Anti-Inflammatory Effects of *Aralia elata* Extract Against Dextran Sodium Sulfate-Induced Colitis in Mice and Raw 264.7 Macrophage Cells Exposed to Lipopolysaccharide: First Report

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**Abstract**

*Aralia elata* (AE) is an anti-inflammatory, polyphenolic containing medicinal plant. However, little is known about AE and its application to ulcerative colitis (UC). This study aimed to confirm AE extract’s antioxidant and anti-inflammatory effects *in vivo* and *in vitro*. The *in vitro* antioxidant activity was evaluated by measuring total polyphenol and flavonoid content in AE extract. AE extract (10 000 mg/L) contained 186.8 mg GAE/g polyphenol and 81.9 mg QE/g flavonoid. Mice were divided into 6 groups, including control, which received normal saline, and treatment groups, which received dextran sodium sulfate (DSS) with or without AE extract (250, 500, and 1000 mg/kg). RAW 264.7 macrophage cells were divided into 2 groups: control and treatment. RAW 264.7 macrophage cells treated with sterile double distilled water, 1 mg/L lipopolysaccharide (LPS), and AE extracts (25, 50, 75, 100 µg/mL) were used to assess the cytotoxicity and anti-inflammatory activity. High-performance liquid chromatography, enzyme-linked immunosorbent assay (ELISA) kits, and histology were employed to analyze the AE extract contents, nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6, as oxidative stress markers. In addition, the disease activity index (DAI) and cytotoxicity were determined in mice and cells, respectively. High-performance liquid chromatography analysis revealed that AE extract is rich in chlorogenic acid (96 ± 0.01 mg/g). DSS increased the DAI and levels of TNF-α, IL-1β, and immune cell infiltration compared with those of the control animals. Furthermore, LPS eventually reduced cell viability and increased the levels of NO, TNF-α, IL-1β, and IL-6 in contrast to control cells. After treatment, a noticeable reduction was observed in the levels of DAI, NO, TNF-α, IL-1β, and IL-6 compared to those without AE treatments. Overall, AE extract is safe and had anti-inflammatory properties. Therefore, AE extract can be considered a potential pre-treatment supplement for UC.

**Keywords**

*Aralia elata*, inflammatory bowel disease, polyphenols, TNF-α, ulcerative colitis

Received: May 16th, 2022; Accepted: August 25th, 2022.

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Introduction

The gastrointestinal tract (GIT) is constantly at risk because of exposure to numerous pathogens and toxins of food and environmental origins.1,2 Ulcerative colitis (UC) is one of the most common GIT disorders.3 UC is an inflammatory bowel disease (IBD) characterized by weight loss, severe abdominal pain, dehydration, anemia, fever, and colon ulceration. This ulceration makes the GIT unable to absorb nutrients and finally leads to bloody diarrhea and mucus.4 UC is life-threatening because once ulceration becomes severe, it might develop into colorectal cancer.5

The pathogenesis of UC involves several pro-inflammatory mediators and cytokines, for instance, tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and nitric oxide (NO).6,7 NO plays an important role in antibacterial action and tumor removal in the body, but NO produced by inducible nitric oxide synthase (iNOS) during inflammation promotes inflammatory reactions such as vascular permeability, edema, and promotes biosynthesis of inflammatory mediators to worsen inflammation.8,9 It has been reported that iNOS is involved mainly in the inflammatory response and is expressed when stimulated with lipopolysaccharide (LPS).10 LPS stimulates innate immune cells such as macrophages and neutrophils, which produce proinflammatory molecules such as IL-1 and TNF, as well as free radicals, resulting in significant sequel inflammation. These disorders result in the destruction of the ultrastructure of colon tissue, manifested by swelling of the mucosa and eruption of the epithelium.11,12 Pro-inflammatory cytokines mentioned above, and NO are important indicators of colitis, which themselves cause oxidative stress. Hence, reducing these inflammatory mediators is the role of antioxidation. RAW 264.7 macrophage cells have been proven to be activated by LPS and release NO, making them the best model for studying inflammation.13

