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ORIGINAL ARTICLE

# Comparison of Efficacy and Safety of Two Tea Tree Oil-Based Formulations in Patients with Chronic Blepharitis: A Double-Blinded Randomized Clinical Trial

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## ABSTRACT

**Introduction:** It was aimed to evaluate the efficacy of two tea tree oil (TTO)-based cleansing gels in chronic blepharitis patients.

**Methods:** Group-1 (basic gel containing 3%(w/w)-TTO) included 50 eyes of 25 patients and group-2 (advanced gel containing 3%(w/w)-TTO plus essential oils and vitamins) included 48 eyes of 24 patients. Ocular Surface Disease Index (OSDI), tear breakup time (TBUT), ocular surface staining pattern, Schirmer's test, impression cytology, Demodex presence and TNF- $\alpha$ , IL-6, IL-1 $\beta$  levels were evaluated at the first visit and 1 month after treatment.

**Results:** In both groups, the mean OSDI score decreased (p1:0.001, p2:0.001), TBUT increased (p1:0.002, p2:0.004). In group-1, Demodex presence decreased from 42% to 27.8%; in group-2 from 54.2% to 20.6% (p1:0.302, p2:0.004). IL-1 $\beta$  and IL-6 decreased in group-2 (p1:0.002, p2:0.050). TNF- $\alpha$  decreased in both groups (p1:0.001, p2:0.001).

**Conclusion:** Both formulations improved ocular surface parameters. Group 2 showed more reduction in tear cytokines and Demodex count.

**Keywords:** Blefar-ex Plus, blepharitis, cleansing gel, demodex, essential oils, tea tree oil

Blepharitis is a chronic inflammatory condition that progresses with the inflammation of the eyelid which contains the eyelash follicles, sebaceous and apocrine glands.<sup>1</sup> Blepharitis with a complex etiology and pathophysiology mostly results in alteration of the tear film, eye irritation, and ocular surface disease.<sup>2,3</sup> One of the causes of severe blepharitis is the presence of Demodex

mites, which are the most common ectoparasites in humans.<sup>4</sup> Blepharitis generally responds well to antibacterial eye pharmaceutical preparations or topical steroids in many cases. However, cases with severe blepharitis are resistant to these treatments, and it is possible that one of the causes of these infections is Demodex mites.<sup>5-9</sup> In fact, the routine cleaning of the periocular area and the

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eyelashes cannot be managed so easily and effectively due to their anatomical features; this reality creates a favorable environment for the development and spread of the Demodex parasite.<sup>10–12</sup>

Blepharitis is an inflammatory disorder of the lid border, which is characterized by ocular surface inflammation leading to tear film abnormalities, itching, redness, burning, dryness, blurred vision, and scaly lashes.<sup>3,10,13,14</sup> These symptoms occur more aggressively in the posterior blepharitis associated with Demodex, and this type of blepharitis is resistant to conventional treatments.<sup>10</sup> Significant differences in some cytokines and chemokines have also been observed in Demodex blepharitis compared to blepharitis not associated with Demodex.<sup>14</sup>

In order to carry out a successful blepharitis treatment, cleaning the roots of the eyelashes is very important.<sup>2</sup> Nevertheless, Demodex has been found to localize in 50% of patients even after cleaning the roots of the eyelashes with baby shampoo for 1 year.<sup>15</sup> In *in vitro* studies, it has been shown that Demodex is resistant to many antiseptic solutions, but is sensitive to tea tree oil (TTO).<sup>13,16</sup> Currently, TTO is used effectively for the eradication of ocular Demodex. Therefore, TTO at a concentration more than 10% has been found to be effective in reducing ocular symptoms as well as inflammation of the eyelids and conjunctiva.<sup>10,11,16</sup> However, when used at high concentrations, TTO causes ocular irritation in some patients.<sup>10,11</sup> Contact dermatitis, allergic reactions, and ocular irritation are reported as common complications of preparations containing TTO. Thus, the TTO toxicity and therapeutic benefits of TTO-containing formulations need to be investigated to develop appropriate cosmeceutical/pharmaceutical preparations. In this respect, a daily used cleansing gel formula as a cosmeceutical preparation including low proportion of TTO would be more efficient and safer than a complex preparation including a high amount of TTO. Blefar-ex Plus is an advanced cleansing gel formulation including 3% (w/w) TTO and additively calendula oil, borage oil, vitamin E and vitamin B5 (less than 5% w/w) which was developed to avoid the irritation and to sensitize the potential of high amount of TTO. This current study was carried out in order to test the hypothesis that Blefar-ex Plus can control blepharitis symptoms and Demodex-induced damage and inflammation while providing effective cleansing of eyelashes. The aim of the study was to investigate the efficacy and safety of the advanced gel, and to compare its effectiveness with the basic gel preparation including only 3% w/w TTO.

