International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2022.794.805

Research Article
Effects of Omega-3 Fatty Acid-Rich *Zanthoxylum schinifolium* Seed Oil in Patients with Dry Eye: A Pilot Randomized, Double-Blind, Placebo-Controlled Trial

[#,1,5,6]In-Chon You, [#,2,6]Su-Jin Jung, [2]Eun-Ock Park, [3,5,6]Byung-Hyun Park, [4]Mun-Yhung Jung, [2]Byung-Ok So, [3]Jin-Woo Park, [2,6]Soo-Wan Chae and [1,4,5]Nam-Chun Cho

[1]Department of Ophthalmology, Research Institute of Clinical Medicine of Jeonbuk National University, Jeonju, Korea
[2]Clinical Trial Center for Functional Foods, Jeonbuk National University Hospital, Jeonju, Korea
[3]Department of Biochemistry and Molecular Biology, Jeonbuk National University Medical School, Jeonju, Korea
[4]Department of Food Science and Biotechnology, College of Food Science, Woosuk University, Wanju, Jeonbuk, Korea
[5]Research Institute of Clinical Medicine, Jeonbuk National University, Jeonju, Korea
[6]Research Institute of Clinical Medicine of Jeonbuk National University, Biomedical Research Institute, Jeonbuk National University Hospital, Jeonju, Korea
[#]These authors contributed equally to this work as first authors

Abstract

Background and Objective: Many clinicians advise the use of supplements of ω-3 FAs, to mitigate symptoms and inflammation in patients with Dry Eye Disease (DED). However, there is little clinical evidence of the ZSO use in DED. The present study was conducted to investigate the potential effect of dietary supplementation with *Zanthoxylum schinifolium* Seed Oil (ZSO), its clinical efficacy and the safety in the patients with DED. **Materials and Methods:** Twenty subjects were randomly assigned to the ZSO (n = 10) or the placebo group (n = 10) and were given 4 g/day of ZSO or placebo (soybean oil) for 10 weeks. The outcomes such as efficacy of relieving dry eye symptoms FA composition of the Erythrocyte Membrane (EM), inflammatory and safety were assessed initially at a baseline and after 10 weeks. Improved ocular surface disease index scores and decreased corneal staining scores and tear interleukin-1β levels were observed in the ZSO group after 10 weeks compared with the baseline ($p<0.05$, respectively). **Results:** Improved Ocular Surface Disease Index (OSDI) scores and decreased corneal staining scores and tear interleukin-1β levels were observed in the ZSO group after 10 weeks compared with the baseline ($p<0.05$). After 10 weeks of ZSO supplementation, the ω-3 FA(α-linolenic acid) level of the erythrocyte membrane increased and the ω-6/ω-3 FA ratio decreased, showing a significant difference compared to the placebo group ($p<0.05$). **Conclusion:** These results suggested that the use of ZSO as a dietary source of omega-3 fatty acids might have a favorable effect on the FA composition of the EM and attenuate the symptoms in DED patients.

Key words: Dry eye disease, omega-3 fatty acids, *Zanthoxylum schinifolium* seed oil, inflammation, erythrocyte membrane

Citation: You, I.C., S.J. Jung, E.O. Park, B.H. Park and M.Y. Jung *et al.*, 2022. Effects of omega-3 fatty acid-rich *Zanthoxylum schinifolium* seed oil in patients with dry eye: A pilot randomized, double-blind, placebo-controlled trial. Int. J. Pharmacol., 18: 794-805.

Corresponding Author: Soo-Wan Chae, Jeonbuk National University Hospital, 20 Geonjiro, Jeonju, 54907, Republic of Korea Fax: 82-63-259-3060
Nam-Chun Cho, Department of Ophthalmology, Jeonbuk National University Medical School, 20, Geonjiro, Jeonju-si, Jeollabuk-do, 54907, Republic of Korea Tel: + 82-63-250-1969 Fax: 82-63-259-3060

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Approximately 15% of the population suffer from Dry Eye Disease (DED) globally. The DED is a common chronic disease that severely affects the quality of life of patients and is related to instability of the tear film and activation of the inflammatory response on the eye surface[1,2], but the exact mechanism of the condition is yet to be determined. A trend in treatment of DED is that direct and ultimate treatments reducing inflammation of the lacrimal gland and the eye surface are preferred over passive treatments to mitigate the symptoms. In recent years, studies have been continuously published regarding the effects of supplemental ω-3 FAs in preventing and treating DED; these are Essential Fatty Acids (EFAs) that reduce drug dependence with anti-inflammatory effect[3-10]. There have been several reports on the prevention and treatment of DED by supplementation with the animal (fish)-based ω-3 FAs[3-8,10-12] and plant-based FAs[13,14]. Recently, it is reported in many systematic reviews and meta-analysis that although supplementation with ω-3 FAs and polyunsaturated FAs (PUFAs) is used for DED, it is unclear whether it improves DED[9-11,15,16]. In general, supplementation with PUFAs, which are classified as omega-3 or omega-6 PUFAs, is used in the treatment of several diseases to reduce inflammation[13,17-20]. Dietary supplementation with PUFAs is shown to affect the inflammatory pathways by shifting the balance of the omega-6 to omega-3 FA ratio (ω-6/ω-3 FA ratio), thereby altering the inflammatory components of numerous diseases[4,21]. Therefore, strategies that shift the dietary ω-6/ω-3 FA ratio toward higher levels of ω-3 FAs lower the overall inflammatory state of the body[22]. Investigations on dietary modifications or supplements are becoming popular as these investigations support ocular health and to treat eye diseases, especially macular disease[23,24]. One candidate supplement of interest is *Zanthoxylum schinifolium* Seed Oil (ZSO), the oil obtained from the seeds of *Z. schinifolium* (ZS), a deciduous shrub of the Rutaceae family that is widely grown in Korea, Japan and China. The pericarp of the dried fruits of ZS and related species are known as Chinese pepper or Sichuan pepper and the seed and the pericarp of the dried fruits of ZS have been used as a spice or medicinal agent in the Northeast Asian region since its discovery. In particular, ZSO is rich in Alpha-linolenic Acid (ALA, 28.3%), which is a precursor of Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA), key ω-3 FAs. ZSO contains various other FAs such as omega-9 FA (oleic acid: 29.3%) and omega-6 FA (linoleic acid: 25.6%)[25]. The biological functions of ZSO, including its antioxidant, anti-inflammatory[26-28], antibacterial[29], immunity-boosting[30] and anticancer[31,32] effects, have been reported in preclinical studies. However, there is little clinical evidence supporting its use in DED. Therefore, the present study was conducted to investigate the potential effects of ZSO dietary supplementation in changing the FA composition of the red blood cell membrane, its anti-inflammatory effects and its clinical efficacy in DED patients.

MATERIALS AND METHODS

Study design: This study is a 10 week, randomized, double-blind, placebo-controlled parallel group clinical trial performed according to a computer-generated randomization schedule designed to assign the subjects to the ZSO or placebo groups. A total of 20 subjects were randomly assigned (1:1) to either of the study groups (10 subjects each) using a computer-generated random number, table generated by the randomization program of SAS, version 9.4 (SAS Institute, Cary, NC, USA).

Subjects: Study subjects were recruited and selected from the Clinical Trials Center for Functional Foods (CTCF2) at Jeonbuk National University Hospital (JBUH) from February-July, 2015. All subjects gave written informed consent before their enrollment in the study. The Helsinki Declaration guidelines were applied in this study and the JBUH Institutional Review Board (IRB) for Functional Foods approved all the proceedings (approval No. 2014-01-002); this approval was subsequently transferred to the JBUH IRB. This clinical trial was registered at ClinicalTrials.gov (www.clinicaltrials.gov) under NCT02802150. The criteria for the selection and exclusion of participants in this study are described below.