Polyphenols are known to relieve colitis and prevent colon cancer caused by colitis.14,15 Polyphenols inhibit macrophages and reduce the expression of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6.16 Flavonoids are polyphenolic compounds that have therapeutic effects on IBDs.17 Chlorogenic acids, which can be measured using high-performance liquid chromatography (HPLC), are plant polyphenols that inhibit the synthesis of pro-inflammatory cytokines such as IL-1β.18 Chlorogenic acid functions as an antioxidant by lowering IL-1β.

Aralia elata (Miq.) Seem., family Araliaceae (AE) is a traditional medicine used for inflammatory diseases such as gastroenteritis, rheumatoid arthritis, and nephritis.19 The plant contains polyphenols, flavonoids, and chlorogenic acids.20 One of the most essential components contributing to health is polyphenolic compounds, which have a variety of biological activities such as removing free radicals, preventing oxidation, and mitigating inflammation.5,14 A. elata has various effects, such as antibacterial, anticancer,21 lipid-lowering,22 and antioxidant.23 However, to the best of our knowledge, no study underlines the effects of A. elata on a colitis animal model, and the above-mentioned indicators are expected to be effective in evaluating UC. DSS (dextran sodium sulfate) was found to produce typical UC lesions in mice.24 RAW 264.7 macrophage cells are commonly used to examine the effects of anti-inflammatory compounds.10,13 Therefore, this study aimed to analyze functional compounds in AE extract by HPLC, and to investigate the protective effects of AE extracts in mice that received DSS, and RAW 264.7 cells exposed to LPS.

Methods

Preparation of A. elata Extract

AE was purchased from a local market Sunchang-gun, Jeollabuk-do, Republic of Korea, in May 2020, and the voucher specimen (No. 14100691) was deposited at the Department of Biotechnology, Jeonbuk National University, Iksan, Republic of Korea. The plant was washed with tap water to remove foreign substances, sliced into 2 mm thick pieces, and dried at 60 °C. The dried material was pulverized with a grinder (Philips, HR3752/00), passed through a 20-mesh sieve (0.841 mm), and stored at 4 °C for further use. The dried powder (1 kg) was extracted with distilled water at 80 °C for 5 h. The extract was centrifuged for 10 min (3000×g) and filtered through filter paper (Advantec No. 2, Toyo Roshi Co., Ltd). The filtrate was then frozen and lyophilized at −80 °C using a freeze dryer (FD 8508, IlShin BioBase Co., Ltd). The resultant yield of extract was 12.8% of the dry matter. The extract was completely dissolved in distilled water and used as a stock solution.

Total Polyphenol Content

To estimate the total polyphenol content in AE extract, we adopted the method of Folin-Denis.25 In brief, 0.5 mL of 1 N Folin-Giocatleu’s reagent (Cat No. F9252, Sigma) was added to 1 mL of a diluted sample (Conc. 10 mg/mL) and mixed thoroughly. Next, 1 mL of 5% Na2CO3 (Cat. No. 222321, Sigma) was added to the mixture before incubation in the dark for 1 h, and the absorbance measured at 725 nm was measured using a spectrophotometer (Agilent, Agilent 8453). The total polyphenol content of the extract was expressed as mg gallic acid (Sigma, St. Louis) equivalents (GAE)/g.

