Original Article

Validation of the Anti-Inflammatory Effect of *Tenebrio Molitor* Larva Oil in a Colitis Mouse Model

(*Tenebrio molitor* larva oil / ulcerative colitis / dextran sodium sulphate / inflammatory cytokines / NF-κB pathway)

B. M. PARK[1], J. LEE[2], B. G. JUNG[3*], B. J. LEE[1*]

[1]Department of Veterinary Infectious Diseases, College of Veterinary Medicine, Chonnam National University, Gwangju 61186, Korea
[2]Biology Department at Morrissey College of Arts and Sciences, Boston College, Chestnut Hill, MA 02467, USA
[3]Department of Pulmonary Immunology, Center for Pulmonary and Infectious Diseases Control, University of Texas Health Science Center at Tyler, Tyler, Texas 75708, USA

Abstract. Ulcerative colitis is caused by various external factors and is an inflammatory disease that causes decreased intestinal function. *Tenebrio molitor* larvae contain more than 30 % fat, and the fat component consists of 45 % oleic acid, 20 % linoleic acid and 20 % polyunsaturated fatty acids. In this study, after administering *Tenebrio molitor* larva oil (TMLO) in a dextran sodium sulphate (DSS)-induced ulcerative colitis mouse model, the pathological findings and inflammatory markers of colitis were analysed to assess whether a colitis mitigation effect was achieved. In the TMLO-administered group, the colon length increased, the spleen weight decreased, and the body weight increased compared with that in the DSS group. In addition, the disease activity index level decreased, the mRNA expression level of inflammatory cytokines in the colon decreased, and the myeloperoxidase activity level significantly decreased. Also, the activity of the NF-κB pathway involved in the regulation of the inflammatory response was lower in the TMLO group than in the DSS group. Taken together, these results suggest that TMLO suppresses occurrence of acute ulcerative colitis in the DSS mouse model. Therefore, TMLO has the potential to be developed as a health food for the prevention and treatment of ulcerative colitis.

Received December 12, 2021. Accepted April 13, 2022.

Corresponding authors: Bong Joo Lee, Department of Veterinary Infectious Diseases, College of Veterinary Medicine, Chonnam National University, 77 Yongbong-ro, Buk-gu, Gwangju, 500-757, Korea. E-mail: bjlee@chonnam.ac.kr. Bock Gie Jung, Department of Pulmonary Immunology, Center for Pulmonary and Infectious Diseases Control, University of Texas Health Science Center at Tyler, Tyler, Texas 75708, USA. E-mail: skulljung@gmail.com

Abbreviations: DAI – disease activity index, DSS – dextran sodium sulphate, IBD – inflammatory bowel disease, IFN – interferon, IL – interleukin, MPO – myeloperoxidase, PUFA – polyunsaturated fatty acid(s), TMLO – *Tenebrio molitor* larva oil, UC – ulcerative colitis.

Introduction

Inflammatory bowel disease (IBD) causes a chronic bowel function decline and is classified into two types: Crohn's disease and ulcerative colitis (UC). It causes symptoms such as abdominal pain, diarrhoea, bloody stool, and anaemia (Abraham and Cho, 2009; Adams and Bornemann, 2013). UC is an idiopathic disease that causes continuous mucositis extending from the rectum to the proximal colon. UC has the most frequent onset between the ages of 20 and 30 and the second most frequent onset between the ages of 50 and 80. An incidence of 1.2–20.3 cases per 100,000 persons has been reported, with typical symptoms including mucinous diarrhoea and bloody stools, intermittent abdominal pain, and thickening of the intestinal wall. UC can be caused by genetic factors, environmental factors, autoimmune reactions, and intestinal microflora (Gajendran et al., 2019). Continuous exposure of the colonic environment to inflammatory mediators such as harmful substances, toxins, and bacteria causes inflammation in the intestinal tract and damages the tissues as it progresses, causing characteristic symptoms (Xavier and Podolsky, 2007). Steroid-based drugs, including mesalamine, thiopurines and methotrexate, have been used as treatments for UC. However, they inevitably have serious adverse effects (Nadpara, 2020). In patients receiving mesalamine, a representative colitis treatment, lung damage along with dry cough and mild fever has been reported (Kotsiou and Gourgoulianis, 2019). With regard to thiopurines, the response to treatment is slow, it cannot be used alone, and liver toxicity occurs when the drug is metabolized (Berends et al., 2019). Many existing drugs

have limitations in their use owing to their mild-to-serious side effects.

As food intake is part of the cause of UC (Głąbska et al., 2019), active research is being conducted to identify natural substances with anti-inflammatory effects, and various foods and additives are being considered as potential treatments for UC. Insects are selected as candidates for future foods, as they have abundant fatty acids, proteins and microelements. They are ingested in their original form or in the form of an additive (Zielińska et al., 2017). In addition, various studies have been conducted on insect resources as potential health supplements, such as studies on antioxidants, anti-inflammatory treatments using animal models, and development of medicinal substances (Nowakowski et al., 2022).

Tenebrio molitor (TM) is an edible insect found worldwide and rich in chitin; it contains 30 % fat and 70 % protein, drawing attention as a high-protein food (Zhao et al., 2016; Li et al., 2019). The fat component of TM contains 45 % oleic acid, 20 % linoleic acid and 20 % polyunsaturated fatty acids (PUFA), which have a higher composition ratio than those of animal fat. Although it is an insect oil, the ratio of omega-3 to omega-6 is similar to that of vegetable oil (Son et al., 2020). Among the studies using fatty acids extracted from insects as test substances, the oleic acid extracted from *Cryptotympana pustulata* was effective in alleviating inflammatory cell infiltration, decreasing airway hyperresponsiveness and reducing secretion of inflammatory cytokines (Kim et al., 2021). Oleic acid increases the levels of anti-inflammatory bacterial genera and effectively decreased the disease activity index (DAI), macroscopic score of colitis, inflammatory cell density, and myeloperoxidase (MPO), interleukin (IL)-17 and interferon (IFN)-γ levels in a rat colitis model (Fernández et al., 2020). Women who consume a large amount of n-3 PUFA tend to have a reduced risk of UC compared with those who do not (Ananthakrishnan et al., 2014). In addition, a number of studies have reported the anti-inflammatory effects of vegetable oils. These oils alleviate the effect of colitis by reducing the excessive expression of cytokines caused by the intake of plant and fish oils (Yorulmaz et al., 2019; Tanideh et al., 2020). In mouse models, the use of natural dietary supplements such as yarrow oil (Mohamed et al., 2021), which alleviates colitis by regulating the NF-κB and PPAR-α pathways, has been reported (Parian and Limketkai, 2016). A previous study also reported that the acute inflammatory reaction in colitis models was alleviated by administering cotton seed oil, which restored the colon length and weight and reduced expression of inflammatory cytokines in the tissues (Park et al., 2019).