The inclusion criteria were as follows:

- Male and female patients aged between 20 and 70 and having DED
- Low Tear-film Break-up Time (TBUT) (<10 sec) or low Schirmer score (<10 mm for 5 min upon application of a local anesthetic) or presence of corneal and conjunctival damage at the time of screening
- Blood triglyceride (TG) levels greater than 150 mg dL^{-1} or High Density Lipoprotein (HDL) cholesterol levels <40 mg dL^{-1} (for men) or 50 mg dL^{-1} (for women)

The exclusion criteria were as follows:

- Current treatment with anti-inflammatory eye drops for dry eye (topical steroid and topical cyclosporine)
- Use of a cholesterol-lowering medication within 3 months of study entry

- Individuals with a history of chronic disease
- A clinically significant illness that in the opinion of the investigators could risk the patient's safety or influence the interpretation of results
- Participation in any clinical trial using an investigative medication within 2 months prior to the administration of the 1st dose in the present study
- Known allergy or hypersensitivity to any of the ingredients in the test products
- Pregnancy or breastfeeding
- History of alcoholism or drug abuse
- History of medical or psychological conditions that the investigators deemed would interfere with successful participation in the study

Participants who responded and met the entry criteria as determined during a telephone screening interview were scheduled for a baseline visit.

Test supplements: The ZS is deposited as a raw food material in the database of the Ministry of Food and Drug Safety (MFDS) of Korea. ZS was obtained at Kurye market (Kurye, Chonnam, Korea) in October, 2013. After removing ZS skin, only the ZS seeds were selected and washed and then dried in the shade to remove moisture for 5 days. ZS seeds were crushed with a grinder, steamed for 2 min using boiling water steam and steamed ZO was pressed in a milking machine preheated to 95 for 30 min and filtering to obtain ZSO (essential oil) (Goldpress, National Engineering Co., Goyang, Korea). The yield rate of ZSO is 34%.

Soybean Oil (SBO) was used as a placebo and was obtained commercially (Haepyo soybean oil, Sajo Haepyo Co., Seoul, Korea). The daily intake of ω-3 FA (DHA+ EPA), the raw material of MFDS's functional foods, ranged from 0.5-2 g. It was calculated by applying the amount of linolenic acid (18:3 n-3) of ZSO that can be a precursor of ω-3 FA among the ingredients contained in ZSO (test product for human application) to the intake range of ω-3 FA, a functional ingredient. At this time, it was in the range of 1.76-7.06 g/day as ZSO. Therefore, the daily intake dose of Sancho in this study was set to 4 g/day. During the 10 week intervention period, test materials (ZSO or SBO) were supplied as prepackaged 2 g doses to be administered at approximately the same time every morning and evening after meals (for a total of 4 g/day). The ZSO and the placebo materials were supplied in identical containers for masking purposes. Sequentially numbered containers were provided to participants in a double-blinded manner.

Gas chromatography analysis of the FAs of test supplements: Both ZSO and SBO were extracted and methylated prior to analysis. The resulting FA Methyl Ester (FAME) samples (1 µL each) were injected in splitless mode into a gas chromatograph equipped with a flame ionization detector (GC 2010; Shimadzu, Tokyo, Japan)[33,34]. The column used in this study was a highly polar cyanopropyl siloxane-phase fused silica capillary GC column (SP2560; 100 m×0.25 mm I.D.; Supelco Inc., Bellefonte, PA, USA). Helium was used as the carrier gas with a head pressure of 300 kPa. The injector and detector temperatures were 230 and 260°C, respectively. The oven temperature was ramped as follows: Initial oven temperature of 60°C for 5 min, increased to 175°C at 10°C min^{-1}, increased to 230°C at 2°C min^{-1} and then maintained at 230°C for 20 min. Peaks were identified by comparison with FAME standards and the integrated peak area of each resolved peak was used to calculate the percentage of each FA. The analytical results of the test and placebo supplements used were listed in Table 1.

Outcome measurements: The efficacy and safety evaluation parameters at baseline and after 10 weeks for the assigned diet were analyzed prior to participation in this study. The primary outcome is selected to be the variations of Ocular Surface Disease Index (OSDI) score, Tear Breakup Time (TBUT), Schirmer's test score and corneal staining score at baseline, 5 and 10 weeks after the initial treatment. The secondary outcome is selected to be variations in serum and tear inflammatory marker profiles (IL-1β, IL-13 and IL-17), blood levels of antioxidant index based on malondialdehyde and oxidized LDL, blood lipid profiles [TG, Total Cholesterol (TC), High-Density Lipoprotein Cholesterol (HDL-C), Low-Density Lipoprotein Cholesterol (LDL-C)] and fatty acid composition of the erythrocyte membrane. The secondary outcome was evaluated at baseline and 10 weeks after the initial treatment.

Table 1: FA compositions of ZSO and placebo supplements (GC)

Components	Test capsule ZSO (%)	Placebo supplement SBO (%)
Palmitic (C16:0)	13.4	13.8
Palmitoleic (C16:1)	1.8	-
Stearic (C18:0)	1.6	5.7
Oleic (C18:1)	28.1	28.3
Iso-oleic (C18:1cis)	1.2	1.8
Linoleic (C18:2)	25.6	44.1
Linolenic (C18:3)	28.3	6.0
Paprika extract color	-	0.10
Gardenia blue pigment	-	0.20
Total	100	100

GC: Gas chromatography analysis

Clinical assessment of DED: The subjective symptoms of DED were graded using the OSDI score (0-100); higher scores represent greater disability[35]. TBUT and Schirmer testing were conducted according to previously described methods[36,37]. All examinations were performed by a single investigator (I.C.Y.) who was blinded to the patient's clinical information. In brief, 2 µL of a 1% fluorescein solution was instilled into the inferior palpebral conjunctiva. The interval between the last blink and the appearance of the first precorneal hypofluorescent spot, streak or other irregularity interrupting the normal homogenous fluorescein pattern was recorded as the TBUT (s). Schirmer testing was performed using a standard test strip placed in the lateral canthus for 5 min with the eyes closed. The wetting length of the strip was measured in millimeters. Corneal staining with fluorescein was scored using the Oxford Schema[38], using a range from zero (no staining) to 5 points (severe staining).

Sample preparation (extraction and methylation) for FA analysis: Red Blood Cells (RBCs) (300 µL) were collected by centrifugation of blood samples and placed in Eppendorf tubes. Purified water was added to each tube. Membrane lipids were then extracted with 1 mL of solvent mixture (chloroform:methanol 2:1, v/v) using the Folch method[39]. The chloroform layer of each sample was collected into a glass tube. This extraction step was repeated once. The collected solvent extract was washed with the same volume of purified water. Next, the solvent was removed by nitrogen flushing, after which 2 mL of H_2SO_4 solution (2% in methanol) was added to the tube. The tube was heated at 80°C for 120 min. Finally, 2 mL of saturated sodium chloride solution was added, followed by the addition of hexane to extract the FA methyl esters (FAMEs).

Biochemical analyses: Fasting blood samples were collected at the beginning and at the end of the trial to determine the efficacy parameters. Basal tear samples were collected a traumatically from the inferior tear meniscus of both eyes using 10 µL glass micropipettes as previously described[40]. Tear samples were obtained from each subject on the day of enrollment and diluted with PBS. Serum and tear cytokine levels, including interferon-γ (IFN-γ), interleukin (IL)-1β, IL-13 and IL-17 levels were measured using Luminex fluorescent bead human cytokine immunoassays (MILLIPLEX MAP; Millipore Corp., Billerica, MA, USA). Serum malondialdehyde (MDA) and oxidized Low-Density Lipoprotein (LDL) contents were measured with enzyme-linked immunosorbent assays (Mercodia, Uppsala, Sweden). Blood lipid profiles, including TC, TG, HDL-C and LDL-C levels, were analyzed using a Hitachi 7600-110 analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan).