Total Flavonoid Content

Moreno’s technique was used to determine the total flavonoid content of AE.26 Simply, a mixture was made of 0.5 mL of the extract (10 mg/mL), 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 M potassium acetate, and 4.3 mL of ethanol (Daejung Chem. and Metal Co. Ltd). The reaction mixture was incubated at room temperature for 40 min, and the absorbance measured at 415 nm. The flavonoid content was determined using the standard curve of quercetin (Sigma) (25-200 μL/mL). The extract’s total flavonoid content was reported as mg quercetin equivalents (QE)/g.
Analysis of Chlorogenic Acid in AE Extract

Chlorogenic acid in the AE extract was measured using an Agilent 1200 series system (Agilent Technologies) consisting of a vacuum degasser, quaternary pump, autosampler, DAD (diode array detector), and Agilent ZORBAX Eclipse Plus C18 column (5 μm, 250 × 4.6 mm). Samples and standard solutions were filtered through a 0.2 μm filter using HPLC-grade solvents prior to analysis. Gradient elution was conducted with solvent A; 1% formic acid (Cat. No. F0507, Sigma) in water and solvent B; methanol (Daejung Chem. and Metal Co. Ltd), as follows: (0 min) 75% A:25% B, (20 min), 50% A:50% B, (25 min), and 20% A:80% B. The HPLC injection volume was 20 μL (20 °C), and the flow rate was 1.0 mL/min. The absorption spectrum of chlorogenic acid was recorded at 325 nm.

Animal Study Design

Sixty male C57BL6/J mice (20-22 g) were obtained from Hanil Laboratory Animal Center. The animals were kept at 23 ± 1 °C, humidity 50 ± 10%, and with a 12-h light/dark cycle; feed and water were provided ad libitum. The animals were divided into 6 groups (n = 10) as follows: the control group (Cont) received normal saline orally; AE control received 1000 mg/kg of AE extract; DSS control received only DSS in drinking water; and the remaining 3 groups received DSS and 250, 500, and 1000 mg/kg AE extract, respectively. The A elata extract was dissolved in saline and given by oral gavage (100 μL) daily. The treatments continued for 14 days. UC was induced by treating the drinking water with DSS at a concentration of 2.5%, according to a previous study.11 Bodyweight was taken 3 times a week. The study design was based on earlier research, with minor modifications.10,12 The data that support the study are accessible upon reasonable request from the corresponding author. All tests were performed following international and national regulations. The study was performed in accordance with Guidelines for Ethical Conduct in the Care and Use of Animals at Jeonbuk National University Laboratory Animal Center. JBNU 2020-0134 (http://iac.honamlife.com/) is the approved number.

For sacrifice, the mice were anaesthetized with urethane (700 mg/kg) to minimize suffering. Then the blood was collected from the left ventricle. Serum was separated after centrifugation at 3000 rpm for 10 min and stored at −80 °C for further analysis. The liver, spleen, and colon were collected immediately after sacrifice. The length of the colon and the weight of the spleen were measured. Disease activity index (DAI) was evaluated based on the clinical progression of colitis, calculated by combining the degree of bloody stool, stool softness, and weight loss, according to Park’s method.25

Histopathology

The liver and colon were fixed in 4% paraformaldehyde. The samples were dehydrated, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E). The slides were then examined under a light microscope (100×).

Immunohistochemistry

The colons were fixed in 4% paraformaldehyde. Dehydrated samples were embedded in paraffin, sectioned at 5 μm, and incubated for 1 h at 60 °C. Afterwards, the samples were deparaffinized with xylene, rehydrated with ethanol, the antigen retrieved with citrate buffer, quenched with H2O2 and methanol, and a blocking solution was applied. The primary antibodies were then applied and incubated overnight at 4 °C (TNF-α, IL-1β diluted in PBS 1:500). Labelling was removed under a high-power field microscope (400×) after the application of the secondary antibody. A microscope was used to examine the slides. The free software ImageJ v.1.80 and the Color Deconvolution2 plug-in were obtained from the NIH website (https://imagej.nih.gov/ij) for immunoreactivity analysis.