## MATERIALS AND METHODS

### Patients, Randomization and Study Protocol

This study, which was initiated with the approval of Marmara University Cosmetics Clinical Research

Studies Ethics Committee (27/06/2016, No: 2), and approval of the Ministry of Health, Turkish Medicines and Medical Devices Agency (01.07.2016, No: 58307721–512-99–86341), is designed as a prospective, randomized, double-blind clinical study, and includes patients with chronic blepharitis presented to Ankara Numune Training and Research Hospital, Ophthalmology Clinic between October 2016–June 2017 with complaints of burning, stinging, and heaviness sensations in the eyes. Patients with oily secretion and cylindrical dandruff in the eyelashes and who age between 18 and 65 years were enrolled in the study. Patients who; (i) use systemic anti-inflammatory drugs, (ii) are pregnant, (iii) are nursing, (iv) are using contact lenses, (v) had ocular surgery within the last 3 months or (vi) received treatment other than artificial tear preparations were excluded from the study.

The patients were divided into two groups by simple randomization. Both gel formulations were packaged in the same box except for their lot numbers (Figure 1). The first group received the formulation with the lot number BP01 and the second group received the formulation with the lot number BP02. Examining researchers and patients were not informed about which gel formulation was in which package until statistical analyses were completed.

### Cleansing Gel Formulations

#### Lot Number BP01

A basic washing gel formulation containing 3% (w/w) TTO (Organic *Melaleuca alternifolia* Leaf Oil) as bioactive ingredient.

#### Lot Number BP02

Blefar-ex Plus cleansing gel formulation containing 3% (w/w) TTO (Organic *Melaleuca alternifolia* Leaf Oil) plus calendula oil, borage oil, vitamin E, vitamin B5 less than 5% (w/w).

Patients were examined twice; one on their first visit, and the other after using the provided gel twice a day for 1 month.

### Follow-up and Clinical Assessment

1. The subjective complaints of patients:  
A Ocular Surface Disease Index (OSDI) questionnaire

Objective examination findings:

- A. Fluorescein tear breakup time (TBUT): Saline moistened fluorescein strips were placed in the



FIGURE 1. Representation of the gel bottles used in the study. A basic washing gel formulation was packaged in the box with lot number BP01 and Blefar-ex Plus cleansing gel formulation was packaged in the box with lot number BP02.

lower fornix without topical anesthesia prior to asking patients to blink several times to encourage its distribution. The interval between the last blink and the appearance of the first corneal dry spot was measured under blue cobalt filter. This measurement was repeated three times and the mean value was recorded.

B. Ocular surface staining pattern, lissamine green (Oxford Scheme): Saline moistened lissamine green strips were placed in the lower fornix prior to asking patients to blink several times to encourage its distribution. The staining of nasal and temporal conjunctiva was graded according to Oxford Scheme.

C. Schirmer's test: The Schirmer's test was performed without topical anesthesia using standardized Whatman filter paper placed on the lower lateral one-third of conjunctival fornix for 5 min.

Histological analyses:

- A. Presence of Demodex in eyelash samples
- B. Conjunctival changes (Impression cytology)

1. Tear analysis for inflammatory markers (TNF- $\alpha$ , IL-6, IL-1 $\beta$ )

### Detection of Demodex in Eyelash Specimens

Two eyelashes, from each lower eyelid, with intensely oily secretion and cylindrical dandruff were collected from the patients in order to be examined under the light microscope. Specimens were placed individually on a clean slide and a drop of 15% potassium hydroxide (KOH) solution was added on top. After 15–20 min of incubation with KOH, the chitin layer on the samples was solubilized. Coverslips were placed on the samples, which were now transparent, and the periphery of the coverslips was covered with liquid paraffin. Finally, the presence of *Demodex folliculorum* in the specimens was identified using binocular light microscopy (Figure 2).