Safety assessment: Safety parameters were based on the electrocardiogram, hematology and laboratory tests (i.e., white blood cell count, red blood cell count, hemoglobin, hematocrit, liver enzymes, platelet count, albumin, glucose, total bilirubin, BUN, creatinine, urine specific gravity and urine pH at screening and 10 weeks of intervention), blood pressure and pulse (vital signs testing) and the personal report obtained at each visit. Serious Adverse Effects (SAEs) were defined according to the harmonized tripartite guidelines of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. All biochemical analyses were performed by the clinical pathology department of JBNUH.

Measurement of dietary intake: During the 10 week study period, participants were instructed to continue their usual diets and physical activity and to not ingest any other functional foods or dietary supplements. Dietary intakes were monitored by a dietician according to data obtained from each visit to evaluate each subject's 3 days usual diet. Dietary intake analysis was conducted on this 3 days data using CAN-pro 4.0 software (Computer-Aided Nutritional Analysis Program, The Korean Nutrition Society Forum, Seoul, Korea) to estimate the average daily calorie and nutrient intake.

Statistical analysis: Statistical analyses were performed using SAS version 9.3 software (SAS Institute, Cary, NC, USA). The data were presented as Mean±Standard Deviation (SD) or median with interquartile range (25th-75th percentile), depending upon the normalcy of data distribution. Efficacy and safety parameters were analyzed within the Intention-To-Treat (ITT) group. This study was conducted as a pilot study; the minimum required sample size was estimated to be 10 subjects per group (total n = 20). Thirty-four people were recruited for the study and 20 participants were enrolled. To detect a 5.0 mm [SD: 4.0 mm] difference in the Schirmer test level or a 3 sec (SD: 2.0 s) difference in TBUT between groups with 80% power with up to 10% drop-out. A chi-squared test was performed to determine the differences between the baseline values and frequencies of categorized variables between the groups. Paired student's t-tests were used to assess the differences in continuous measures before and after the 10 weeks intervention period. A linear mixed-effects

model was applied to repeated measures for each continuous outcome variable according to the satisfaction of normality. Fixed effects included the treatment group, treatment visits and interaction between the treatment group and visits. The significance was statistically significant at the level of $p<0.05$.

RESULTS

Participant demographic characteristics and compliance: A flowchart showing enrollment, allocation, follow-up and the analysis of participants was given in Fig. 1. The general characteristics of the study participants were shown in Table 2. No significant differences in age, sex, weight or height were observed between the treatment arms at baseline. All participants completed the 10 weeks study and no moderate or serious AEs were reported during the study period. Concerning to adverse reactions, 5 cases of slight abnormalities occurred in 5 subjects who ingested placebo among the total 20 subjects {cold (2), flu (1), skin damage (1), indigestion (1)}; however, this difference was not statistically significant ($p>0.05$). The compliance rates, based on capsule count, were 92.8±10.6 and 90.9±9.4% in the placebo and ZSO groups, respectively and these were not significantly different ($p>0.05$).

Change in the RBC membrane FA composition: The compliance of the participants was confirmed by measuring the change in the RBC membrane FA composition. As listed in Table 3, the membrane contents of palmitic acid (C16:0, $p = 0.024$), ALA (C18:3, $p = 0.001$) and arachidonic acid (C20:4, $p = 0.036$) were significantly increased, while those of stearic acid (C18:0, $p = 0.024$) and arachidic acids (C20:0, $p = 0.018$) were significantly decreased in the zso group after the treatment period; the ω-6/ω-3 FA ratio was also decreased ($p = 0.032$). Additionally, EPA (C20:5, $p = 0.057$) and DHA (C22:6, $p = 0.057$) showed nonsignificant, but increasing trends in the ZSO group alone, suggesting endogenous conversion of ALA into these compounds. No significant changes were observed in the placebo group with the exceptions of arachidic acid ($p = 0.029$) and behenic acid

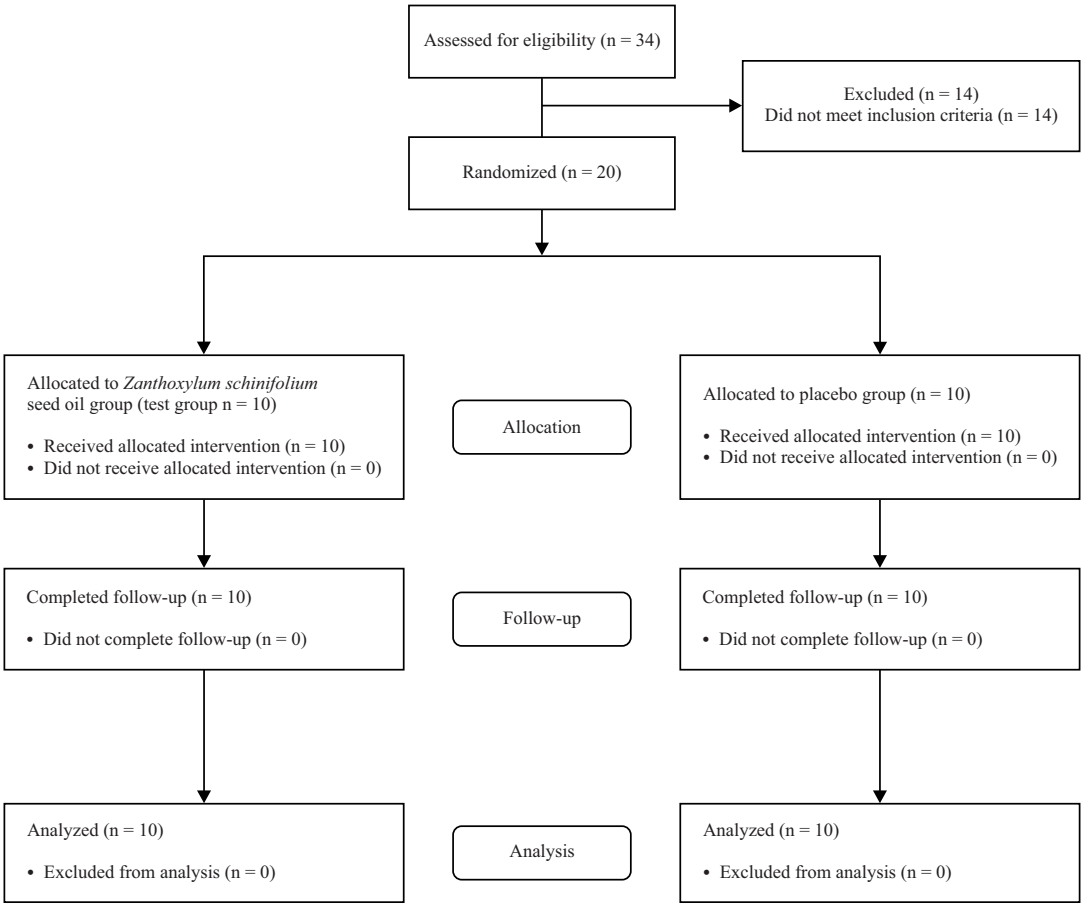

Fig. 1: Flow diagram showing participant demographic characteristics and compliance

