had been elaborately explained, informed consent was obtained from each participant prior to participation.

**Treatments.** A total of 52 participants were randomly divided into two groups using a random number table. The okra group (n=27) received okra eyelid patch treatment (YourGa®; Shanghai YourGa Co., Ltd.; [http://www.ganyanzheng.com/?\\_l=zh\\_CN](http://www.ganyanzheng.com/?_l=zh_CN)), and the TTO group (n=25) received TTO eye care patch treatment (YourGa; Shanghai YourGa Co., Ltd.; Fig. 1). The treatment was performed by participants at home every night before sleep for three months. The procedure was as follows: After the eyelid margin was cleaned by eye rinse (YourGa), the eyelid was heated to 42°C from the outside by a Moisture Chamber (YourGa) for 20 min. Finally, eyelid patches (okra or TTO) were applied to both eyes for 15 min and then removed.

The order of examinations performed was as follows: Ocular surface disease index (OSDI), slit-lamp biomicroscopic examination, Schirmer I test (SIT), tear film break-up time (TBUT), MG assessment and corneal fluorescein staining (CFS). Each participant was subjected to the examinations three times, namely on the day prior to treatment and 1 and 3 months after treatment. All examinations were performed by a skilled researcher (WL) to reduce operational error. In addition, both eyes were examined and treated, but only the data of the right eye were analyzed.

**OSDI.** The OSDI questionnaire, a 12-item questionnaire with a scale of 0-100, has been designed to rapidly evaluate ocular discomfort symptoms (e.g. soreness, light sensitivity, blurred vision). The OSDI provides an assessment of vision-related dyspraxia (difficulty reading, driving, operating a computer and watching TV). There is a positive correlation between OSDI scores and the severity of ocular discomfort, with higher scores representing greater ocular discomfort (21). Prior to treatment, the OSDI questionnaire was completed by participants to set the baseline.

**SIT.** After a sterile dry strip (Jingming®) was inserted into the lateral canthus of the lower eyelid away from the cornea for 5 min, the wetted length of the strip absorbed with tears was then measured to assess tear production. Potential SIT scores ranged from 0 to 30 mm.

**TBUT.** A fluorescein strip (Jingming) moistened with preservative-free saline solution gently touched the central lower lid margin the patient was then requested to blink several times to ensure adequate coating of the complete corneal dye. After several natural blinks, the patient was required to rapidly open the eye and the time of eye opening was recorded as the starting point (time=0 sec). TBUT was defined as the interval between the starting point and the first black spot appearing in the stained tear film with a cobalt blue filter and slit lamp microscope. The test was repeated three times on each patient and the average TBUT was calculated (22).

**MG expressibility (MGE).** A total of 5 consecutive MGE measurements of the central lower eyelid were assessed with an MG evaluator (MGE-1000; TearScience). The patient was instructed to look upwards and the lower eyelid gland orifices were gently cleaned with a cotton swab. The MG evaluator was held in position and pressure was maintained for 10-15 sec to evaluate whether each gland secreted or not. The MGE score was recorded according to the number of secretory glands: 0,