### Histological Follow-up for Impression Cytology

Impressions were taken by pressing strip of cellulose acetate filter papers (pore diameter: 0.22 mm) dull side down onto the medial conjunctiva by applying gentle pressure with a blunt, smooth tipped forceps for 5 s and then the strip was peeled off with a forceps. The



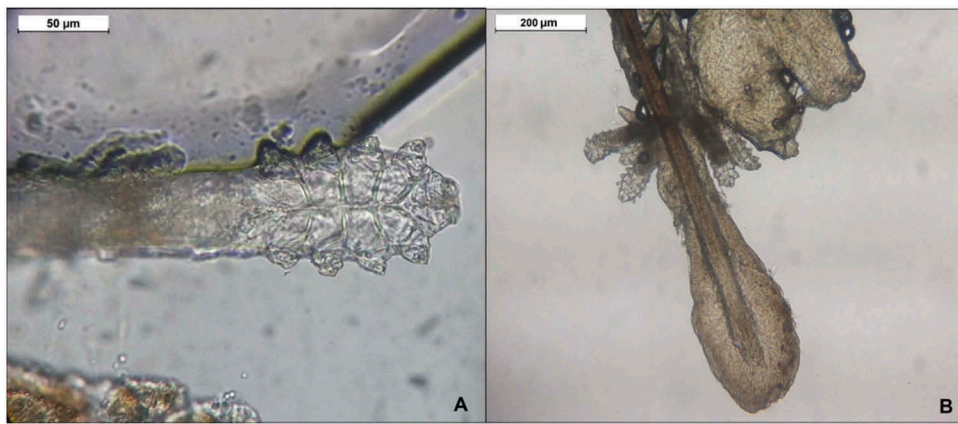


FIGURE 2. Demodex mite. Demonstrative image for Demodex parasite detected in the eyelash follicle of one of our patients (A X400; B X100; light microscopy).

samples collected for impression cytology were stored for 10 min in a solution containing 1:1:20 acetic acid, formaldehyde (37%) and ethyl alcohol (70%) as their fixations are provided. Filter papers were stained using Periodic Acid-Schiff (PAS) protocol, and their photographs were taken using the LAS program in Leica DCM 4000 (Germany) computer-aided imaging system.

### Periodic Acid-Schiff Staining (PAS) Protocol

The PAS reaction method is used to show the mucopolysaccharides in the tissue.<sup>17</sup> As instructed in the PAS staining protocol, the samples were incubated for 10 min in periodic acid solution, then washed with dH<sub>2</sub>O and were incubated for an additional 20 min in Schiff's solution. Then, following two incubations for 2 min in sodium metabisulphite, the samples were washed under running water for 5 min. Next, nuclear staining was performed by incubating the samples for 5 min in Mayer's hematoxylin. At the end of the staining procedure, the filter papers were transferred on glass and were washed with increasing percentages of alcohol and xylol to ensure dehydration, and then were covered with the intellinal medium.

### PAS Grading (according to Nelson's Method)<sup>18</sup>

*Grade 0:* Epithelial cells are small, in close adherence to each other, rounded and have a large nucleus (1:2); Goblet cells abundant, plump, oval shaped, PAS ++.

*Grade 1:* Epithelial cells are slightly large, polygonally shaped and have slightly small nuclei (1:3); lesser Goblet cells, plump, oval shaped, PAS ++.

*Grade 2:* Epithelial cells are large, polygonally shaped and have small nucleus (1:4); Goblet cells are much lesser, small, PAS + (difficult to distinguish).

*Grade 3:* Epithelial cells are very large and convoluted, the nucleus is pyknotic or too small; Goblet cells are completely diminished, small, PAS-.

### Tear Analysis for Inflammatory Markers

Unstimulated tear sampling was the first step of the examinations. For this analysis, horizontally placed capillary tubes to lateral conjunctiva were used. Tear samples collected were transferred to 1.5 ml eppendorf tubes. A total of approximately 150–200 µL teardrop samples were pooled and stored at –80°C until the time of the study.