Table 2: Baseline clinical characteristics of the participants

	Test (ZSO) group (n = 10)	Placebo (SBO) group (n = 10)	Total (n = 20)	p-value[1]
Age (years)	53.5±5.8	53.0±5.7	53.3±5.6	0.849
Sex (M/F)	1/9	3/7	4/16	0.582[2]
Height (cm)	158.5±4.2	161.6±7.1	160.1±5.9	0.250
Weight (kg)	62.1±9.2	64.3±7.9	63.2±8.4	0.572
BMI (kg m^{-2})	24.7±3.5	24.6±1.6	24.6±2.6	0.903
SBP (mmHg)	122.6±13.2	121.4±14.2	122.0±13.4	0.847
DBP (mmHg)	78.6±8.5	77.1±10.7	77.9±9.4	0.732
Pulse (BPM)	70.7±13.0	66.7±7.0	68.7±10.4	0.404
Alcohol (Y/N)	4/6	4/6	8/12	>0.999[2]
Alcohol (unit)[3]	1.3±1.6	1.3±2.0	1.3±1.7	>0.999
Smoking (Y/N)	0/10	0/10	0/20	>0.999[2]
Schirmer test (mm)	10.9±7.8	14.1±10.2	12.5±9.0	0.601
TBUT (s)	6.7±3.8	5.4±2.7	6.1±3.3	0.309
Corneal staining	0.9±0.6	0.6±0.5	0.8±0.5	0.325
OSDI (0-100 score)	42.2±14.8	41.3±11.6	41.7±12.9	0.881
Tear inflammatory				
IL-13 (pg mL^{-1})	808.6±195.9	190.1±312.5	499.3±254.2	0.337
IFN-g (pg mL^{-1})	36.5±25.8	27.4±19.0	31.9±22.5	0.380
IL-1β (pg mL^{-1})	50.3±32.2	25.9±18.1	30.1±28.4	0.055
IL-17 (pg mL^{-1})	43.0±23.8	34.9±26.8	38.9±25.0	0.486
MDA (μmol g^{-1})	2.9±0.3	3.9±2.0	3.4±1.5	0.148
Oxidized LDL	51402.6±10378.1	48521.6±14197.7	49962.1±12193.6	0.611
TC (mg dL^{-1})	205.2±38.3	188.9±27.9	197.1±33.7	0.291
TG (mg dL^{-1})	159.5±88.1	189.8±78.1	174.7±82.5	0.426
HDL-C (mg dL^{-1})	42.7±5.3	41.1±7.6	41.9±6.4	0.592
LDL-C (mg dL^{-1})	130.8±33.1	110.0±29.9	120.4±32.5	0.158

Values are presented as Means±SD or number, [1]Analyzed with an independent t-test, [2]Analyzed with a chi-squared test, [3]Alcohol: 1 unit = 10 g/12.5 mL, M: Male, F: Female, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, BPM: Beats per minute, TBUT: Tear film break-up time, OSDI: Ocular surface disease index, IL: Interleukin, IFN: Interferon, MDA: Malondialdehyde, TC: Total cholesterol, TG: Triglyceride, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol

Table 3: Fatty acid composition of erythrocyte membranes of the ZSO and placebo groups measured at baseline and 10 weeks

	Test (ZSO) group (n = 10)			Placebo (SBO) group (n = 10)			
	Baseline	10 weeks	p-value[1]	Baseline	10 weeks	p-value[1]	p-value[2]
Palmitic acid (C16:0)	26.2±0.8	27.0±1.0	0.024	27.1±2.7	26.6±1.5	0.692	0.298
Stearic acid (C18:0)	30.9±3.3	27.4±2.4	0.024	31.0±4.9	28.2±4.5	0.142	0.753
Oleic acid (C18:1)	11.1±0.9	10.9±1.2	0.619	11.0±0.8	11.3±1.3	0.452	0.380
Linoleic acid (C18:2)	11.8±3.0	12.3±1.8	0.533	10.5±2.5	11.9±2.3	0.074	0.414
α-Linolenic acid (C18:3)	0.3±0.1	0.6±0.2	0.001	0.4±0.2	0.4±0.1	0.271	0.0004
Arachidic acid (C20:0)	0.3±0.1	0.2±0.1	0.018	0.3±0.1	0.2±0.1	0.029	0.774
Arachidonic acid (C20:4)	10.5±0.9	11.7±1.4	0.036	10.4±2.4	11.3±0.8	0.255	0.728
EPA (C20:5)	2.1±0.8	2.7±0.9	0.057	2.1±0.6	2.5±0.7	0.204	0.532
Behenic acid (C22:0)	1.2±0.2	1.1±0.3	0.498	1.1±0.3	1.2±0.2	0.018	0.061
DHA (C22:6)	5.7±0.7	6.2±0.8	0.057	6.1±1.5	6.6±0.7	0.364	0.872
ω-6/ω-3 fatty acid ratio	2.8±0.6	2.5±0.5	0.032	2.5±0.6	2.5±0.5	0.262	0.048

Values are presented as Means±SDs, [1]Analyzed using the paired t-test, [2]Analyzed using the linear effects model for repeated measures, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid

(C22:0, p = 0.018). The membrane level of ALA was significantly increased, while the ω-6/ω-3 FA ratio was significantly decreased in the ZSO group than in the placebo group ($p<0.05$).

Clinical parameters of DED: Significant improvement in the OSDI score was not observed in the placebo group during the study (p = 0.061), the ZSO group contrastingly showed significant differences after follow-ups at 5 and 10 weeks (p = 0.005). The OSDI score did exhibit significant changes in either the test or placebo group during the follow-up period (p = 0.030 and p = 0.018) and significant differences were not observed between the two groups ($p>0.05$).

As listed in Table 4, Schirmer test scores and TBUTs did not exhibit significant changes in either the test or placebo group during the follow-up period (p = 0.431 and p = 0.390 vs. p = 0.685 and p = 0.268, respectively) and significant differences were not observed between the 2 groups

Table 4: Tear film and ocular surface parameters, serum and tear inflammatory levels, antioxidant marker levels and blood lipid profiles of subjects

	Test (ZSO) group (n = 10)				Placebo (SBO) group (n = 10)				
	Baseline	5 weeks	10 weeks	p-value[1]	Baseline	5 weeks	10 weeks	p-value[1]	p-value[1]
Tear film and ocular surface parameters									
Schirmer test (mm)	10.9±7.8	10.3±8.1	7.6±5.0	0.431	14.1±10.2	11.5±5.7	10.5±9.0	0.390	0.847
TBUT (s)	6.7±3.8	6.8±3.6	7.6±3.6	0.685	5.4±2.7	4.2±1.6	4.9±2.3	0.268	0.508
Corneal staining	0.9±0.6	0.6±0.5	0.2±0.4	0.014	0.6±0.5	0.3±0.5	0.4±0.5	0.387	0.121
OSDI (0-100 score)	42.2±14.8	41.4±13.2	30.4±10.6	0.005 0.030[2]	41.3±11.6	33.3±14.5	27.6±10.0	0.061 0.0.18[2]	0.515
Tear inflammatory									
IL-13 (pg mL^{-1})	808.6±195.9	-	241.0±198.9	0.384	190.1±312.5	-	171.3±306.3	0.902	0.401
IFN-g (pg mL^{-1})	36.5±25.8	-	25.2±15.5	0.143	27.4±19.0	-	23.4±7.6	0.557	0.463
IL-1β (pg mL^{-1})	50.3±32.2	-	27.1±13.2	0.031	25.9±18.1	-	23.4±7.2	0.701	0.073
IL-17 (pg mL^{-1})	43.0±23.8	-	29.5±17.6	0.079	34.9±26.8	-	25.5±8.6	0.300	0.711
Serum inflammatory									
IL-13 (pg mL^{-1})	139.2±81.1	-	132.0±64.8	0.810	160.4±91.4	-	137.9±54.2	0.543	0.742
IFN-g (pg mL^{-1})	3.6±0.5	-	3.5±0.5	0.388	3.5±0.4	-	3.3±0.5	0.275	>0.999
IL-1β (pg mL^{-1})	5.8±0.5	-	5.6±0.2	0.426	5.6±0.3	-	5.6±0.4	0.906	0.557
IL-17 (pg mL^{-1})	0.21±0.3	-	0.13±0.0	0.324	0.13±0.0	-	0.13±0.0	-	0.311
Blood levels of antioxidant markers									
MDA (μmol g^{-1})	2.9±0.3	-	3.0±0.5	0.546	3.9±2.0	-	3.8±1.6	0.306	0.226
Oxidized LDL	51402.6±10378.1 49495.5 (36715.8-64705.5)	-	46479.5±11666.3 43007.3 (33652.0-68645.4)	0.178	48521.6±14197.7 42467.8 (32272.4-81095.4)		50917.6±9665.7 50171.5 (35984.1-64831.4)	0.492	0.141
Blood lipid profile									
TC (mg dL^{-1})	205.2±38.3	-	215.0±25.7	0.244	188.9±27.9	-	212.7±26.5	0.006	0.193
TG (mg dL^{-1})	159.5±88.1	-	159.0±76.3	0.981	189.8±78.1	-	211.2±133.4	0.425	0.512
HDL-C (mg dL^{-1})	42.7±5.3	-	43.9±4.6	0.483	41.1±7.6	-	44.9±8.7	0.044	0.275
LDL-C (mg dL^{-1})	130.8±33.1	-	139.3±28.9	0.279	110.0±29.9	-	139.5±28.9	0.009	0.106