However, to date, no studies have confirmed the anti-inflammatory effect of TMLO in colitis. In this study, the effect of TMLO in UC was evaluated, and the possibility of developing effective food additives for UC using TMLO was investigated. TMLO was orally administered to a mouse colitis model induced by dextran sodium sulphate (DSS). This study aimed to investigate the efficacy of TMLO in alleviating UC symptoms by comparing the gross pathological and histopathological findings and the expression levels of inflammatory cytokines and NF-κB-related proteins in the tissues of mice.

Material and Methods

Experimental animals and DSS-induced colitis

Nine-week-old male Institute of Cancer Research (ICR) mice from Damul Science (Daejeon, Korea) were used in this study, while TMLO was provided by the Korea Institute of Useful Insects (Goksong, Korea). The provided TMLO is an oil that accounts for 30 % of the TM larvae. TMLO was extracted using supercritical extraction. The oil had 36 % saturated fatty acids and 64 % unsaturated fatty acids. The major unsaturated fatty acids were oleic acid at 45.8 % and palmitic acid at 29.7 %, showing a high ratio of the two fatty acids. In this study, colitis was induced using DSS (MP Biomedicals, Solon, OH), which is widely used as an inducer in UC mouse models (Chassaing et al., 2014). The mice were randomly divided into three groups, with each group comprising five mice: DSS group (DSS + physiological saline oral administration), TMLO 50 group (DSS + TMLO 50 µl oral administration), and TMLO 100 group (DSS + TMLO 100 µl oral administration). To investigate the colitis prevention ability of TMLO, TMLO was orally administered to mice for seven days before DSS administration. The prescribed amount of TMLO was orally administered daily in each group using a needle catheter. DSS was dissolved in drinking water at a concentration of 3.5 % within seven days after TMLO administration so that it could be drunk freely (Fig. 1). Body weight was measured on the first and last days of drinking DSS, and changes in body weight were compared between the three groups. Environmental conditions were maintained at 22 ± 1 °C, with relative humidity of 50 ± 5 % and in an alternating 12-h light/dark cycle. The animal experiments in this study were approved by the Animal Experiment Ethics Committee of Chonnam National University (CNUIACUC-YB-2021-13).

Evaluation of the disease activity index score

Determination of the DAI score is a method of comprehensively scoring the degree of induction of colitis, weight change, faecal dilution, and bloody stool (Sann et al., 2013). The DAI score ranges from 0 to 4:

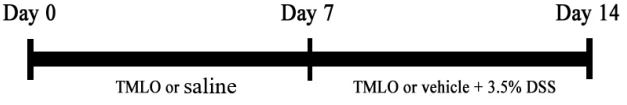

Fig 1. Overview of the experimental dietary administration DSS-induced colitis was triggered by 7 days of TMLO treatment alone followed by 7 days of TMLO treatment and administration of 3.5% DSS.

0: normal stool, no bloody stool, no anal haemorrhage, and a weight loss rate of less than 2 %;
1: slightly diluted stool, red stool, no anal haemorrhage, and a weight loss rate of 2 % or more but less than 5 %;
2: watery stools, bloody stools, anal haemorrhage, and a weight loss rate of 5 % or more but less than 9 %;
3: diarrhoea, clear red bloody stool, anal haemorrhage, and a weight loss rate of 9 % or more but less than 17 %;
4: diarrhoea, clear red bloody stool, anal haemorrhage, and a weight loss rate of 17 % or more.

Three observers calculated the average DAI score of each group.

Tissue sample collection

The mice were anesthetized via intraperitoneal injection of 2% 2,2,2-tribromoethanol (Sigma-Aldrich, St. Louis, MO). After administration of anaesthesia, the mouse was cut from the cecum to the rectum to measure its length. For histological analysis, a portion of the colon that was cut 1 cm from the rectum was collected and fixed in 10% neutral formalin (Sigma-Aldrich), while the remaining tissue was stored at −80 °C until analysis. A blood sample was collected from the hearts of anesthetized mice. The blood sample was stored at 4 °C for 24 h and then centrifuged at 5,000 × *g* at 4 °C for 15 min to separate the serum. The spleen was extracted, weighed, and stored at −80 °C until analysis.

Histology

The tissue fixed in formalin was sampled, cut to a thickness of 4 μm, dyed with haematoxylin and eosin, and assessed for the degree of colon mucosa proliferation and the histological state of the crypt using a microscope at 100× magnification. Three observers calculated the average score of the measured values for each group. The degree of tissue damage was determined using the modified version of the method used in the study by Kim et al., 2012. The method used to obtain a score was as follows: state of the crypt (normal: 0; severe damage to the crypt and loss of form: 3), degree of inflammatory cell infiltration (normal: 0; dense inflammatory infiltrate: 3), goblet cell statement (normal: 0; depleted: 1).

Myeloperoxidase content in the colon tissue

The MPO activity in the colon tissue was used as an indicator of leukocyte infiltration (Murakami et al., 2018). For MPO measurements, the mouse colon tissue was homogenized in buffer and then tested using an MPO colorimetric activity assay kit (Sigma-Aldrich) according to the manufacturer's instructions. The colons were dissected, rinsed with phosphate-buffered saline and cut into small pieces. The tissue was then homogenized. Supernatants were collected after centrifugation (12,000 × *g*, 10 min, 4 °C). The MPO activity was calculated by substituting the standard curve and measured using a Multiskan EX microplate reader (Thermo Fisher Scientific, Waltham, MA) at 540 nm. The level of MPO activity in the tissue was expressed as U/g tissue.