Cell Line Preparation

The RAW 264.7 cells were taken from the Korean Cell Line Bank (KCLB). The cells were grown in Dulbecco’s Modified Eagle’s Medium (Cat. No. 0030034DJ) with 10% fetal bovine serum (Cat. No.10082147) and 1% penicillin/streptomycin (Cat. No. 15070063) at 37 °C with 5% CO2. The cells that were cultured in trypsin (0.025%, Cat. No. R001100) and PBS (phosphate-buffered saline, Cat. No. 28348) were isolated and used throughout the experiment. All cell culture reagents were obtained from Gibco. Cell preparation protocols were performed as reported in a previous study.12

Cytotoxicity

To assess the cytotoxicity, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Cat. No. M2128, Sigma) was used.13 Briefly, cells were seeded into a 96-well plate (1 × 105 cells/well) and treated with 100 μL of diluted A elata samples (25, 50, 75, 100, and 150 μg/mL). Following that, 0.5 mg/mL MTT was added to each well and incubated for 24 h at 37 °C. Then, the supernatants were removed, and the residue dissolved in 200 μL DMSO. The absorbance was measured at 570 nm using a microplate reader (Tecan infinite M200Pro, Männedorf).

Nitric Oxide Assay

Cells were treated with AE extract (25, 50, 75, 100, and 150 μg/mL) for 2 h and then LPS (1 μg/mL) was added and incubated for 24 h. Afterwards, 100 μL of the supernatant was mixed with 100 μL of Griess reagent, Cat No. MAK367-1KT (0.1% N-(1-naphthyl) ethylenediamine and 1% sulfanilamide 1:1) in a new 96-well plate and permitted to react for 10 min. Absorbance was measured at 570 nm using a microplate reader (Tecan infinite M200Pro, Männedorf).
Measurement of Inflammatory Mediators

The cells were treated with AE extract (25, 50, 75, 100 µg/mL) for 1 h and then exposed to LPS (1 µg/mL) for 18 h. Commercial ELISA kits were used to quantify the concentrations of tumor necrosis factor-alpha (TNF-α), Cat No. RAB0477; interleukin-1β (IL-1β), Cat No. RAB0275-1KT; and interleukin-6 (IL-6), Cat No. RAB0308-1KT, according to the manufacturer’s (Sigma) instructions. Additionally, the morphology of cells treated with LPS (1 µg/mL) and AE extract (25, 50, 100, and 150 µg/mL) was examined under an optical microscope (400x).

Statistical Analysis

The results are stated as mean ± standard error of the mean (SEM). Statistical analysis was performed using Prism 5.0 (Graphpad Software Inc.). The Bonferroni post-hoc test following one-way ANOVA was used for multiple comparisons. All tests were judged statistically significant at P-values less than .05.

Results

Analysis of AE Extract

To explore the active components of the AE extract, polyphenols, flavonoids, and chlorogenic acid were measured. The regression equations obtained indicate slight differences between the data, $y = 0.0055x - 0.0318$ ($R^2 = 0.9969$) and $y = 0.0014x + 0.0011$ ($R^2 = 0.9989$) for polyphenols and flavonoids, respectively. AE extract at concentrations of 10,000, 5,000, 2,500, 1,250, 625, and 312.5 mg/mL revealed 186.8, 105.4, 62.7, 38.7, 21.4, and 1.3 mg GAE/g polyphenols, respectively, and 81.9, 40.2, 23.9, 12.2, 6.8, and 3.5 mg QE/g flavonoid, correspondingly. The result signifies that polyphenols and flavonoid content are related proportionally to the concentration of AE extract.

Chlorogenic acid is one of the polyphenols abundant in vegetables and fruits and has antioxidant and cytoprotective effects.26 Our HPLC analysis showed that a solid peak of chlorogenic acid was observed at a retention time of 7.921 min, consistent with the standard peak. The chlorogenic acid content in the A. elata extract was 0.96 ± 0.01 mg/g.

Disease Activity Index

Table 1 demonstrates that AE extract ingestion at concentrations of 250, 500, 750, and 1000 mg/kg significantly reduced the DAI compared to that in the DSS group. This result indicates the beneficial effects of AE extract when given to mice with DSS-induced colitis.