Values are presented as Means±SD or median (interquartile range), [1]Analyzed using the linear effects model for repeated measures and paired t-test, [2]p-value were obtained by paired t-test (baseline and 10 weeks), TBUT: Tear film break-up time, OSDI: Ocular surface disease index, IL: Interleukin, IFN: Interferon, MDA: Malondialdehyde, TC: Total cholesterol, TG: Triglyceride, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol

(p = 0.847 and p = 0.508, respectively). Fluorescein corneal staining scores showed a significant improvement in the test group at the end of the study period compared to the baseline scores (p = 0.014), whereas no significant change was observed in the placebo group (p = 0.387). Also, no significant differences were observed between the two groups during the follow-up period (p = 0.121).

Inflammatory markers in serum and tears: The levels of cytokines, including IL-1β, IL-13, IL-17 and IFN-g, were measured in both serum and tears after 10 weeks of supplementation. As shown in Table 4, no significant changes were observed in the serum levels. However, daily consumption of ZSO (4 g) showed a decrease in the levels of IL-1β (p = 0.031) and IL-17 (p = 0.071), which represent the inflammatory index in tears and the IL-1β level of ZSO showed a decreasing tendency in the comparison between groups (p = 0.073), although the change did not reach statistical significance in the comparison between groups. The serum IL-17 concentration was below the detection limit.

Antioxidant markers and blood lipid profile: The blood levels of antioxidant markers MDA and oxidized LDL were determined and are summarized in Table 4. No significant changes were detected in either group after 10 weeks. However, oxidized LDL levels showed a moderate decreasing trend in the ZSO group (p = 0.178) as compared with the increase noted in the placebo group. No changes in blood TG level were observed in either group during the study. In the placebo group, TC, HDL-C and LDL-C levels increased significantly (p = 0.006, p = 0.044 and p = 0.009, respectively). However, in the ZSO group, no changes were observed in any of these parameters by the end of the 10 weeks period.

Dietary intake: Dietary intake results are summarized in Supplementary Table 1. No significant dietary intake differences (calories, carbohydrates, fat and fiber) were observed between the groups during the intervention period. However, there was a statistically significant difference in protein intakes between groups (p<0.05).

Safety and adverse events: Subjects in this study showed no significant changes or differences in safety indicators such as laboratory tests, electrocardiograms, or vital signs (BP and pulse) during their participation (p>0.05). All laboratory test items were within the normal range and no side effects were Supplementary Table 2.

DISCUSSION

DED is a multifactorial disorder that affects the surface of the eye and the tear layer, causing vision impairment, discomfort and damage to the surface of the eye. In particular, DED increases osmosis of the tear layer, causing inflammation of the surface of the eyeball[41]. Based on this factor, the objective of this study was to evaluate the effects and safety of ZSO supplementation comprising a large proportion of PUFA with a balanced ratio of ω-3 FA and ω-6 FA (1:1) on inflammation, signs and symptoms of the disease in patients with DED. This study had limitations in verifying the efficacy of ZSO due to the design as a pilot study (small number of subjects).

Daily supplementation of 4 g of ZSO in DED patients for 10 weeks showed the potential to improve the degree of inflammation index; OSDI, which is a subjective symptom of DED and the objective sign of corneal staining 10 weeks after baseline. In the ZSO group, the baseline corneal staining was higher than that of the placebo group. However, there was no significant difference between the groups compared to the placebo group. During this study, the average OSDI score change in the ZSO group which had PUFA without other eye treatments and in the placebo group (SBO) decreased significantly (ZSO group: -12 and placebo: -14 points), indicating no significant difference between the groups. In particular, the average OSDI of the active group of 3 g of fish origin ω-3 FA (DHA, EPA) was similar to the placebo group (olive oil), with results of -13.9 and -12.5, respectively, showing no significant difference between the groups. Also, there was no evidence of the usefulness of ω-3 FA supplementation[16] the effects of dietary supplementation of ω-3 FA and associated improvement of symptoms of DED remain ambiguous. According to meta-analysis of 13 RCTs (from 2003 to March 2019) on DED conducted by Chi *et al.*[9], the effect of PUFAs combined with other eye treatments (oral drugs and eyewash treatments) could not be determined[16]. However, PUFA intake alone produced a significant improvement in DED in a sub-analysis[9].

In a clinical study, the fish origin ω-3 FA (DHA, EPA) is proposed to reduce symptoms and inflammation in patients with DED[3,5-8,10-12] and large quantities of ω-3 FA and ω-6 FAs as plant-based PUFA sources, 3FA (ALA) and ω-6 FAs (LA, GLA), respectively, have been confirmed to improve DED[13,14,42-46]. As reported in the preceding study, where plant-based PUFA was reported to show improvements in DED, the lack of improvement of OSDI compared to the placebo group in the

present study was probably because the compositions of ZSO and SBO were similar except for the ALA content (28.3 vs. 8.0%). In addition, linoleic acid and oleic acid compositions of ZSO were similarly high in the placebo group. That is, these factors cannot be ruled out that the verification of the biological effectiveness of ZSO for DED may represent limitations. In general, thromboxane A2, leukotriene B_4, IL-1β, IL-6, TNF_α and CRP are associated with chronic diseases and increase with increased consumption of ω-6 FAs, but decrease with increased consumption of ω-3 FAs. However, the ratio of ω-6:ω-3 FA, not just the quantitative concept of intake of ω-3 FAs, is important in expression of inflammatory response genes[17,47]. In particular, the biological activity and systemic body inflammatory state of PUFA are affected by the ratio of ω-6:ω-3 FA and the recommended EFA ratio of ω-6/ω-3 FA is to be <4:1 dietary intake[48]. Interestingly, PUFA accounted for about 85% of the FA composition of ZSO at a ω-6/ω-3 ratio of 1:1 and was far below the recommended level, while SBO showed a ratio of 7.4:1. In the current study, the omega-3 FA (ALA) level of RBC increased significantly (baseline of 0.3±01%→0.6±0.2% after 10 weeks) through ZSO supplementation and the decrease in FA ratio of ω-6/ω-3 (baseline of 2.8±0.6→2.5±0.5 after 10 weeks) was significant compared to the lack of change in the placebo group. It was reported that supplementation of omega-3[13,49] increases ω-3 FA and decreases the ratio of ω-6/ω-3 FA in RBC. The tendency of increasing EPA and DHA levels in the ZSO group suggests a metabolism from the compounds of ALA[50]. The mechanism of action of PUFA in DED is not clear, but the supplement improves DED through anti-inflammatory action that inhibits IL-1 and TNF-α generation, lipid mediators (prostaglandins, thromboxanes and leukotrienes) and arachidonic acid[51]. In support of the preceding preclinical study, the results of the present study show that ZSO supplementation has an anti-inflammatory effect[28,52] and omega-3 FA supplementation[53-56] has a similar effect in reducing corneal inflammatory and pro-inflammation cytokines.