Determination of cytokine expression in the mouse colon

The frozen colon tissues were homogenized using a Precellys 24 homogenizer (MP Biomedicals) at 50 mg each, and RNA was extracted using a Nucleospin RNA Plus kit (Macherey-Nagel, Duren, Germany). The RNA was synthesized as cDNA using a cDNA Synthesis Master Mix (LeGene Biosciences, CA) and stored at −20 °C until analysis. The synthesized cDNA (100 ng/ul) was analysed to determine the mRNA expression levels using TOPreal™ qPCR 2X PreMIX (SYBR Green with low ROX, UDG plus) (Enzynomics, Daejeon, Korea). The primer sequences used were as follows,
IL-1β F: CAACCAACAAGTGATATTCTCCATG
IL-1β R: GATCCACACTCTCCAGCTGCA
IL-4 F: TCAACCCCCAGCTAGTTGTC
IL-4 R: TGTTCTTCGTTGCTGTGAGG
IL-6 F: CAGAGATACAAAGAAARGATGGATGCT
IL-6 R: CAGAAGACCAGAGGAAATTTTCAATA
TNF-α F: CCACGCTCTTCTGTCTACTGAACTT
TNF-α R: TGAGAGGGAGGCCATTTGG
IFN- γ F: TGGCATAGATGTGGAAGAAAAGA
IFN- γ R: TGGCATAGATGTGGAAGAAAAGAG
GAPDH F: CTGGAGAAACCTGCCAAGTA
GAPDH R: AGTGGGAGTTGCTGTTGAAG.

Western blot

The mouse colon tissue was homogenized using a Precellys 24 homogenizer (MP Biomedicals), and the proteins were extracted using radioimmunoprecipitation assay buffer (Sigma-Aldrich). The proteins were mixed with Novex™ Tris-Glycine SDS sample buffer (2×) (Thermo Fisher Scientific) and incubated at 95 °C for 5 min. The proteins were electrophoresed in 10% sodium dodecyl sulphate polyacrylamide gel. The separated proteins were transferred to a polyvinylidene transfer membrane (Thermo Fisher Scientific). The membrane was blocked with 5% skim milk in 0.05% Tris-buffered saline with Tween 20 (TBST). The membrane was incubated at 4 °C for 12 h by diluting the primary antibody in TBST at a concentration of 1 : 1,000. The antibodies used in this study were anti-PCNA antibody (Abcam, Waltham, MA), anti-NF-κB p65 antibody (Abcam), and anti-phospho-NF-κB p65 antibody (Abcam). After completion of the reaction with the primary antibody, the membrane was washed three times in TBST for 15 min and mixed with anti-rabbit IgG conjugated with HRP and anti-mouse IgG conjugated with HRP in TBST at a concentration of 1 : 10,000 and reacted for 2 h. The membrane was washed three times in TBST for 15 min and visualized using a Pierce™ ECL Western blotting substrate (Thermo Fisher Scientific) and UV. The intensity of protein bands was analysed using the ImageJ software (NIH Image, MD). PCNA was used as the loading control.

Statistical analysis

The experimental results obtained from each sample were tested for significance by conducting Duncan's multiple tests at a P value of < 0.05 using the analysis of variance performed in GraphPad Prism software version 5.0. The test results were displayed graphically as mean and standard deviation.

Results

Clinical signs, body weight change, DAI score, colon length, and spleen weight

Following DSS administration, the mice started producing loose stools after three days and showed signs of bloody stools. On the 7th day after the administration of DSS, all groups showed symptoms of bloody stools and diarrhoea. After autopsy, when the large intestine was dissected and observed visually, the degree of intestinal bleeding was relatively severe in the DSS group, while it was relatively decreased in the TMLO 50 and TMLO 100 groups (data not shown). Body weight changes were compared by measuring the weight before DSS feeding and at the end of the experiment. At the end of the experiment, the DSS group weighed 29.0 (± 3.9) g, the TMLO 50 group weighed 36.4 (± 1.1) g, and the TMLO 100 group weighed 34.9 (± 1.5) g. Compared with the values before the DSS treatment, the weight loss rates at the end of the experiment were 18 % in the DSS group, 1.1 % in the TMLO 50 group and 2.6 % in the TMLO 100 group. Compared with the DSS group, the weight loss was significantly inhibited in the TMLO 50 and TMLO 100 groups (Fig. 2A). The DAI scores were 3.7 (± 0.6) in the DSS group, 1 (± 1.2) in the TMLO 50 group and 0.7 (± 0.6) in the TMLO 100 group. Compared with the DSS group, the DAI score was significantly decreased in the TMLO 50 and TMLO 100 groups (Fig. 2B). The length of the colon removed from the mouse was measured; the average colon lengths were 5.3 (± 1.2) cm in the DSS group, 8.4 (± 1.8) cm in the TMLO 50 group and 9.2 (± 1.5) cm in the TMLO