AE Extract Protects Against Histological Changes in DSS-Induced Colitis

To prove whether TE extract possesses hepatotoxic effects, the liver tissues of all animals were examined. Normal liver structures are composed of small lobules of a roughly hexagonal shape arranged around the portal vein (Figure 1A, B, C, D, and F). This result proves that the AE extract is harmless to the liver.

To examine the protective effects of AE extract on DSS-induced colitis, histological analysis was performed. Cont (Figure 2A) and AE-1000 (Figure 2B) revealed normal tissue architecture (different layers clearly appear), while DSS induced inflammation in the colon (Figure 2C) was shown by immune cells infiltration in the mucosa and submucosa layers with hypertrophy of the muscle layer and loss of the crypt. However, AE-treated groups exhibited less damage (relative appearance of crypt cells, Figure 2D; relative reduction in immune cell infiltration, Figure 2E; and no hypertrophy, Figure 2F) compared with those in the DDS group. The result indicates a dose-dependent effect of the AE extract.

To confirm further the protective effects of AE extract, pro-inflammatory cytokines were quantified in the colon using immunohistochemistry. The results demonstrated that there were no substantial differences in TNF-α and IL-1β levels between the control and AE-1000 groups. TNF-α and IL-1β levels, on the other hand, were noticeably higher in the DSS group compared to the control group ($P$ value <.05 vs Con). However, as shown in Figures 3 and 4A-F, the AE-treated group showed significantly reduced TNF-α and IL-1β compared to the DSS group ($P$ values <.05 vs mice treated with DSS alone). Notably, these results reveal that AE extract prevents DSS-induced colitis.

AE Extract Protects Against Cell Death and NO Overproduction

MTT was performed, in RAW 264.7 cells, to evaluate the viability of cells treated with 25, 50, 75, 100, and 150 µg/mL of AE extract. The result showed that cells treated with AE concentrations of 25 to 100 µg/mL concentration or less. NO inhibitory activity was measured as well. Excessive generation of NO can lead to various inflammatory diseases.27 The AE suppressive effects of NO were tested in RAW 264.7 cells. As shown in Figures 5B and C, the percentage of NO inhibitory activity in cells treated with different concentrations of NO inhibition

Table 1. Disease Activity Index.

| DSS (mg/kg) | 250 | 500 | 1000 |
|------------|-----|-----|------|
| 10.6 ± 0.5 | 8.3 ± 0.9* | 7.2 ± 1.4*** | 4.8 ± 1.3*** |

The data are reported as means ± SD (n = 6).

*: $P$ <.05; ** and ***: $P$ <.001, Bonferroni post-hoc test following one-way ANOVA dextran sulfate sodium (DSS) versus mice treated with 250, 500, and 1000 mg/kg of A. elata (AE) extract, respectively.
Increased with AE extract concentration, with AE extract concentrations of 0, 25, 50, 75, and 100 g/mL inhibiting nitric oxide by 0, 23.1, 27.4, 33.5, and 44.1%, respectively. This result suggests that AE treatment prevents cell death and NO overrun during the cell cycle. AE-treated cells showed significant inhibition in NO% compared with those untreated cells.

**AE Extract Curbs Pro-Inflammatory Cytokines Secretion**

To figure out whether AE extract reduces the pro-inflammatory markers, RAW 264.7 cells were pretreated with LPS and then treated with AE extracts (25, 50, 75, 100 µg/mL). Cytokines were measured using commercially available ELISA kits. The levels of TNF-α, IL-1β, and IL-6 significantly increased in LPS-treated cells compared to those in the Cont cells (P values < .001 vs Con). However, AE-treated cells remarkably inhibited the levels of TNF-α, IL-1β, and IL-6 versus LPS-treated cells (P values < .05 vs LPS-treated cells) except for TNF-α in cells treated with 25 µg/mL, which showed a P value > .05, as shown in Figure 6A-C. Hence, AE extract shows anti-inflammatory properties in RAW 264.7 cells pretreated with LPS.