The tear samples were collected to measure the levels of proinflammatory cytokines by using commercial kits. ELISAs for human IL-1 $\beta$  (17 kDa), IL-6 (28 kDa) and TNF- $\alpha$  (17 kDa) were performed according to the manufacturer's instructions (Diacclone SAS, France). In brief, 50 µl of tear samples from each patient was diluted in assay diluent (twofold) and dispensed in antibody-embedded 96-well plates. After all the assays were performed, absorbance was read by an automatic microplate reader (PowerWave XS2; BioTek Instruments Inc., USA). All the samples were tested in duplicate. The concentrations of the inflammatory markers were calculated via the standard-curve obtained from kit standards and multiplied by the dilution factor.

### Statistical Analyses

Continuous variables (OSDI, Schirmer, BUT, IL levels) were summarized by their mean  $\pm$  standard deviation (median) and nominal-categorical variables (Demodex, Oxford, Nelson) were summarized by frequency and percentage. For each group; continuous variables were evaluated by using Wilcoxon signed-rank test and nominal-categorical variables were evaluated by using McNemar or Chi-square

tests. Mann–Whitney U and Chi-square tests were used for intergroup comparisons of quantitative and qualitative data, respectively. For all statistical analysis, a level of 5% ( $p < 0,05$ ) was considered as statistically significant. Normal distribution of the values was examined with the Kolmogorov–Smirnov and Shapiro–Wilk tests.

## RESULTS

A total of 50 eyes of the 25 patients in the first group and a total of 48 eyes of the 24 patients in the second group were evaluated. The demographic data of the two groups are shown in Table 1. There were no significant differences in the ages and sex among the two groups.

### The Subjective Complaints of Patients

A Ocular surface disease index (OSDI) questionnaire: The mean OSDI scores were significantly decreased in both groups after treatment ( $p_1$ : 0.001,  $p_2$ : 0.001) (Table 2).

TABLE 1. Demographics of the patients in both groups.

	Sex n (%)		Age mean±SD (median)
	Female	Male	
<b>Group 1</b>	12 (48.0)	13 (52.0)	48.80 ± 13.22 (49)
<b>Group 2</b>	15 (62.5)	9 (37.5)	53.16 ± 9.59 (56)
<b>P value</b>	0.307		0.293

TABLE 2. OSDI, TBUT, SCHIRMER values in the first and second examinations of patients in both groups.

	OSDI mean ±SD (median)		TBUT mean ±SD (median)		SCHIRMER mean ±SD (median)	
	First examination (25 patients)	Second examination (18 patients)	First examination (50 eyes of 25 patients)	Second examination (36 eyes of 18 patients)	First examination (50 eyes of 25 patients)	Second examination (36 eyes of 18 patients)
<b>Group 1</b>	36.49 ± 17.84 (34.40)	18.65 ± 14.95 (13.50)	9.86 ± 5.79 (8.00)	13.25 ± 5.79 (12,00)	9,96 ± 7,48 (10,00)	9,24 ± 5,05 (9,50)
<b>P value</b>	0.001		0.002		0.934	
<b>Group 2</b>	First examination (24 patients)	Second examination (17 patients)	First examination (48 eyes of 24 patients)	Second examination (34 eyes of 17 patients)	First examination (48 eyes of 24 patients)	Second examination (34 eyes of 17 patients)
	44,34 ± 22,53 (44,70)	24,01 ± 16,13 (18,20)	9,02 ± 5,92 (6,50)	13,76 ± 6,58 (13,50)	8,71 ± 5,02 (7,50)	8,30 ± 5,56 (6,00)
<b>P value</b>	0.001		0.004		0.975	

### Objective Examination Findings

- A. Fluorescein tear breakup time (TBUT): Tear breakup time significantly increased in both groups ( $p_1$ : 0.002,  $p_2$ : 0.004) (Table 2).
- A. Ocular surface staining pattern: There were no significant changes in the ocular surface staining patterns (Oxford Scheme) in both groups ( $P > .05$ ) (Table 3).
- B. Schirmer's test: There were no significant changes in the Schirmer's test values in both groups ( $P > .05$ ) (Table 2).