Recently, it was reported that the ω-6:ω-3 ratio of tear lipids in DED patients showed high positive correlation with a degree of corneal staining and ω-3 deficiency in tear film lipids could cause chronic ocular surface inflammation[57]. Similar to the present study, the study of Pinazoet *et al.*[58] showed that oral intake of combinations of antioxidants and omega-3 FA in DED patients produced significant decreases in levels of IL-1β, IL-6 and IL-10 in tears. Therefore, present study results were similar to the preceding preclinical studies[55,56] and clinical study[57,58], indicating decreases in corneal staining and lL-1β cytokines of tears in the ZSO group. Thus, ZSO supplementation with balanced ω-3(ALA):ω-6 FA increases the level of omega-3 FA (ALA) in the blood and indicates that decreasing the ω-6 to ω-3 FA ratio may have a beneficial effect on a hordeolum that may help improve the overall symptoms of DED[13,22]. Furthermore, in SBO consuming placebo group, total, HDL- and LDL-cholesterol were significantly increased. However, in ZSO group, no significant change of blood lipid parameters were observed at the end of 10 weeks consumption. Triglyceride was not affected in both the groups. These results suggest that ZSO is an edible oil which can supplement dietary omega-3 FA without worsening the blood lipid profiles.

Therefore, the beneficial effect of ZSO on DED, as well as the other anti-inflammatory and metabolic effects observed in the ZSO group, may be due to both the higher omega-3 FA and essential oil content in this group[28]. Fresh ZS seed has a very high content of essential oil, up to 11% and 120 aromatic compounds have been identified, including linalool (29%), limonene (14%) and sabinene (13%) as major components[59]. These results demonstrated that blood oxidized LDL, an antioxidant marker, showed insignificant but somewhat decreasing trend only in ZSO group, suggesting the possible antioxidant potential of ZSO. The various pharmacologic effects of ZSO are usually mediated by these essential oil components. Thus, the possible beneficial effects of ZSO upon DED may actually be due to the actions of its essential oil components which require further investigation[27].

The present study has several limitations to consider. First, it was performed as a pilot study and comprised only a small number of participants. Thus, generalization of the results of this study to other subject groups must be performed carefully. Second, a follow-up of at least 1 year is required for accurate diagnosis of DED to help minimize or elucidate the effects of seasonal factors and a short period of 10 weeks in this study may be a limit upon the accuracy of evaluating DED. In addition, there is a need for studies considering changes in patient characteristics, such as age, sex, menopause, diet and DED severity. We can expect more reliable results if the number of subjects is increased in the future and a confirmatory clinical trial with a longer research period.

CONCLUSION

The results of this study suggested that ZSO is effective in treating patients with mild dry eye disease. We demonstrated that daily supplementation of ZSO has a favorable effect on the FA composition of RBC membranes and decreases inflammatory cytokines in tears without any noticeable side effects.

SIGNIFICANCE STATEMENT

This study discovers that improved Ocular Surface Disease Index (OSDI) scores and decreased corneal staining scores and tear interleukin-1β levels were observed in the ZSO group after 10 weeks compared with the baseline. After 10 weeks of ZSO supplementation, the ω-3 FA (α-linolenic acid) level of the erythrocyte membrane increased and the ω-6/ω-3 FA ratio decreased, showing a significant difference compared to the placebo group.

ACKNOWLEDGMENT

We would like to thank all the participating researchers and our study staff. This study was supported by a grant (2014-001) from the Biochemical Research Institute, Jeonbuk National University Hospital.

REFERENCES

1. Gayton, J.L., 2009. Etiology, prevalence and treatment of dry eye disease. Clin. Ophthalmol., 3: 405-412.
2. Lemp, M.A. and G.N. Foulks, 2007. The definition and classification of dry eye disease: Report of the definition and classification subcommittee of the international dry eye workshop (2007). Ocul. Surf., 5: 75-92.
3. Rand, A.L. and P.A. Asbell, 2011. Nutritional supplements for dry eye syndrome. Curr. Opin. Ophthalmol., 22: 279-282.
4. Miljanović, B., K.A. Trivedi, M.R. Dana, J.P. Gilbard, J.E. Buring and D.A. Schaumberg, 2005. Relation between dietary n-3 and n-6 fatty acids and clinically diagnosed dry eye syndrome in women. Am. J. Clin. Nutr., 82: 887-893.
5. Kangari, H., M.H. Eftekhari, S. Sardari, H. Hashemi, J. Salamzadeh, M. Ghassemi-Broumand and M. Khabazkhoob, 2013. Short-term consumption of oral omega-3 and dry eye syndrome. Ophthalmology, 120: 2191-2196.
6. Bhargava, R., P. Kumar, M. Kumar, N. Mehra and A. Mishra, 2013. A randomized controlled trial of omega-3 fatty acids in dry eye syndrome. Int. J. Ophthalmol., 6: 811-816.
7. Brignole-Baudouin, F., C. Baudouin, P. Aragona, M. Rolando and M. Labetoulle *et al*., 2011. A multicentre, double-masked, randomized, controlled trial assessing the effect of oral supplementation of omega-3 and omega-6 fatty acids on a conjunctival inflammatory marker in dry eye patients. Acta Ophthalmol., 89: e591-e597.
8. Wojtowicz, J.C., I. Butovich, E. Uchiyama, J. Aronowicz, S. Agee and J.P. McCulley, 2011. Pilot, prospective, randomized, double-masked, placebo-controlled clinical trial of an omega-3 supplement for dry eye. Cornea, 30: 308-314.
9. Chi, S.C., H.I. Tuan and Y.N. Kang, 2019. Effects of polyunsaturated fatty acids on nonspecific typical dry eye disease: A systematic review and meta-analysis of randomized clinical trials. Nutrients, Vol. 11. 10.3390/nu11050942.
10. Giannaccare, G., M. Pellegrini, S. Sebastiani, F. Bernabei and M. Roda *et al*., 2019. Efficacy of omega-3 fatty acid supplementation for treatment of dry eye disease: A meta-analysis of randomized clinical trials. Cornea, 38: 565-573.
11. Liu, A. and J. Ji, 2014. Omega-3 essential fatty acids therapy for dry eye syndrome: A meta-analysis of randomized controlled studies. Med. Sci. Monit., 20: 1583-1589.
12. Deinema, L.A., A.J. Vingrys, C.Y. Wong, D.C. Jackson, H.R. Chinnery and L.E. Downie, 2017. A randomized, double-masked, placebo-controlled clinical trial of two forms of omega-3 supplements for treating dry eye disease. Ophthalmology, 124: 43-52.
13. Macsai, M.S., 2008. The role of omega-3 dietary supplementation in blepharitis and meibomian gland dysfunction (an AOS thesis). Trans. Am. Ophthalmol. Soc., 106: 336-356.
14. Larmo, P.S., R.L. Järvinen, N.L. Setälä, B. Yang and M.H. Viitanen *et al*., 2010. Oral sea buckthorn oil attenuates tear film osmolarity and symptoms in individuals with dry eye. J. Nutr., 140: 1462-1468.
15. Molina-Leyva, I., A. Molina-Leyva and A. Bueno-Cavanillas, 2017. Efficacy of nutritional supplementation with omega-3 and omega-6 fatty acids in dry eye syndrome: A systematic review of randomized clinical trials. Acta Ophthalmol., 95: e677-e685.
16. TDEA and MSRG., 2018. N-3 fatty acid supplementation for the treatment of dry eye disease. New Engl. J. Med., 378: 1681-1690.
17. Calder, P.C., 2003. N-3 polyunsaturated fatty acids and inflammation: From molecular biology to the clinic. Lipids, 38: 343-352.
18. Kristensen, S., E.B. Schmidt, A. Schlemmer, C. Rasmussen, M.B. Johansen and J.H. Christensen, 2017. Beneficial effect of n-3 polyunsaturated fatty acids on inflammation and analgesic use in psoriatic arthritis: A randomized, double blind, placebo-controlled trial. Scand. J. Rheumatol., 47: 27-36.
19. Allaire, J., P. Couture, M. Leclerc, A. Charest and J. Marin *et al*., 2016. A randomized, crossover, head-to-head comparison of eicosapentaenoic acid and docosahexaenoic acid supplementation to reduce inflammation markers in men and women: The Comparing EPA to DHA (ComparED) Study. Am. J. Clin. Nutr., 104: 280-287.
20. Hussain, M., R.M. Shtein, M. Pistilli, M.G. Maguire, M. Oydanich and P.A. Asbell, 2020. The dry eye assessment and management (DREAM) extension study-A randomized clinical trial of withdrawal of supplementation with omega-3 fatty acid in patients with dry eye disease Ocul. Surf., 18: 47-55.