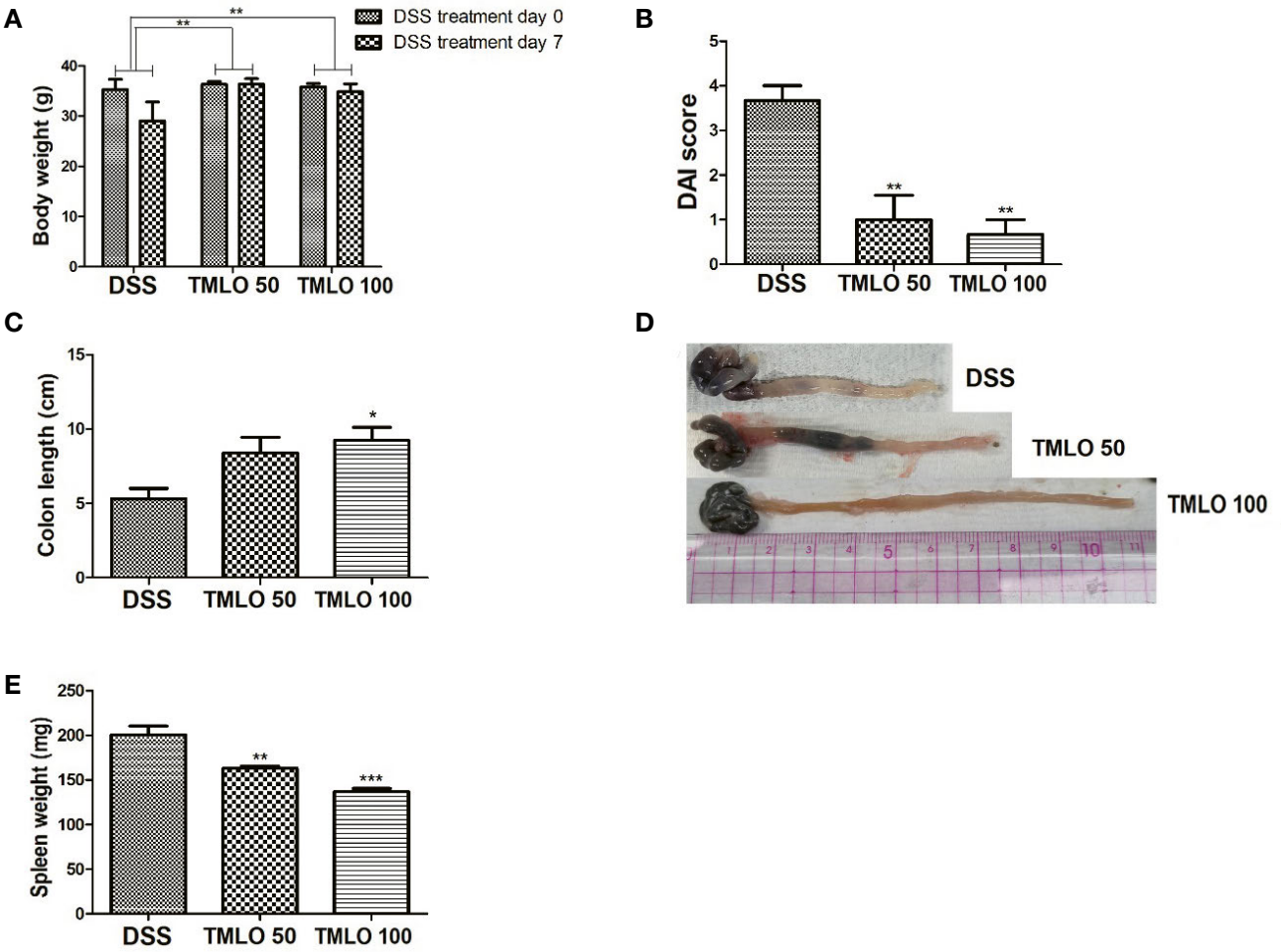

Fig. 2. Body weight, DAI score, colon length, and spleen weight in colitis-induced mouse models treated with TMLO (**A**) Mouse weight loss after treatment, (**B**) DAI score, (**C**) comparison of colon length, (**D**) representative colonic length from each group, (**E**) spleen weight. Data are presented as mean ± SD; the results of the TMLO groups are compared with those of the DSS group using Dunnett's multiple comparison test. *P < 0.05, **P < 0.05, and ***P < 0.01.

100 group. The shortening of the colon length was significantly inhibited in the TMLO 50 and TMLO 100 groups compared with that in the DSS group in a dose-dependent manner (Fig. 2C and 2D). Next, the weight of the spleen removed from the mouse was measured; the spleen weight values were 200 (± 17.2) g in the DSS group, 163 (± 4.6) g in the TMLO 50 group and 137 (± 6.3) g in the TMLO 100 group. Compared with the DSS group, the spleen weight was significantly reduced in the TMLO 50 and TMLO 100 groups (Fig. 2E).

Histology

The intestinal tissue was observed using a light microscope. The DSS group was noted to have damage to the colonic tissue, inflammatory reaction and ulceration. In the DSS group, general oedema was observed in the intestinal tissue, the epithelial layer was severely lost, and the normal shape of the crypt was no longer maintained owing to severe infiltration of inflammatory cells into the mucosal layer. Compared with the DSS group, the TMLO 50 group showed less damage with the crypt shape partially maintained, while the TMLO 100 group was able to maintain a near-normal crypt shape (Fig. 3A). Histologic scores were 3.8 (± 0.4) in the DSS group, 2.2 (± 2.0) in the TMLO 50 group and 1.9 (± 1.9) in the TMLO 100 group. Compared with the DSS group, the TMLO 50 and 100 groups showed a significant decrease in the histologic scores (Fig. 3B).

Myeloperoxidase content in the colon tissue

In this study, the MPO activity was measured to determine the degree of neutrophil tissue infiltration. The MPO levels in the mouse colon tissues were 26.9 (± 4.9) U/g in the DSS group, 23.4 (± 4.8) U/g in the TMLO 50 group and 19.1 (± 1.8) U/g in the TMLO 100 group. Compared with the DSS group, the TMLO 50 and 100 groups showed a decreasing trend in the MPO levels (Fig. 4).

Determination of cytokines in the mouse colon

The expression levels of cytokines in the colon tissue of mice were compared with that of the housekeeping gene to show the differences between the three groups. The TMLO groups showed changes in the expression levels of cytokines compared with the DSS group. The expression of IL-1β was significantly decreased in the TMLO 50 group compared with that in the DSS group (Fig. 5A) ($P < 0.05$). The expression of IL-6 was significantly decreased in the TMLO 100 group compared with that in the DSS group (Fig. 5B) ($P < 0.01$). The expression level of TNF-α was decreased in the TMLO 100 group compared with that in the DSS group, showing a significant difference (Fig. 5C) ($P < 0.05$). The expression levels of IL-4 and IFN-γ were not significantly different (Fig. 5D and 5E).

Western blot

To compare the progression of colitis through the NF-κB pathway in the colon tissue of mice, the expres-