### Histological Analyses

- A. Presence of Demodex in eyelash specimens: While there were no statistically significant post-treatment changes in the presence of Demodex in the first group, a significant decrease was detected in the second group ( $p_1$ : 0.302,  $p_2$ : 0.004) (Table 4). When additional analyses of subjects with Demodex were carried out; although there were no significant differences among the groups, the presence of Demodex persisted in one-third of the eyes in group 1, while it was one-fourth in group 2 (Table 5).
- B. Conjunctival changes (impression cytology): The number of mucopolysaccharide-secreting Goblet cells increased significantly in both groups ( $p_1$ : 0.030,  $p_2$ :

TABLE 3. Percentage of grades according to Oxford Scheme and Nelson's method in the first and second examinations of patients in both groups.

Group 1	Grades	OXFORD (%)		NELSON (%)	
		First examination (50 eyes of 25 patients)	Second examination (36 eyes of 18 patients)	First examination (50 eyes of 25 patients)	Second examination (36 eyes of 18 patients)
	0	48	75	5.6	16.7
	1	26	25	5.6	55.6
	2	26	-	44.4	22.2
	3	-	-	44.4	5.6
<b>P value</b>		-		<b>0.030</b>	

Group 2	Grades	1st examination (48 eyes of 24 patients)		2nd examination (34 eyes of 17 patients)	
		1st examination (48 eyes of 24 patients)	2nd examination (34 eyes of 17 patients)	1st examination (48 eyes of 24 patients)	2nd examination (34 eyes of 17 patients)
	0	31.3	44.1	5.9	52.9
	1	31.3	38.2	5.9	29.4
	2	29.2	14.7	41.2	11.8
	3	8.3	2.9	47.1	5.9
<b>P value</b>		0.279		<b>0.030</b>	

TABLE 4. Percentage of Demodex presence at the first and second examinations of patients in both groups.

Group 1		Presence of Demodex (%)	
		First examination (50 eyes of 25 patients)	Second examination (36 eyes of 18 patients)
	Yes	42	27.8
	No	58	72.2
<b>P value</b>		0.302	

Group 2		Presence of Demodex (%)	
		First examination (48 eyes of 24 patients)	Second examination (34 eyes of 17 patients)
	Yes	54.2	20.6
	No	45.8	79.4
<b>P value</b>		<b>0.004</b>	

0.030). The increase in Grade 0 is greater in the second group (Table 3). An example photo was taken from the impression cytology of our patients for each grade and was presented in Figure 3 to demonstrate the Nelson's method (Figure 3).

TABLE 5. Percentage of Demodex presence at the second examination of Demodex (+) eyes at the first examination.

	Presence of Demodex n (%)		Total
	+	-	
<b>Group 1</b>	5 (33.3)	10 (66.7)	15
<b>Group 2</b>	5 (26.3)	14 (73.7)	19
<b>P value</b>	0.656		

## Tear Analysis Using ELISA

IL-1 $\beta$  levels, which are indicative of the first inflammatory response, decreased significantly after the application of the gel number BP02 (p: 0.002) (Table 6). IL-6 levels, which are indicative of the first inflammatory response, decreased significantly after the application of the gel number BP02 (p: 0.050) (Table 6). TNF- $\alpha$  levels, which are indicative of the chronic inflammatory response, significantly decreased in both groups (p1: 0.001, p2: 0.001) (Table 6).

## DISCUSSION

Blepharitis is a frequently encountered chronic inflammatory condition of the eyelids, associated with ocular surface irritation, eyelid notching, dry eye development, loss of eyelashes and corneal complications. The symptoms often persist despite treatment, but routine hygiene of eyelid has been reported to be a determining factor of therapeutic efficacy of the drug therapy.<sup>19</sup>

The comparative studies of soap, shampoo versus eyelid cleansing products have revealed that both cleansing regimens improve some symptoms but the clinical evidence for eyelid cleansing products is not of the standard usually expected for pharmaceutical products. Nevertheless, at least some of these products have been studied clinically, and are preferred by patients against baby shampoo and soap.<sup>20,21</sup> In this context, TTO-containing cleansing formulations establish a useful tool for the ophthalmologist who is encouraging their patients to persist with eyelid hygiene regimens.<sup>22,23</sup> However, risks of side effects