21. Simopoulos, A.P., 2000. Human requirement for N-3 polyunsaturated fatty acids. Poult. Sci., 79: 961-970.
22. Hwang, D., 2000. Fatty acids and immune responses-A new perspective in searching for clues to mechanism. Annu. Rev. Nutr., 20: 431-456.
23. Jalbert, I., 2013. Diet, nutraceuticals and the tear film. Exp. Eye Res., 117: 138-146.
24. Chiu, C.J., M.L. Chang, F.F. Zhang, T. Li, G. Gensler, M. Schleicher and A. Taylor, 2014. The relationship of major American dietary patterns to age-related macular degeneration. Am. J. Ophthalmol., 158: 118-127.e1.
25. Oh, M. and M.S. Chung, 2014. Effects of oils and essential oils from seeds of *Zanthoxylum schinifolium* against foodborne viral surrogates. Evidence-Based Complementary Alt. Med., Vol. 2014. 10.1155/2014/135797.
26. Cao, L.H., Y.J. Lee, D.G. Kang, J.S. Kim and H.S. Lee, 2009. Effect of Zanthoxylum schinifolium on TNF-α-induced vascular inflammation in human umbilical vein endothelial cells. Vasc. Pharmacol., 50: 200-207.
27. Li, R., J.J. Yang, Y.X. Shi, M. Zhao and K.L. Ji *et al*., 2014. Chemical composition, antimicrobial and anti-inflammatory activities of the essential oil from maqian (*Zanthoxylum myriacanthum* var. pubescens) in xishuangbanna, SW China. J. Ethnopharmacol., 158: 43-48.
28. Lee, J.H., K.M. Chang and G.H. Kim, 2009. Composition and anti-inflammatory activities of *Zanthoxylum schinifolium* essential oil: Suppression of inducible nitric oxide synthase, cyclooxygenase-2, cytokines and cellular adhesion. J. Sci. Food Agric., 89: 1762-1769.
29. Diao, W.R., Q.P. Hu, S.S. Feng, W.Q. Li and J.G. Xu, 2013. Chemical composition and antibacterial activity of the essential oil from green huajiao (*Zanthoxylum schinifolium*) against selected foodborne pathogens. J. Agric. Food Chem., 61: 6044-6049.
30. Seo, J.W., D.H. Park, Y.C. Li, H.J. Kang and H.D. Xu *et al*., 2012. The *Zanthoxylum schinifolium* seed oil modulates immune function under the biological safety level. Mol. Cell. Toxicol., 8: 179-185.
31. Li, W., Y.N. Sun, X.T. Yan, S.Y. Yang, E.J. Kim, H.K. Kang and Y.H. Kim, 2013. Coumarins and lignans from *Zanthoxylum schinifolium* and their anticancer activities. J. Agric. Food Chem., 61: 10730-10740.
32. Wang, C.F., L. Fan, M. Tian, X.S. Qi and J.X. Liu *et al*., 2014. Radiosensitizing effect of schinifoline from *Zanthoxylum schinifolium* sieb et zucc on human non-small cell lung cancer A549 cells: A preliminary *in vitro* investigation. Molecules, 19: 20128-20138.
33. Woo, S.J., K. Lim, S.Y. Park, M.Y. Jung and H.S. Lim *et al*., 2015. Endogenous conversion of n-6 to n-3 polyunsaturated fatty acids attenuates K/BxN serum-transfer arthritis in *fat-1* mice. J. Nutr. Biochem., 26: 713-720.
34. Jung, M.Y. and M.O. Jung, 2002. Identification of conjugated linoleic acids in hydrogenated soybean oil by silver ion-impregnated HPLC and gas chromatography-ion impacted mass spectrometry of their 4,4-dimethyloxazoline derivatives. J. Agric. Food Chem., 50: 6188-6193.
35. Schiffman, R.M., M.D. Christianson, G. Jacobsen, J.D. Hirsch and B.L. Reis, 2000. Reliability and validity of the ocular surface disease index. Arch. Ophthalmol., 118: 615-621.
36. Yoon, K.C., S.K. Im, H.G. Kim and I.C. You, 2011. Usefulness of double vital staining with 1% fluorescein and 1% lissamine green in patients with dry eye syndrome. Cornea, 30: 972-976.
37. Yoon, K.C., H. Heo, S.K. Im, I.C. You, Y.H. Kim and Y.G. Park, 2007. Comparison of autologous serum and umbilical cord serum eye drops for dry eye syndrome. Am. J. Ophthalmol., 144: 86-92.e2.
38. Bron, A.J., V.E. Evans and J.A. Smith, 2003. Grading of corneal and conjunctival staining in the context of other dry eye tests. Cornea, 22: 640-650.
39. Folch, J., M. Lees and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Bio. Chem., 226: 497-509.
40. Yoon, K.C., C.S. Park, I.C. You, H.J. Choi and K.H. Lee *et al*., 2010. Expression of CXCL9, -10, -11 and CXCR3 in the tear film and ocular surface of patients with dry eye syndrome. Invest. Opthalmol. Visual Sci., 51: 643-650.
41. Baudouin, C., M. Irkeç, E.M. Messmer, J.M. Benítez-del-Castillo and S. Bonini *et al*., 2018. Clinical impact of inflammation in dry eye disease: Proceedings of the ODISSEY group meeting. Acta Ophthalmol., 96: 111-119.
42. Wu, D., M. Meydani, L.S. Leka, Z. Nightingale, G.J. Handelman, J.B. Blumberg and S.N. Meydani, 1999. Effect of dietary supplementation with black currant seed oil on the immune response of healthy elderly subjects. Am. J. Clin. Nutr., 70: 536-543.
43. Barabino, S., M. Rolando, P. Camicione, G. Ravera, S. Zanardi, S. Giuffrida and G. Calabria, 2003. Systemic linoleic and γ-linolenic acid therapy in dry eye syndrome with an inflammatory component. Cornea, 22: 97-101.
44. Kokke, K.H., J.A. Morris and J.G. Lawrenson, 2008. Oral omega-6 essential fatty acid treatment in contact lens associated dry eye. Contact Lens Anterior Eye, 31: 141-146.
45. Aragona, P., C. Bucolo, R. Spinella, S. Giuffrida and G. Ferreri, 2005. Systemic omega-6 essential fatty acid treatment and pge1tear content in Sjögren's syndrome patients. Invest. Opthalmol. Visual Sci., 46: 4474-4479.
46. Pinna, A., P. Piccinini and F. Carta, 2007. Effect of oral linoleic and γ-linolenic acid on meibomian gland dysfunction. Cornea, 26: 260-264.
47. Simopoulos, A.P., 2006. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: Nutritional implications for chronic diseases. Biomed. Pharmacother., 60: 502-507.