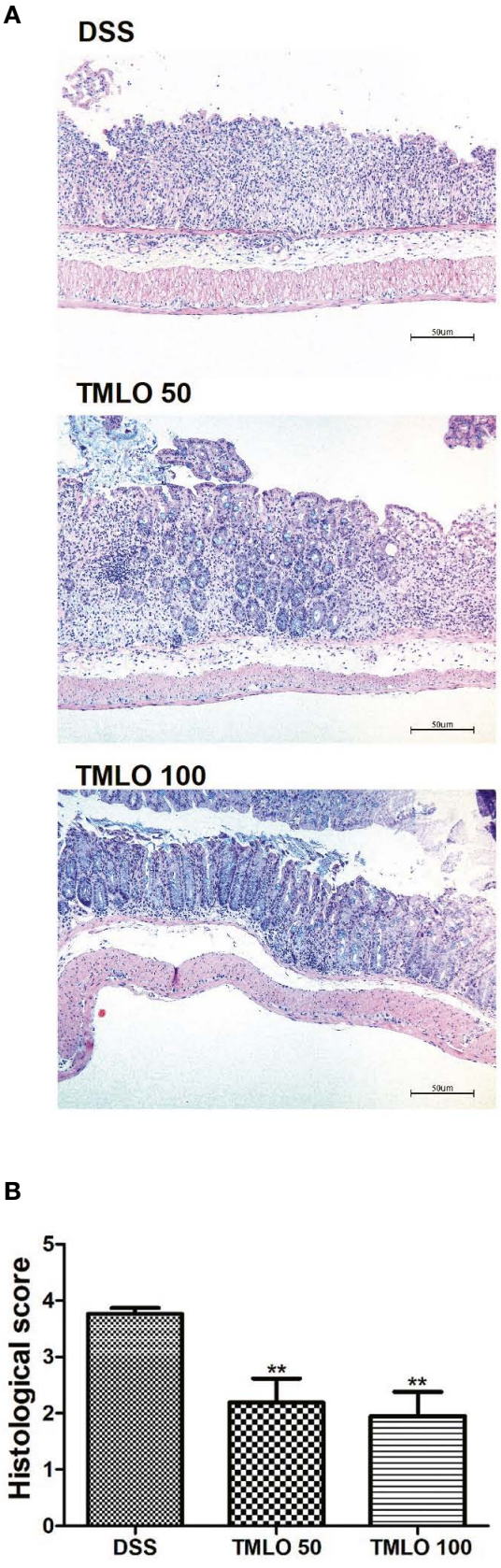

Fig. 3. Histological features and score
(**A**) Histology images showing the effects of TMLO. (**B**) Histological disease score of DSS-induced colitis in mice. Data are presented as mean ± SD; the results of the TMLO groups are compared with those of the DSS group using Dunnett's multiple comparison test. **$P < 0.01$.

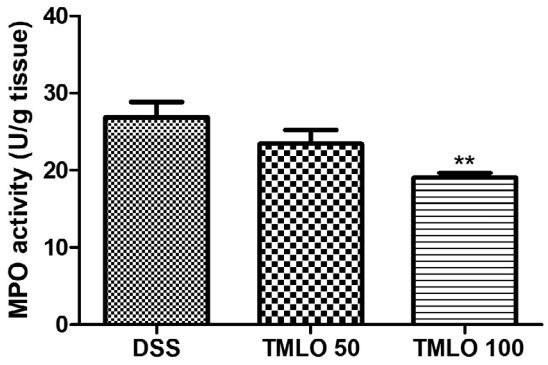

Fig. 4. Measurement of MPO in the colon of mice with DSS-induced colitis
Data are presented as mean ± SD; the results of the TMLO groups are compared with those of the DSS group using Dunnett's multiple comparison test. **P < 0.01.

sion levels of NF-κB and phospho-NF-κB proteins were analysed using Western blotting. The level of NF-κB protein expression was significantly decreased in the TMLO 50 and TMLO 100 groups compared with that in the DSS group (Fig. 6B). The level of phospho-NF-κB protein expression was significantly decreased in the TMLO 50 group compared with that in the DSS group and decreased with a high significance in the TMO 100 group (Fig. 6C).

Discussion

When the mice were fed with DSS to induce colitis, bloody stool, weight loss and colon length reduction were reported, and inflammatory cells penetrated the colonic stroma, destroying the glands and collagen fibres, causing severe oedema and damage to the cell morphology

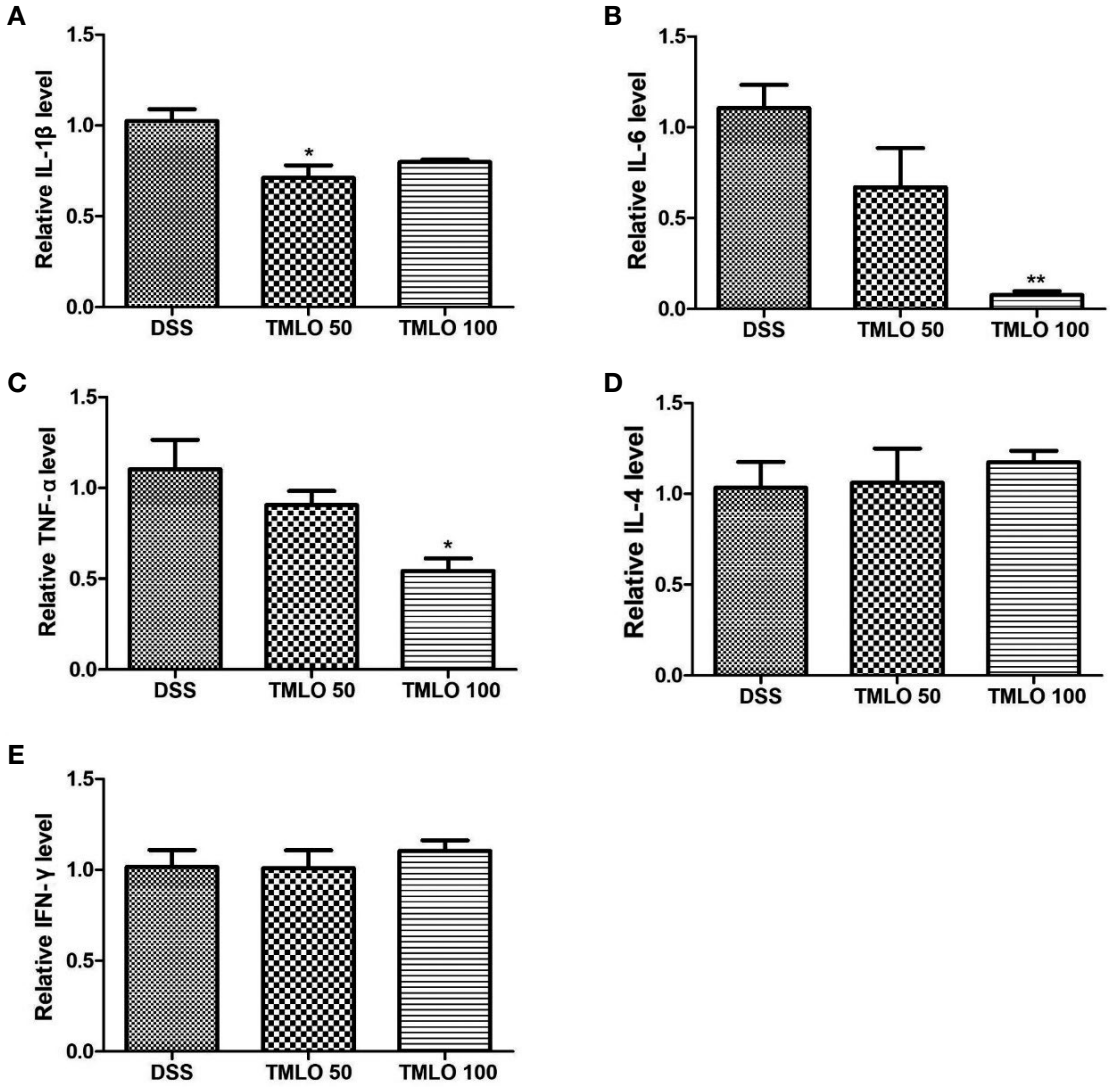

Fig. 5. Expression of cytokine mRNA during the DSS treatment in the colon
(**A**) Relative IL-1β level, (**B**) relative IL-6 level, (**C**) relative TNF-α level, (**D**) relative IL-4 level, and (**E**) relative IFN-γ level. Data are presented as mean ± SD; the results of the TMLO groups are compared with those of the DSS group using Dunnett's multiple comparison test. *P < 0.05 and **P < 0.01.