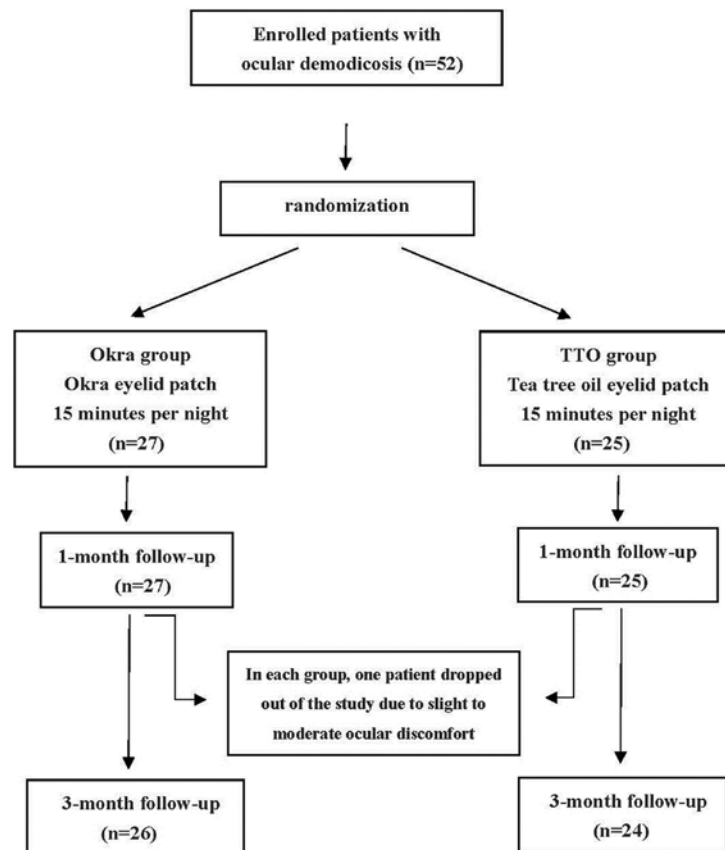


Figure 1. Flow chart indicating the total numbers of eyes enrolled, treated and observed during the follow-up period of the study. TTO, tea tree oil.

all five glands; 1, three to four glands; 2, one to two glands; and 3, none of the glands (7).

**Meibum quality.** The meibum quality of eight central MGs of the upper eyelid was also assessed with the MG evaluator. The assessment procedure was the same as above. According to the secretion characteristics of each gland, the grade was ranked on a scale of 0 to 3 for each gland: 0, clear liquid; 1, colored/cloudy liquid; 2, cloudy with debris (granular); and 3, thick like toothpaste (total grade range, 0-24 scores) (7).

**CFS.** The inspection method of CFS was similar to that for the assessment of the TBUT. The cornea was divided into five zones (central, superior, temporal, nasal and inferior). Corneal epithelial injury was graded on a scale from 0 to 3: 0, no epithelial injury; 1, <30 corneal punctate stains; 2, >30 corneal punctate stains but not fusion; and 3, fusion of corneal staining or ulcer. The total CFS score ranged from 0 to 15 (23).

**Tolerance.** During the treatment, any symptoms regardless of the cause were promptly recorded by the researcher (WL). Common symptoms may include eye redness, pain, allergy, irritation and foreign body sensation. Rare symptoms may involve blurred vision and acute ocular infection. If the participant requested withdrawal from the study due to discomfort, the study will be terminated immediately.

**Survival time (ST) of *Demodex*.** Written informed consents have been obtained from all patients before eyelashes have

been extracted. After eyelashes have been extracted from the participants' eyelid in each group at room temperature, these eyelashes with demodex mites were instantly placed on a glass slide. A total of 60 mites were then divided randomly into three groups (20 mites in each group). Okra and TTO eyelid patch extractions were then dripped onto the glass with a micropipette respectively. Saline was used as the blank group. As *Demodex* is more vulnerable at an earlier stage of life, only adult *Demodex* with four pairs of well-developed legs and a stumpy body were tested (8). After the eyelash had been extracted from eyelid, the movements of the *Demodex* body and legs were observed immediately and continuously under a microscope at a magnification of x40. The ST was defined as the duration from the time-point of eyelid patch extraction dripped on the body to the cessation of movement (Fig. 2). The average ST of *Demodex* mites was compared among the control (n=20 mites), okra (n=20 mites) and TTO (n=20 mites) groups.

**Statistical analysis.** Randomization method was used to designate participants into Okra and TTO groups. Data were analyzed using SPSS v.17.0 software (SPSS Inc.). Categorical data (sex) between two groups were evaluated for statistical significance using the chi-square test. Continuous variables were presented as the mean  $\pm$  standard deviation. The normal distribution test (Kolmogorov-Smirnov) was performed to check whether the numerical variables were normally distributed. Data on age and ocular parameters between the two groups were evaluated for statistical significance using Student's t-test. The data