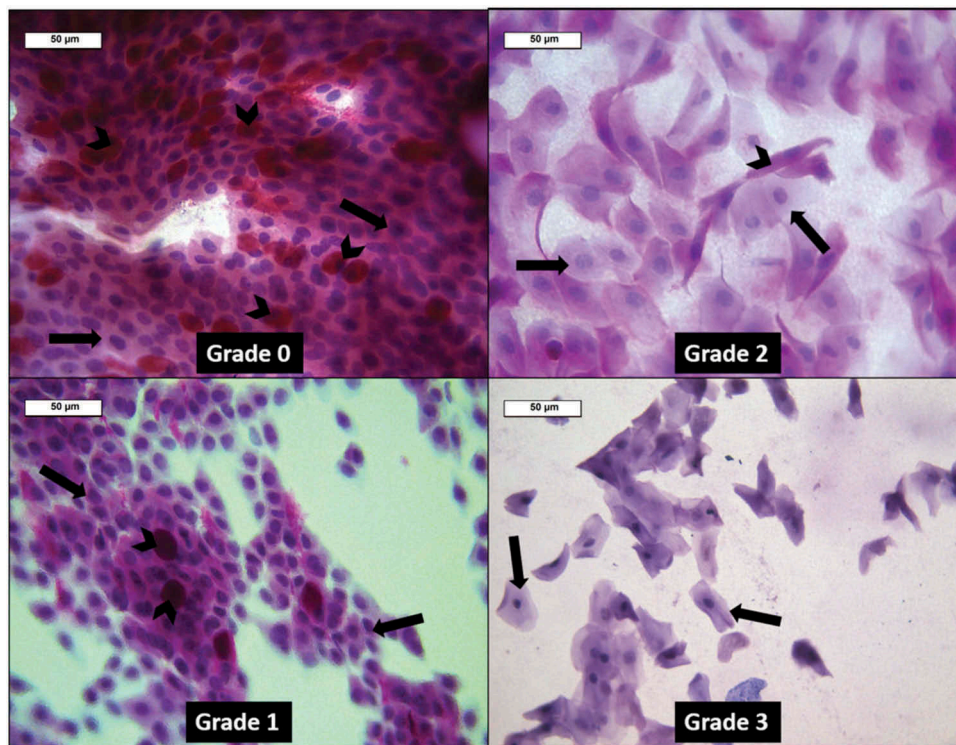


FIGURE 3. Conjunctiva impression cytology specimens of our patients and their grading according to Nelson's method (X400; PAS staining; light microscopy). The number of Goblet cells decreases from top left to bottom right, the cells transform into a polygonal shape from an oval shape, and the size of their nucleus becomes smaller. Goblet cells; Epithelial cells

TABLE 6. Tear IL-1 $\beta$ , IL-6 and TNF- $\alpha$  levels in the first and second examinations of the patients in both groups.

	IL-1 $\beta$ (pg/mL) mean $\pm$ SD (median)		IL-6 (pg/mL) Mean $\pm$ SD (median)		TNF- $\alpha$ (pg/mL) Mean $\pm$ SD (median)	
	First examination	Second examination	First examination	Second examination	First examination	Second examination
Group 1 18 patients	138.332 $\pm$ 14.647 (135.078)	128.568 $\pm$ 9.765 (125.313)	17.492 $\pm$ 8.330 (14,009)	33.394 $\pm$ 56.262 (13.933)	56.979 $\pm$ 19.181 (54.158)	42.311 $\pm$ 3.385 (42.311)
<i>P</i> value	0.038		0,831		0.001	
Group 2 17 patients	143215 $\pm$ 24.412 (136.705)	136.705 $\pm$ 24.412 (130.195)	25.670 $\pm$ 14.312 (23.096)	18.779 $\pm$ 5.831 (17,719)	50.774 $\pm$ 6.770 (53.594)	43.440 $\pm$ 5.077 (40,055)
<i>P</i> value	0.002		0.050		0.001	

of high ratio of TTO as a bioactive component in the formulations necessitate prudent clinical judgment surrounding their use and highlight the need for alternative management strategies to be developed.