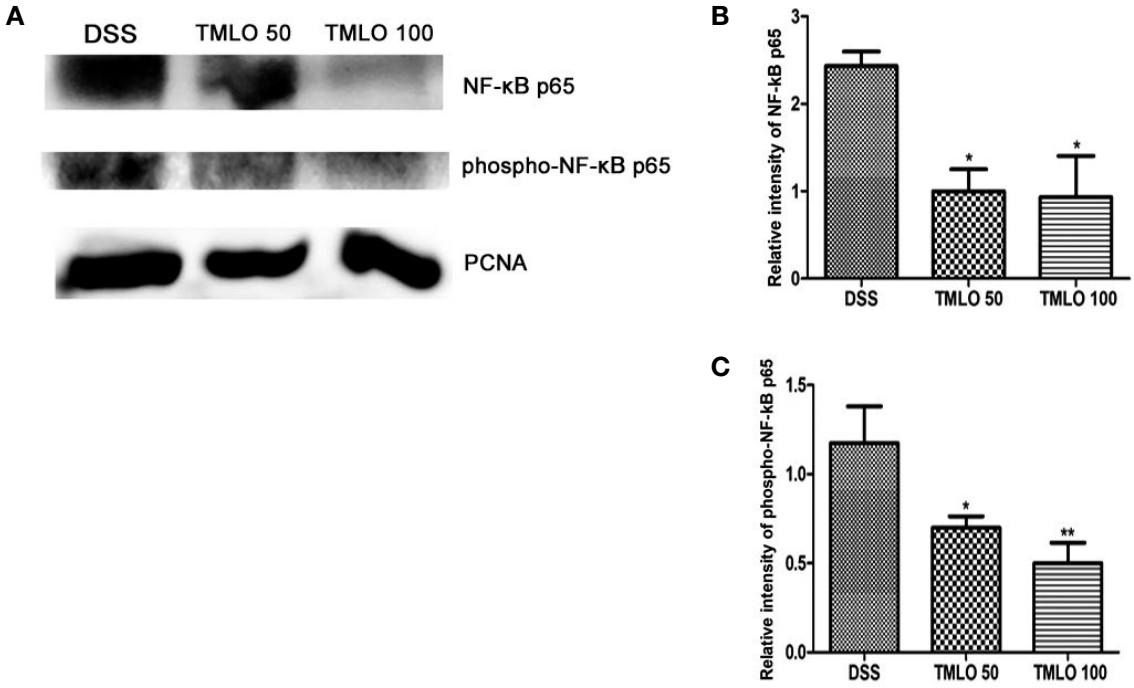

Fig. 6. Western blot analysis of activation of NF-κB during the DSS treatment in mice
(**A**) Detection of NF-κB activation. PCNA was used as the loading control. (**B**) Relative intensity of NF-κB p65. (**C**) Relative intensity of phospho-NF-κB p65. Data are presented as mean ± SD; the results of the TMLO groups are compared with those of the DSS group using Dunnett's multiple comparison test. *P < 0.05 and **P < 0.01.

(Adams and Bornemann, 2013; Yoda et al., 2014; Xu et al., 2020). In this study, the efficacy of TMLO was investigated by comparing the gross pathological findings, inflammatory cytokine expression levels, NF-κB-related protein levels in the colon tissue, and the degree of histopathological damage after administering TMLO to a DSS-induced colitis mouse model. In this study, all the DSS-fed mice demonstrated characteristic diarrhoea, bloody stools, a decrease in colon length, and a reduction in body weight, but showed an increase in the DAI score, indicating DSS-induced colitis. However, the TMLO 50 and TMLO 100 groups showed reduction in diarrhoea and bloody stools, alleviation of colon length reduction, alleviation of splenomegaly, and a decrease in the DAI score.

Various immune cells are involved in the immune responses in the large intestine, but T cells, such as Th1, Th2 and Th17, have a more significant role. Inflammation in the colonic mucosa increases expression of inflammatory chemokines in tissue capillaries and attracts lymphocytes, leading to abnormal inflammatory reactions and stimulating the innate and acquired immune systems as well as excessive secretion of cytokines such as IFN-γ, TNF, IL-1, IL-4, and IL-6 (Abraham and Cho, 2009; Blumberg, 2009). In the case of colitis caused by DSS, TNF-α, IL-6 and IL-17 produced by Th1 and Th17 cells are over-expressed in acute colitis; in the case of chronic colitis, the expression of IL-4 and IL-10 of Th2 cells is increased, while that of TNF-α, IL-6 and IL-17 is decreased (Cooper et al., 1993; Alex et al., 2009; Geremia et al., 2014). In this study, the expression levels of TNF-α, IL-1β and IL-6, which were increased in the DSS group, decreased in the colon tissues of the TMLO 50 and TMLO 100 groups, indicating that the treatment with TMLO alleviated acute colitis in the mice. These results are similar to those reported in other studies. Son et al. (2020) suggested that TM larva powder has potential antioxidant and anti-inflammatory effects owing to the reduced NO production of taurine, polyphenol, chitosan, glucosamine, and unsaponifiable lipids in TM oil.

MPO is a peroxidase that is mainly found in neutrophils and monocytes. MPO is associated with inflammatory diseases, as it is a mediator of various inflammatory injuries and diseases, and its levels increase when oxidative stress increases (Aratani, 2018; Ndrepepa, 2019). Inflammatory disease in tissues leads to neutrophil infiltration. MPO is an enzyme expressed in large amounts in neutrophils and is used as an indicator of inflammatory response (Krawisz et al., 1984). MPO expressed in neutrophils plays an important role in killing microorganisms, and it is a local mediator of tissue damage and various inflammatory diseases in several animal models (Aratani, 2018). In this study, the level of MPO in the colon was also significantly decreased in the TMLO 50 and TMLO 100 groups, suggesting that TMLO may alleviate tissue damage by reducing the degree of neutrophil or monocytic inflammation.