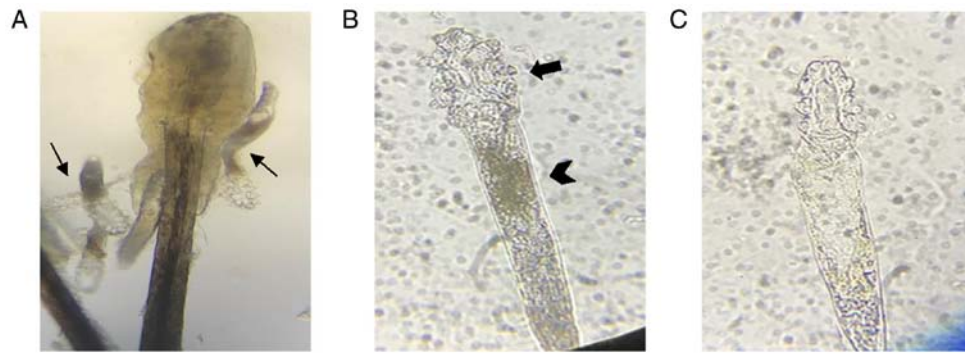


Figure 2. Microscopic examination of *Demodex* mites. (A) Several *Demodex* mites were detected under microscopic examination of the epilased lash (magnification, x20; thin arrows). (B and C) The one of the *Demodex* mite from (A) was photographed for live and dead status under a microscope (magnification, x40). (B) The *Demodex* mite was determined to be 'alive' if marked movements of the legs (thick arrow) and body (thick arrowhead) was present. (C) By contrast, the *Demodex* mite was determined to be 'dead' when movements of the leg ceased and the body became transparent.

at the baseline and at 1 and 3 months after treatment in the Okra group were homoscedastic and normally distributed and the differences between various time-points were analyzed by repeated-measures ANOVA followed by a least-significant differences test. The results are indicated as P-values, where  $P < 0.05$  was considered to indicate a statistically significant difference. According to a previous study, the *Demodex* eradication rate of TTO therapy is  $\sim 90\%$  (20). In the present study, it was previously estimated that there would be a 25% relative difference between the Okra and the TTO groups (20), which meant that a sample size of 23 patients in each group was required to achieve a statistical power of 80% for a significance level of 0.25% with a two-tailed test.

## Results

**Patient baseline characteristics.** A total of 27 participants underwent okra eyelid patch treatment (13 males and 14 females, aged  $46.21 \pm 13.03$  years), while 25 participants received TTO eyelid patch treatment (13 males and 12 females, aged  $40.54 \pm 10.39$  years). No significant differences in terms of sex ( $\chi^2 = 0.103$ ,  $P = 0.749$ ) and age ( $P = 0.160$ ) were determined between the two groups. There were no differences in the *Demodex* count, OSDI, meibum quality, MGE, SIT, TBUT and CFS between the okra and control groups prior to treatment ( $P > 0.05$ ; Table I).

***Demodex* eradication and ocular parameters.** The *Demodex* counts and ocular parameters at 1 and 3 months in the okra group were compared with those at baseline (Table II). Okra eyelid patch treatment can significantly eradicate *Demodex* mites from  $10.15 \pm 4.53$  to  $1.31 \pm 1.41$  on patients with ocular demodicosis after 3 months ( $P < 0.01$ ). The average OSDI score in the okra group decreased from baseline ( $40.51 \pm 10.85$ ) to the end of treatment ( $23.67 \pm 10.71$ ;  $P < 0.01$ ). Other ocular parameters, including SIT, TBUT, CFS, meibum quality and MGE, were also compared before and after 1 and 3 months of treatment, respectively. Statistically significant improvements in TBUT ( $P = 0.007$ ), CFS ( $P < 0.01$ ) and meibum quality ( $P < 0.01$ ) have been observed in Okra group by 3 months. Blepharitis of 13 patients was observed to be improved from slight to obvious degrees after Okra eyelid treatment. Among these patients, the sign of eyelid margin in one patient who was improved markedly (Fig. 3).

The *Demodex* counts and ocular parameters at 1 and 3 months of treatment were also compared between the okra and TTO groups (Table III). The average *Demodex* count in the okra group had decreased by  $-8.85 \pm 3.84$  after 3 months of treatment, whilst the *Demodex* count in the TTO group decreased  $-9.36 \pm 6.03$  after 3 months of treatment ( $P = 0.716$ ). There was no significant difference in the anti-demodectic effects between the two groups at 3 months ( $P = 0.716$ ). Compared with that in the TTO group, the anti-demodectic effect of okra was slightly milder at 1 month, but no significant difference was observed between the two treatments at 1 and 3 months. The absolute *Demodex* eradication rate in the okra group (11/27, 40.74%) was slightly lower than that in the TTO group (12/25, 48%). The improvements in each of ocular parameters between okra and TTO groups did not exhibit significant difference, except for SIT ( $P = 0.035$ ) and CFS ( $P = 0.023$ ) at 3 months.

**Tolerance.** Any ocular discomfort, including allergic reaction, irritation and pruritus, acute ocular infection and visual acuity loss, was recorded during treatment. No allergic reactions or acute ocular infections were reported during treatment in either group. Of the 27 participants in the okra group, 26 (96.3%) did not report any adverse events or tolerability issues during the treatment period. However, one participant (3.7%) reported transient ocular pruritus and discomfort following application of the okra eyelid patch close to the eyelash margin and surrounding skin. This participant was then dropped out of the study and lost to follow-up. Of the 25 patients in the TTO group, four (16%) reported slight to moderate irritation with conjunctival congestion. Among these four participants, one was subsequently lost to follow-up due to dropping out.