**AE Extract Improves Cell Morphology Deteriorated by LPS Exposure**

As demonstrated in Figure 7, control cells showed a round, regular shape while LPS-treated cells were elongated, with
granulated and separated cytoplasm and bigger nuclei. However, cells treated with AE extract showed substantial improvements in morphology and size, in a dose-dependent manner.

**Discussion**

UC is a global disease that threatens the lives of people worldwide. To slow the progression of the disease, doctors recommend a variety of treatments, including antibiotics, immunosuppressive drugs, and corticosteroids. However, there are concerns about their potential side effects. Polyphenols and flavonoids contribute to a wide range of biological activities, including anti-inflammatory, immune regulation, antioxidant, cardiovascular protection, and anticancer. The current study sought to identify the anti-inflammatory properties of AE extract in mice and RAW 264.7 cells exposed to DSS and LPS, respectively.

In this study, polyphenol and polyphenol-based substances (flavonoid and chlorogenic acid) were detected as key components of the AE extract. Perhaps DSS is the most used substance to induce colitis in animals because of its simplicity and effectiveness. The extract is safe in the animal experiment using C57BL/6 mice as no hepatotoxicity was observed. When AE extract was applied to a DSS-induced colitis mouse model, the DAI was decreased, damage to the colon tissue was suppressed, and expression of pro-inflammatory cytokines (TNF-α, IL-1β) was increased compared to the DSS group, confirming that the AE extract has an attenuating effect on colitis. In the cell experiment using RAW 264.7 cells, concentrations with low cytotoxicity were selected. The inflammation was modulated and the antioxidant effect through the NO inhibitory activity was confirmed.

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81.9 QE/mg, respectively. According to our results in Table 1, the result showed that the AE extract polyphenol contest is comparable with previous studies by Nikolovska et al.\textsuperscript{31} and Aboelmaati et al.\textsuperscript{32} investigating natural products with antioxidant activity. Moreover, polyphenols are substances with antioxidant activity\textsuperscript{33} and, hence, the protective effects can be attributed to this property. All treated groups showed an index compared with the DSS group. Besides, the length of the colon decreased less than that of the DSS group, and the weight of the spleen tended to increase less than that of the DSS group, confirming the colitis attenuating effect of AE extract.

As shown in Figure 2, H & E staining of the colon slides demonstrated the inflammatory cells and damage to the epithelial tissue in the colitis groups. However, in the AE extract groups, fewer inflammatory cells and less damage were observed in a dose-dependent manner, showing that the AE extract appears to have colitis mitigating effects. These effects are attributed to the polyphenol- and flavonoid-rich AE extracts.\textsuperscript{32,33} It has been reported that Aralia species possess anti-inflammatory effects in rat models via reducing pro-inflammatory cytokines.\textsuperscript{34} Furthermore, although DSS and LPS administration induced TNF-\(\alpha\) and IL-1\(\beta\) overexpression in mice and cells, the AE-treated group downregulated these inflammatory mediators. Chasseing and co-workers concluded that DSS primarily induces immune cells to release TNF-\(\alpha\), and IL-1\(\beta\) release is the main feature of UC.\textsuperscript{11} Therefore, TNF-\(\alpha\) is the primary target for treatment protocols due to its importance.\textsuperscript{35} TNF-\(\alpha\) plays a role in inflammation and cytotoxicity as a result of immune cell release. TNF-\(\alpha\) then attaches to its receptor, TNFR1 (tumor necrosis factor receptor 1), producing a number of immune complexes. These complexes