For histological analysis, in Fig. 3, the colon tissue was stained with haematoxylin and eosin. In the DSS group, the colonic epithelial cells were damaged owing to inflammation caused by DSS administration, and infiltration of inflammatory cells, proliferation of mucosal cells and damage to the crypt were evident. In the TMLO groups, the crypt form was restored and the infiltration of inflammatory cells was reduced.

NF-κB, which plays an important role in the activity of immune cells, allows expression of various immune-related genes involved in the regulation, cell proliferation and survival regulation of inflammatory responses (Yamamoto and Gaynor, 2004). When the cells are stimulated by pro-inflammatory cytokines such as TNF-α and IL-1, the NF-κB complex enters the inflammation-inducing pathway to promote acute inflammatory reactions, stimulates disease progression and generates the pathological findings of UC (Baeuerle, 1998). NF-κB exists in various dimeric forms; in most cells, it exists in an inactive state bound to the IKB protein. The inactive NF-κB complex is activated by the IKK complex. When cells are stimulated, the IKK complex phosphorylates and degrades IKB bound to the NF-κB dimer and separates and activates the NF-κB dimer from IKB. The isolated NF-κB enters the nucleus, binds to DNA, and then proceeds with gene transcription. It promotes the inflammatory response by activating chemokines, cytokines, tumour induction, and inflammatory mediators through gene transcriptional activity (Makarov, 2000; Lawrence, 2009; Liang et al., 2018; Zusso et al., 2019). In this study, the expression levels of NF-κB and phospho-NF-κB in mouse colon tissues were measured by Western blotting. The expression of NF-κB and phospho-NF-κB was decreased in the TMLO group compared with that in the DSS group. This finding shows that the administration of TMLO inhibits the expression of NF-κB and the phosphorylation activity of NF-κB, thereby preventing initiation of the intestinal inflammatory response caused by the DSS feeding and inhibiting progression by triggering an excessive inflammatory response.

In this study, we confirmed our initial hypothesis that administration of TMLO to a DSS-induced colitis mouse model alleviated the decrease in the colon length in the TMLO 100 group, the spleen enlargement and weight loss in the TMLO 50 and 100 groups; reduced the frequency of diarrhoea and bloody stool; and improved the histological findings in the TMLO 50 and 100 groups. In addition, significant reductions in MPO in the TMLO 50 group, TNF-α (TMLO 100), IL-1β (TMLO 50) and IL-6 (TMLO 100), and reduction in the inflammatory responses through inhibition of the NF-κB pathway activity were confirmed in the TMLO 50 and 100 groups. The present study was the first to report that TMLO administration markedly suppressed the symptoms of acute UC in a DSS mouse model, suggesting that TMLO can be effective for the treatment of UC. Therefore, TMLO could be developed as a health food for the prevention and treatment of acute UC.

Acknowledgments

We would like to thank Editage (www.editage.co.kr) for English language editing.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

B. M. Park and J. Lee equally contributed to this work.

References

Abraham, C., Cho, J. H. (2009) Inflammatory bowel disease. *N. Engl. J. Med.* **361**, 2066-2078.

Adams, S. M., Bornemann, P. H. (2013) Ulcerative colitis. *Am. Fam. Physician.* **87**, 699-705.

Alex, P., Zachos, N. C., Nguyen, T., Gonzales, L., Chen, T. E., Conklin, L. S., Centola, M., Li, X. (2009) Distinct cytokine patterns identified from multiplex profiles of murine DSS and TNBS-induced colitis. *Inflamm. Bowel Dis.* **15**, 341-352.

Ananthakrishnan, A. N., Konijeti, H. K. G. G., Higuchi, L. M., Silva, P. D., Fuchs, C. S., Willett, W. C., Richter, J. M., Chan, A. T. (2014) Long-term intake of dietary fat and risk of ulcerative colitis and Crohn's disease. *Gut* **63**, 776-784.

Aratani, Y. (2018) Myeloperoxidase: its role for host defense, inflammation, and neutrophil function. *Arch. Biochem. Biophys.* **640**, 47-52.

Baeuerle, P. A. (1998) Pro-inflammatory signaling: last pieces in the NF-κB puzzle? *Curr. Biol.* **8**, 19-22.

Berends, S. E., Strik, A. S., Löwenberg, M., D'Haens, G. R., Mathôt, R. A. A. (2019) Clinical pharmacokinetic and pharmacodynamic considerations in the treatment of ulcerative colitis. *Clin. Pharmacokinet.* **58**, 15-37.

Blumberg, R. S. (2009) Inflammation in the intestinal tract: pathogenesis and treatment. *Dig. Dis.* **27**, 455-464.

Chassaing, B., Aitken, J. D., Malleshappa, M., Kumar, M. V. (2014) Dextran sulfate sodium (DSS)-induced colitis in mice. *Curr. Protoc. Immunol.* **104**, 15.25.1-15.25.14.

Cooper, H. S., Murthy, S. N., Shah, R. S., Sedergran, D. J. (1993) Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab. Invest.* **69**, 238-249.

Fernández, J., Fuente, V. G. D. L., García, M. T. F., Sánchez, J. G., Redondo, B. I., Villar, C. J., Lomb, F. (2020) A diet based on cured acorn-fed ham with oleic acid content promotes anti-inflammatory gut microbiota and prevents ulcerative colitis in an animal model. *Lipids Health Dis.* **19**, 28.

Gajendran, M., Loganathan, P., Jimenez, G., Catinella, A. P., Ng, N., Umapathy, C., Ziade, N., Hashash, J. G. (2019) A comprehensive review and update on ulcerative colitis. *Dis. Mon.* **65**, 1-37.

Geremia, A., Biancheri, P., Allan, P., Corazza, G. R., Sabatino, A. D. (2014) Innate and adaptive immunity in inflammatory bowel disease. *Autoimmun. Rev.* **13**, 3-10.

Głąbska, D., Guzek, D., Lech, G. (2019) Analysis of the nutrients and food products intake of Polish males with ulcerative colitis in remission. *Nutrients* **11**, 1-12.