**ST of *Demodex*.** The average ST of *Demodex* in the different groups is provided in Fig. 4. The average ST in the okra group was 115.25 min, which was significantly lower compared with the average ST of 378.75 min in the blank group ( $P < 0.01$ ). Compared with that in the TTO group (106.7 min), the ST in the okra group was slightly but significantly longer ( $P = 0.042$ ).

## Discussion

Increased attention has been paid to *Demodex* blepharitis by ophthalmologists in the past two decades. Several pathogenic



Table I. Comparison of demographic data, baseline data between the okra and TTO treatment groups.

Item	Okra (n=27)	TTO (n=25)	P-value
Age (years)	46.21±13.03	40.54±10.39	0.160
Females/males	14/13	12/13	0.749
<i>Demodex</i> mites	10.15±4.53	11.24±5.88	0.455
OSDI score	40.51±10.85	35.86±12.77	0.162
Meibum quality score	8.56±4.26	6.68±4.72	0.138
MGE	1.15±0.66	1.44±0.51	0.082
SIT (mm/5 min)	9.63±5.13	7.20±6.70	0.147
TBUT (sec)	4.59±1.74	5.12±1.88	0.298
CFS	2.22±1.60	1.40±1.68	0.077

Values are expressed as the mean ± standard deviation or n. CFS, corneal fluorescein staining; MGE, Meibomian gland expressibility; OSDI, ocular surface disease index; SIT, Schirmer I test; TTO, tea tree oil; TBUT, tear break-up time.

Table II. Variation in the parameters from baseline to 1 and 3 months after treatment in the Okra group.

Item	Prior to treatment	1 month	3 months	F	P1	P2
<i>Demodex</i> mites	10.15±4.53	3.26±2.03	1.30±1.41	125.55	<0.01	<0.01
OSDI score	40.51±10.85	29.55±12.15	23.67±10.71	29.74	<0.01	<0.01
Meibum quality score	8.56±4.26	5.85±4.29	5.48±4.08	16.39	<0.01	<0.01
MGE	1.15±0.66	0.93±0.62	1.04±0.71	1.44	0.056	0.449
SIT	9.63±5.13	10.74±4.71	11.22±4.53	3.03	0.119	0.014
TBUT (sec)	4.59±1.74	5.56±1.50	5.96±1.58	12.74	0.006	<0.01
CFS	2.22±1.60	1.11±1.12	0.78±0.93	43.54	<0.01	<0.01

Values are expressed as the mean ± standard deviation. P-values: P1, pretreatment vs. 1 month; P2, pretreatment vs. 3 months. F, variance values among three groups; CFS, corneal fluorescein staining; MGE, Meibomian gland expressibility; OSDI, ocular surface disease index; SIT, Schirmer I test; TBUT, tear break-up time.

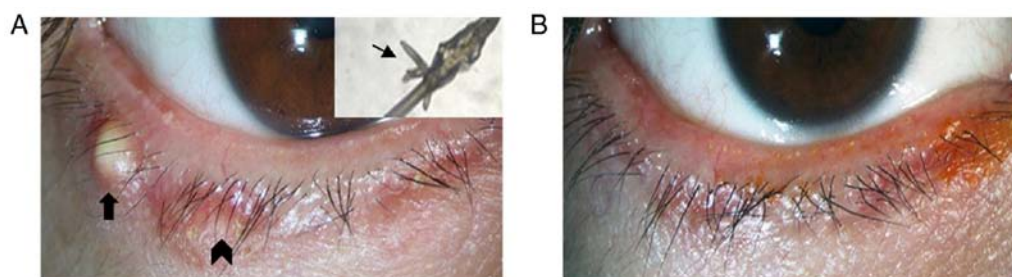


Figure 3. Photographs of the palpebral margin of a representative case (male, 40 years old) in the okra group prior to and after treatment. (A) Prior to okra eyelid treatment, *Demodex* was strongly positive (thin arrowhead), with blepharitis (thick arrowhead) and local hordeolum (thick arrow; magnification, x16). (B) After 3 months of okra treatment, *Demodex* had been eradicated and symptoms had significantly improved.

mechanisms of *Demodex* blepharitis have been postulated in previous studies. First, *Demodex* mites may significantly damage the habitat where they live by continuous movement and invasion. Furthermore, *Demodex* mites may block the hair follicles and sebaceous ducts mechanically to induce epithelial hyperplasia and hyperkeratinization, while debris or waste from *Demodex* mites may elicit inflammatory responses or an innate immune response (24). In addition to the mechanisms described above, the pathogenic role of other

microbial infections, including *Streptococci*, *Staphylococci*, *Propionibacterium acnes* associated with *Demodex* infestation that leads to MGD and dry eye has also gained increasing attention.