48. Cortina, M.S. and H.E. Bazan, 2011. Docosahexaenoic acid, protectins and dry eye. Curr. Opin. Clin. Nutr. Metab. Care, 14: 132-137.
49. Witte, T.R., A.J. Salazar, O.F. Ballester and W.E. Hardman, 2010. RBC and EBC fatty acid composition following consumption of an omega 3 supplement: Lessons for future clinical trials. Lipids Health Dis., Vol. 9. 10.1186/1476-511x-9-31.
50. Das, U.N., 2011. Influence of polyunsaturated fatty acids and their metabolites on stem cell biology. Nutrition, 27: 21-25.
51. James, M.J., R.A. Gibson and L.G. Cleland, 2000. Dietary polyunsaturated fatty acids and inflammatory mediator production. Am. J. Clin. Nutr., 71: 343s-348s.
52. Kim, B.A., 2014. Anti-oxidant and anti-inflammatory activities of *Zanthoxylum schinifolium* essential oil. J. Korean Soc. Applied Sci. Technol. 31: 440-445.
53. Rashid, S, Y. Jin, T. Ecoiffier, S. Barabino, D.A. Schaumberg and M.R. Dana, 2008. Topical omega-3 and omega-6 fatty acids for treatment of dry eye. Arch. Ophthalmol., 126: 219-225.
54. Serhan, C.N. and N.A. Petasis, 2011. Resolvins and protectins in inflammation resolution. Chem. Rev., 111: 5922-5943.
55. Erdinest, N., H. Ovadia, R. Kormas and A. Solomon, 2014. Anti-inflammatory effects of resolvin-D1 on human corneal epithelial cells: *In vitro* study. J. Inflammation, Vol. 11. 10.1186/1476-9255-11-6.
56. Li, N., J. He, C.E. Schwartz, P. Gjorstrup and H.E.P. Bazan, 2010. Resolvin E1 improves tear production and decreases inflammation in a dry eye mouse model. J. Ocul. Pharmacol. Ther., 26: 431-439.
57. Walter, S.D., K. Gronert, A.L. McClellan, R.C. Levitt, K.D. Sarantopoulos and A. Galor, 2016. ω-3 tear film lipids correlate with clinical measures of dry eye. Invest. Opthalmol. Visual Sci., 57: 2472-2478.
58. Pinazo-duran, M., C. Galbis-Estrada, Pons-Vázquez, J. Cantu-Dibildox, C. Marco-Ramirez and F. Benitez-del-castillo, 2013. Effects of a nutraceutical formulation based on the combination of antioxidants and ω-3 essential fatty acids in the expression of inflammation and immune response mediators in tears from patients with dry eye disorders. Clin. Interventions Aging, 8: 139-148.
59. Yang, X., 2008. Aroma constituents and alkylamides of red and green huajiao (*Zanthoxylum bungeanum* and *Zanthoxylum schinifolium*). J. Agric. Food Chem., 56: 1689-1696.

Supplementary Table 1: Major nutrient intakes of the ZSO and placebo groups measured at baseline and at 10 weeks

	ZSO group (n = 10)			Placebo group (n = 10)			
Variables	Baseline	Week 10	p-value[1]	Baseline	Week 10	p-value[1]	p-value[2]
Energy (kcal)	1808.2±687.1	1613.0±415.6	0.405	1328.1±465.1	1587.5±340.4	0.142	0.116
Carbohydrates (g)	286.8±72.6	280.5±81.9	0.837	235.8±58.5	258.9±38.4	0.347	0.443
Lipids (g)	46.6±35.8	34.7±15.6	0.343	26.6±19.5	34.4±17.4	0.262	0.163
Protein (g)	69.1±33.9	55.3±18.7	0.240	43.8±19.6	64.8±28.2	0.021	0.017
Fiber (g)	27.8±9.2	24.2±7.7	0.163	21.2±9.2	19.1±6.1	0.493	0.695

Values are presented as Means±SDs, [1]Analyzed by a paired t-test, [2]Analyzed by a linear mixed model of differences between groups

Supplementary Table 2: Laboratory profiles of study subjects

	ZSO group (n = 10)			Placebo group (n = 10)			
Laboratory profiles (standard range)	Baseline	Week 10	p-value[1]	Baseline	Week 10	p-value[1]	p-value[2]
WBC (4.8-10.8×10^3 μL^{-1})	5.4±1.1	5.2±1.5	0.166	5.7±1.4	5.7±1.3	0.909	0.455
RBC (4.2-5.4×10^6 μL^{-1})	4.5±0.2	4.4±0.3	0.039	4.5±0.4	4.5±0.5	0.387	0.031
Hemoglobin (12-16 g dL^{-1})	13.5±1.1	13.3±0.9	0.118	13.5±1.3	13.8±1.6	0.061	0.014
Hematocrit (37-47%)	40.0±2.7	38.8±1.3	0.011	39.7±3.0	39.7±3.8	0.977	0.030
Platelet (130-450×10^3 μL^{-1})	260.0±35.2	256.2±31.4	0.651	259.7±57.4	265.9±58.9	0.627	0.507
ALP (45-129 IU L^{-1})	78.3±16.9	72.1±10.1	0.032	74.5±13.3	72.5±11.1	0.315	0.190
GGT (8-48 IU L^{-1})	15.5±5.9	15.6±4.8	0.985	34.5±43.7	32.2±29.2	0.678	0.663
AST (12-33 IU L^{-1})	24.7±5.3	23.4±5.0	0.579	25.0±11.7	23.3±13.4	0.561	0.913
ALT (5-35 IU L^{-1})	24.4±7.6	21.5±5.9	0.340	22.2±8.9	20.5±6.0	0.597	0.780
Total bilirubin (0.2-1.2 mg dL^{-1})	0.74±0.2	0.77±0.2	0.790	0.96±0.4	1.03±0.4	0.503	0.730
Total protein (6.7-8.3 g dL^{-1})	7.2±0.3	7.3±0.2	0.520	7.4±0.3	7.7±0.3	0.001	0.080
Albumin (3.5-5.3 g dL^{-1})	4.3±0.2	4.3±0.2	0.121	4.4±0.2	4.4±0.1	0.678	0.147
BUN (8-23 mg dL^{-1})	13.9±3.4	14.4±3.9	0.610	12.6±2.7	14.5±3.2	0.085	0.318
Creatinine (0.7-1.7 mg dL^{-1})	0.6±0.1	0.6±0.1	0.835	0.6±0.1	0.6±0.1	0.317	0.579
Glucose (74-106 mg dL^{-1})	91.1±5.9	88.3±8.3	0.178	88.9±9.5	89.8±12.6	0.650	0.189
eGER (-mL/min/L)	106.9±9.0	105.9±6.0	0.528	106.7±10.1	105.2±7.7	0.391	0.812
SG (1.005-1.030)	1.02±0.0	1.02±0.01	0.714	1.02±0.0	1.02±0.01	0.552	0.814
pH (4.5-9.0)	6.2±0.8	6.3±0.9	0.891	6.2±0.9	5.8±0.4	0.121	0.302

Values are presented as Means±SDs, [1]Analyzed by a paired t-test, [2]Analyzed by a linear mixed model of differences between groups, WBC: White blood cell, RBC: Red blood cell, ALP: Alkaline phosphatase, GGT: Gamma glutamyl transferase, AST: Aspartate transaminase, ALT: Alanine transaminase, BUN: Blood urea nitrogen, SG: Specific gravity