Kim, J. J., Shajib, M. D. S., Manocha, M. M., Khan, W. I. (2012) Investigating intestinal inflammation in DSS-induced model of IBD. *J. Vis. Exp.* **1**, 3678.

Kim, S. H., Hong, J. H., Yang, W. K., Kim, H. J., An, H. J., Lee, Y. C. (2021) Cryptotympana pustulata extract and its main active component, oleic acid, inhibit ovalbumin-induced allergic airway inflammation through inhibition of Th2/GATA-3 and interleukin-17/RORγt signaling pathways in asthmatic mice. *Molecules* **26**, 1854.

Kotsiou, O. S., Gourgoulianis, K. I. (2019) A case report of mesalazine-induced lung injury: a reversible drug side effect. *Respir. Med. Case Rep.* **27**, 100865.

Krawisz, J., Sharon, P., Stenson, W. F. (1984) Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. *Gastroenterology* **87**, 1344-1350.

Lawrence, T. (2009) The nuclear factor NF-κB pathway in inflammation. *Cold Spring Harb. Perspect. Biol.* **1**, a001651.

Li, N., Xiong, X., Ha, X., Wei, X. (2019) Comparative preservation effect of water-soluble and insoluble chitosan from *Tenebrio molitor* waste. *Int. J. Biol. Macromol.* **133**, 165-171.

Liang, H., Yang, H., Liu, C., Sun, Z., Wang, X. (2018) Effect of NF-kB signaling pathway on the expression of MIF, TNF-α, IL-6 in the regulation of intervertebral disc degeneration. *J. Musculoskelet. Neuronal Interact.* **18**, 551-556.

Makarov, S. S. (2000) NF-κB as a therapeutic target in chronic inflammation: recent advances. *Mol. Med. Today* **6**, 441-448.

Mohamed, M. E., Elsayed, S. A., Madkor, H. R., Eldien, H. M. S., Mohafez, O. M. (2021) Yarrow oil ameliorates ulcerative colitis in mice model via regulating the NF-κB and PPAR-γ pathways. *Intest. Res.* **19**, 194-205.

Murakami, S., Tasaka, Y., Takatori, S., Tanaka, A., Kawasaki, H., Araki, H. (2018) Effect of Eucommia ulmoides leaf extract on chronic dextran sodium sulfate-induced colitis in mice. *Biol. Pharm. Bull.* **41**, 864-868.

Nadpara, N., Reichenbach, Z. W., Ehrlich, A. C., Friedenberg, F. (2020) Current status of medical therapy for inflammatory bowel disease: the wealth of medications. *Dig. Dis. Sci.* **65**, 2769-2779.

Ndrepepa, G. (2019) Myeloperoxidase – a bridge linking inflammation and oxidative stress with cardiovascular disease. *Clin. Chim. Acta* **493**, 36-51.

Nowakowski, A. C., Miller, A. C., Miller, M. E., Xiao, H., Wu, X. (2022) Potential health benefits of edible insects. *Crit. Rev. Food Sci. Nutr.* **62**, 3499-3508.

Parian, A., Limketkai, B. N. (2016) Dietary supplement therapies for inflammatory bowel disease: Crohn's disease and ulcerative colitis. *Curr. Pharm. Des.* **22**, 180-188.

Park, J. S., Choi, J. W., Hwang, S. H., Kim, J. K., Kim, E. K., Lee, S. Y., Lee, B. I., Park, S. H., Cho, M. L. (2019) Cottonseed oil protects against intestinal inflammation in dextran sodium sulfate-induced inflammatory bowel disease. *J. Med. Food* **22**, 672-679.

Sann, H., Erichsen, J. V., Hessmann, M., Pahl, A., Hoffmeyer, A. (2013) Efficacy of drugs used in the treatment of IBD and combinations thereof in acute DSS-induced colitis in mice. *Life Sci.* **92**, 708-718.

Son, Y. J., Choi, S. Y., Hwang, I. K., Nho, C. W., Kim, S. H. (2020) Could defatted mealworm (*Tenebrio molitor*) and mealworm oil be used as food ingredients? *Foods* **9**, 40-53.

Tanideh, N., Sadeghi, F., Amanat, S., Firoozi, D., Noorafshan, A., Iraji, A., Koohi-Hosseinabadi, O. (2020) Protection by pure and genistein fortified extra virgin olive oil, canola oil, and rice bran oil against acetic acid-induced ulcerative colitis in rats. *Food Funct.* **11**, 860-870.

Xavier, R. J., Podolsky, D. K. (2007) Unravelling the pathogenesis of inflammatory bowel disease. *Nature* **448**, 427-434.

Xu, X., Lin, S., Yang, Y., Gong, X., Tong, J., Li, K., Li, Y. (2020) Histological and ultrastructural changes of the colon in dextran sodium sulfate-induced mouse colitis. *Exp. Ther. Med.* **20**, 1987-1994.

Yamamoto, Y., Gaynor, R. B. (2004) IκB kinases: key regulators of the NF-κB pathway. *Trends Biochem. Sci.* **29**, 72-79.

Yoda, K., Miyazawa, K., Hosoda, M., Hiramatsu, M., Yan, F., He, F. (2014) Lactobacillus GG-fermented milk prevents DSS-induced colitis and regulates intestinal epithelial homeostasis through activation of epidermal growth factor receptor. *Eur. J. Nutr.* **53**, 105-115.

Yorulmaz, E., Yorulmaz, H., Gökmen, E. S., Altınay, S., Küçük, S. H., Zengi, O., Çelik, D. S., Şit, D. (2019) Therapeutic effectiveness of rectally administered fish oil and mesalazine in trinitrobenzenesulfonic acid-induced colitis. *Biomed. Pharmacother.* **118**, 1-7.

Zhao, X., Gutiérrez, J. L. V., Johansson, D. P., Landberg, R., Langton, M. (2016) Yellow mealworm protein for food purposes - extraction and functional properties. *PLoS One* **11**, 1-17.

Zielińska, E., Baraniak, B., Karaś, M. (2017) Antioxidant and anti-inflammatory activities of hydrolysates and peptide fractions obtained by enzymatic hydrolysis of selected heat-treated edible insects. *Nutrients* **9**, 1-14.

Zusso, M., Lunardi, V., Franceschini, D., Pagetta, A., Lo, R., Stifani, S., Frigo, A. C., Giusti, P., Moro, S. (2019) Ciprofloxacin and levofloxacin attenuate microglia inflammatory response via TLR4/NF-kB pathway. *J. Neuroinflammation* **16**, 148-159.