In the study, the efficacy and safety of two formulations were compared; Blefar-ex *Plus* cleansing gel and basic washing gel, containing TTO as a major bioactive ingredient. It was found that, using the advanced gel formula, which also contains additional natural supplements as calendula oil, borage oil, vitamin E and vitamin B5 showed a similar clinical improvement

compared to the patients who used the basic and simple washing gel. In this study, patients who used both TTO gels enjoyed the positive effect shortly after starting the treatment with a significant improvement in OSDI and TBUT. The mean OSDI scores of both treatment groups decreased significantly suggesting that basic washing gel containing only 3% TTO is also as effective as the other formula in reducing the subjective symptoms and complaints of patients. As the objective examination findings demonstrate, TBUT also significantly increased in both groups of patients after treatments, while ocular



surface staining pattern and Schirmer's test were unchanged.

In the study, the cells on the surface of the conjunctiva were examined using impression cytology and were evaluated based on their number and morphology. The decision to take samples from the nasal conjunctiva was made based on the fact that this area has the greatest density of goblet cells.<sup>24</sup> Moreover, due to having taken the tear samples from the lateral side, the lateral side was no longer suitable for impression cytology. When the number of PAS-positive Goblet cells and the morphology of epithelial cells were evaluated, it was seen that the number of Goblet cells increased in both group 1 and group 2 after the treatment and the cell morphology improved. For example, while grade 0 (best eye grade) increased about threefold after the treatment in group 1; in group 2, this increase was about 10-fold. In summary, the increase in the number for the grades 0 and 1 after treatment; and the decrease in the number for the grades 2 and 3 after the treatment, were remarkable. The difference between pre-treatment and post-treatment values was statistically significant in both groups.

TTO is a natural oil distilled from the leaf of *Melaleuca alternifolia* that has antibacterial, antifungal, antiviral, and antiprotozoal properties in addition to anti-inflammatory effects.<sup>25</sup> The tea tree oil eyelid scrub treatment has shown to be highly effective for eliminating ocular Demodex and improving subjective ocular symptoms.<sup>26–30</sup> Gao et al. presented that TTO is effective for killing Demodex in a dose-dependant manner in vitro and in 50% concentration in vivo.<sup>30</sup> In another study; weekly lid scrubs with 50% TTO were performed in the clinic, additionally daily lid scrubs with 10% TTO were advised for 4 weeks and a significant decrease in Demodex count was reported.<sup>14</sup> Although both of these studies revealed that TTO in mentioned concentrations is effective in eradicating Demodex; in this study 3% TTO was used. It was found that there was a significant reduction in Demodex presence in patients using the advanced gel formula, while the presence of Demodex in patients using basic washing gel did not show a statistically significant change. When the eyes with Demodex are assessed, the persistence of Demodex in the second examination was one-third in group 1, while it was one-fourth in group 2. These findings suggest that the TTO concentration of the gel preparations may not be adequate for Demodex eradication alone, but the formula has achieved more reduction in Demodex count as it contains additional bioactive substances in its formulation. Calendula oil and borage oil, which have soothing properties, and Vitamin E and B5, which are known to have moisturizing properties are pre-

sent in the cleansing gel formula, and all might be synergistically interacting with 3% TTO for Demodex eradication. Terpene profile of the used oils might be synergistically effective on Demodex since terpinen-4-ol is the most active ingredient of TTO was shown to eradicate Demodex mites.<sup>11</sup> Additionally, Terpinen-4-ol also possesses anti-inflammatory properties by suppressing superoxide production and pro-inflammatory cytokines.<sup>11,31</sup> TTO was demonstrated to be associated with significant inhibition of iNOS expression, NO production and NF- $\kappa$ B activation in human macrophages.<sup>32</sup>