Different TTO products are now widely applied in *Demodex* blepharitis treatments. A weekly eyelid scrub with 50% TTO proved successful in eradicating ocular *Demodex* infestation and *Demodex* counts as low as zero have been confirmed in the majority of patients after 4 weeks of

Table III. Variation in the parameters from baseline to 1 and 3 months after treatment compared between the two groups.

Item	1 month			3 months		
	Okra	TTO	P1	Okra	TTO	P2
<i>Demodex mite</i> eradication	-6.89±3.24	-7.84±6.24	0.489	-8.85±3.84	-9.36±6.03	0.716
OSDI score	-8.65±11.49	-12.40±9.53	0.627	-16.84±10.17	-17.44±16.05	0.873
Meibum quality score	-2.70±2.30	-1.56±2.45	0.089	-3.07±3.15	-2.48±3.42	0.517
MGE	-0.22±0.58	-0.04±0.20	0.141	-0.11±0.75	-0.32±0.63	0.284
SIT (mm/5 min)	1.11±3.58	-0.36±1.68	0.067	1.59±3.13	0.04±1.81	0.035
TBUT (sec)	0.96±1.68	0.16±1.72	0.095	1.37±1.47	0.76±1.83	0.190
CFS	-1.11±0.89	-0.44±0.87	0.008	-1.44±0.93	-0.72±1.28	0.023

Values are expressed as the mean ± standard deviation. P-values: P1, pretreatment vs. 1 month; P2, pretreatment vs. 3 months. F, variance values among three groups; TTO, tea tree oil; CFS, corneal fluorescein staining; MGE, Meibomian gland expressibility; OSDI, ocular surface disease index; SIT, Schirmer I test; TBUT, tear break-up time.

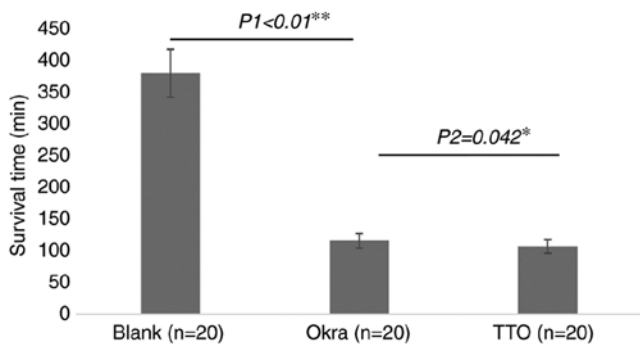


Figure 4. Survival time of *Demodex* mites under different treatments. Each bar represents the mean survival time and error bars represent the standard deviation. \*P<0.05, Okra vs. TTO; \*\*P<0.01, Blank vs. Okra. TTO, tea tree oil.

treatment (25-27). The present study indicated that okra had a similar effect to that of TTO in terms of *Demodex* eradication on the eyelids of patients, and furthermore, it significantly shortened the survival time of *Demodex in vitro*. Therefore, okra is expected to be developed as a potential treatment for *Demodex* blepharitis.

As TTO may exert antibacterial, antifungal and anti-inflammatory actions, its therapeutic effects may not be attributed to killing *Demodex* mites only; TTO also possesses anti-inflammatory and bacterial colonization reduction properties. In previous studies, TTO demonstrated a strong effect on *Demodex* eradication and among a total of 15 major active components in TTO, terpinen-4-ol has been identified as the most potent ingredient (8,26).

Regarding bacterial infection, okra fruit has a high tannin content that may abolish several common types of bacteria, including several Gram-positive and Gram-negative bacteria (28). Furthermore, Lengsfeld *et al* (29) reported that *H. pylori* adhesion on human stomach sections was almost completely inhibited by fresh okra fruit juice. In addition to its antibacterial effects, a number of studies suggested that crude okra acts as an immunomodulator with both immune-stimulatory and immunosuppressive activities (30). Okra is also able to act as an immune modulator by activating phagocytes to

produce more proinflammatory cytokines (e.g. TNF- $\alpha$  and IL-17) to improve the host defense against various bacteria. Among the medicinal herbs, okra is an important plant that is widely distributed worldwide; furthermore, okra is a Chinese medicine and is less pungent than TTO, resulting in minor toxicity and few side effects (31).

Among antiparasitic remedies, polysaccharides from different sources have become a prime research topic. Volatile oils from certain Chinese crude medicines with abundant polysaccharides exerted the most potent effect among various *Demodex* blepharitis treatments (32). Apart from this, sulfated polysaccharides from algae were reported to serve as an alternative to heparin in the treatment of leishmaniasis to significantly reduce the count of the promastigotes of *L. amazonensis* in a dose-dependent manner (33). The possible anti-demodectic mechanism of polysaccharides is to regulate the host's immune system by activating immune cells such as lymphocytes, macrophages and natural killer cells. Furthermore, the attachment between host and parasite may be attenuated (34). Okra contains an abundance of polysaccharides, which are considered to be responsible for effective *Demodex* eradication.

In addition, okra, which contains an abundance of flavonoids, polyphenol and vitamin C, may act as an antioxidant and anti-inflammatory agent with low toxicity and few side effects (35,36). The antioxidant activity of methanolic seed extracts of okra has been reported in several studies, revealing antioxidant activity of okra seed extracts under different conditions (37,38). Furthermore, the reactive oxygen species (ROS) oxidation pathway has been confirmed to involve in the pathogenesis of dry eye disease, whereas okra may interfere with dry eye by inhibiting the ROS oxidation pathway to alleviate ocular discomfort (39,40).

Similar to *Demodex* blepharitis, rosacea is a characteristic cutaneous dermatosis due to the presence of multiple small, dome-shaped erythematous papules and papulopustules and *Demodex* mites may also have a vital role in its pathogenesis (41,42). Certain studies have suggested that rosacea remedies, including antibacterial, anti-inflammatory and immune regulation treatments, have proven curative

effects (43). A number of previous studies suggested that antibiotic treatments, such as oral antibiotics, including tetracycline or topical metronidazole, may obviously alleviate skin inflammation and symptoms of rosacea. Superantigens produced by *Streptococci* and *Staphylococci* may have a role in the induction of rosacea (43). Hence, researchers should also pay more attention to the role of microorganisms in the treatment of *Demodex* infestation (44,45). Due to the reported anti-inflammatory and antioxidant effects of okra (28,37), it could be speculated that the symptoms of *Demodex* blepharitis may be improved by these compounds. In addition, it has been proposed that *Demodex* may be eradicated by changing the living environment of *Demodex* and reducing the colonization of related bacteria in MG in view of the antibacterial, anti-inflammatory and immunoregulatory effects of okra (30,36). In the present study, okra caused less irritation than TTO, was more comfortable and resulted in good compliance. In conclusion, okra may be a potential remedy for the treatment of *Demodex* blepharitis and MGD.

Due to the limitation of the small number of participants in the present study, the efficacy of okra eyelid patches for *Demodex* blepharitis requires further verification in a larger cohort. Although the anti-demodectic effects of the okra eyelid patch in *Demodex* blepharitis are explicit, the mechanisms of the anti-demodectic effects of okra's remain to be fully elucidated. Future studies are required to clarify the anti-demodectic mechanism of okra.

In conclusion, the okra eyelid patch effectively eradicated *Demodex* mites both in patients and *in vitro* and its application was associated with reduced ocular discomfort. The okra eyelid patch presented superior ocular tolerance and may be more comfortable to use than the TTO eyelid patch.

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## Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

## Authors' contributions

LG participated in the project design, sample size calculation and revision of the manuscript. WL was responsible for the enrolment and follow-up of patients and participated in performing the statistical analysis. WL also drafted the manuscript. Both authors confirm the authenticity of the raw data and read and approved the final manuscript.

## Ethics approval and consent to participate

The present study was strictly performed on the basis of the Declaration of Helsinki for research involving human

participants and was approved by the Ethics Committee of the EENT Hospital of Fudan University (Shanghai, China). After the experimental details and potential benefits and risks were explained, written informed consent was obtained from all participants prior to the examination and treatment.

## Patient consent for publication

Oral consent from the patient whose lid margin and *Demodex* photographs are displayed in Fig. 3 was obtained prior to publication.

## Competing interests

The authors declare that they have no competing interests.

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