The natural history of blepharitis is commonly characterized by intermittent episodes of inflammatory exacerbations, and as far as we know, a limited number of studies on tear cytokine analysis in blepharitis and Demodex patients are present. To our knowledge, there were no studies in the literature in which TNF- $\alpha$ , the indicator of the chronic inflammatory response, and IL-6, which is known to increase at the intermediate stage of every type of inflammation, are evaluated before and after TTO treatment in blepharitis patients. It was found that tear TNF- $\alpha$  levels decreased after usage of both gels, while IL-6 levels were reduced significantly only in patients who used the advanced gel formula. Accordingly, previous studies showed that TTO treatment inhibits IL-1 $\beta$  and IL-17 levels in blepharitis patients.<sup>33,34</sup> It has been also reported that IL-1 $\beta$  levels are reduced by the same treatment method as by Gao et al. (Weekly lid scrubs with 50% TTO and daily lid scrubs with 10% TTO).<sup>16,34</sup> In the present study, it was discovered that IL-1 $\beta$  levels declined only by the treatment with the advanced gel formula, but patients treated with basic washing gel showed no significant decrease in the level of IL-1 $\beta$ . This finding once again supports the fact that the combined effect of the bioactive components serves more blepharitis control compared to the basic gel formulation. It is possible, since important benefits like promotion of wound healing, anti-inflammatory and antioxidative effects of topical applications of the calendula oil have been reported.<sup>35,36</sup> There is also another important constituent; Borage oil. It is derived from the seeds of the *Borago officinalis*, and contains high levels of essential fatty acids, linoleic and linolenic acids, which contribute to the therapeutic effects of borage oil.<sup>37</sup> Topical application of borage oil in infants and children with seborrheic dermatitis or atopic dermatitis has shown to normalize skin barrier function. It has also proved to reduce inflammation<sup>38</sup> and exhibit beneficial effects on the function of the skin and on the regulation of skin lipid metabolism.<sup>39,40</sup> On the other hand, the number of patients in the above mentioned two studies<sup>33,34</sup> are quite low compared to this study. Sufficient number of patients and the presence of

different parameters in this study will shed light on the other studies investigating the mechanism of the immunological response to Demodex parasite.

A recent study revealed significant differences in the concentrations of a number of cytokines and chemokines in the blepharitis group with ocular Demodex compared with the blepharitis group without ocular Demodex.<sup>33</sup> Specifically, tear concentrations of IL-7, IL-12, and IL-17 in patients with Demodex blepharitis were increased significantly ( $p < 0,05$ ), but then normalized after TTO treatment. However, mean IL-1 $\beta$  level in the Demodex blepharitis group was not significantly different from those of the Demodex free blepharitis group. The authors concluded that the infestation of Demodex mites induces secretion of IL-17, which can stimulate inflammatory or allergic reactions as well as cause mechanical blockage of follicles, which ultimately results in ocular surface damage.<sup>14,33</sup> In another study of Kim et al.; tear concentrations of IL-1 $\beta$  ( $p: 0.001$ ) and IL-17 ( $p: 0.001$ ) were significantly reduced in Demodex blepharitis patients after TTO treatment.<sup>34</sup> Collectively, these data support the inclusion of TTO and terpinen-4-ol as the most active ingredient in future formulations to treat a number of ocular diseases caused by demodicosis. In conclusion, the improvement in the findings of subjective and objective examinations observed in this study is possible due to the decreased Demodex count and/or due to the anti-inflammatory effects of the substances used in the formulations. The symptoms of the patients relieved due to the direct or indirect effect of gel formulations, yet it is unclear if the gels only help the blepharitis subjects with Demodex or if they help all blepharitis patients. The distinction can be determined through additional detailed studies in which Demodex (+) and Demodex (-) cases are investigated separately. This may seem to be the weakness of the study; however, the study design was founded on the routines of most of the outpatient clinics that do not have the opportunity for microbiological analyses. In cases where mites are embedded to the lash follicles, it is rather difficult to know whether there is Demodex or not in a patient via biomicroscopical examination. However, the clinician may suspect the possibility of Demodex in a chronic blepharitis patient who has also cylindrical dandruff surrounding the lashes. Another issue that may complicate the situation is not having seen the Demodex does not mean this is not a case of a Demodex or vice versa. Thus, this study has enrolled the chronic blepharitis cases, not the Demodex blepharitis patients.

This study has demonstrated that cleansing gel preparations including 3% TTO can improve the severity of ocular surface discomfort in blepharitis patients. The study also showed that the combination of 3% TTO with vitamins and essential plant oils

effectively reduced Demodex presence, ocular inflammation, and blepharitis severity. When compared to basic washing gel, advanced formula was thought to be more effective in improving tear quality, reducing mucinous cell damage and also maintaining epithelial integrity. In addition, none of the adverse effects reported for the existing cosmeceutical/pharmaceutical formulations containing more than 5% TTO were observed in either studied groups. The findings suggest that TTO can be effectively and safely used in blepharitis patients, and the possible side effects of TTO can be reduced by appropriate formulations including anti-inflammatory, antibacterial and antioxidant vitamins and essential oils.

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## DECLARATION OF INTEREST

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