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**Figure 4.** Immunoreactivity of interleukin 1 beta (IL-1\(\beta\)) at 200\(\times\) magnification. Cont (A), dextran sulfate sodium (DSS) (B), Aralia elata (AE)-1000 (C), DSS + AE-250 (D), DSS + AE-500 (E), DSS + AE-1000 (F), and IL-1\(\beta\) (G) Immunoreactivity. Although DSS causes severe elevation of IL-1\(\beta\) levels, the AE-treated animals significantly reduced the IL-1\(\beta\) levels. The data are reported as means \(\pm\) SD (\(n = 6\)). *: \(P < .05\), Bonferroni post-hoc test following one-way ANOVA control (Cont) versus mice treated with 250, 500, and 1000 mg/kg of AE extract, respectively; while #: \(P < .05\), Bonferroni post-hoc test following one-way ANOVA dextran sulfate sodium (DSS) versus mice treated with 250, 500, and 1000 mg/kg of AE extract, respectively.
activate caspase 8, which finally causes cell death. Our results in Figures 3, 4, and 6 proved that AE extract noticeably suppresses TNF-α, IL-1β, and IL-6 in cells and animals. Our findings are consistent with an earlier study that found that AE extract reduced inflammation in LPS-induced macrophages by inhibiting prostaglandin, IL-1β, and IL-6 production. Our result coincides with recent reports that proved that DDS produced a typical UC picture clinically and histologically. Inflammatory mediators evaluated in our study (TNF-α, IL-1β, and IL-6), as well as previous reports, were inhibited by supplementation of AE extract. These beneficial effects are attributed to the presence of chlorogenic acid in the AE extract.

LPS causes toxicity in macrophage cells. We confirmed that LPS reduced cell viability and improved morphology after 18 h. Nonetheless, AE-pretreated cells improved the cell viability in a dose-dependent manner. Furthermore, NO production, overproduced by LPS, was reduced in cells pretreated.
with AE extract. Lee and Kang confirmed that AE extract exhibited a sufficient inhibitory effect on NO production and suppresses inflammatory reactions by regulation of NF-kappa B signaling in macrophage cells.\(^3\) The findings of this experiment are compatible and directly compared with those of a recent report, which, similar to our protocol, assessed AE against LPS-induced inflammation in RAW 264.7 cells and DSS-induced colitis in mice, in which oxidative stress, TNFα, IL-6, IL-1β, and NO production were substantially reduced.\(^4\) Moreover, we confirmed that a single plant extract of AE extracts, not a combined treatment of *Cirsium japonicum* and AE extracts, also improved the colitis animal models. As a result, the mechanism of the AE extract in the treatment of UC is based on its suppression of the inflammatory cytokines TNF-α, IL-6, and IL-1β and hindering NO production in the cells. These modifications resulted in improved colon histology and, as a result, enhanced DAI in treated animals.

**Limitations of This Research**

There are several limitations to this study, including the absence of endoscopy imaging to trace the progression of the treatment, which is due to the unavailability of endoscopy in our unit. Although AE treatment involves controlling the inflammatory mediators, the exact mechanism of action remains undetermined.

**Conclusion**

To sum up, we confirmed the polyphenol and flavonoid content of AE extract and found that it had antioxidant and anti-inflammatory properties by suppressing inflammatory mediators in RAW 264.7 cells and mice. Hence, UC was significantly reduced. These findings suggest that AE extract could be an effective phytomedicine with clinical applications in the treatment of colitis. More research is needed to determine the underlying molecular mechanism.

**Authors’ Contributions**

EL, SWL, GOA, and HJT conceived and wrote the manuscript; YJY, HYS, DA, and THJ performed the experiments; BTO, BYP, ISK, SHL, and JHL analyzed the data; HJY and JHL supported the study. SHL, CE, and HGO revised the manuscript. All authors have agreed to be held accountable for all aspects of the manuscript.

**Declaration of Conflicting Interests**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Sunchang Research Institute of Health and Longevity; National Research Foundation of Korea (NRF) grant funded by the Korean government (NRF-2019R1A6A1A03033084); Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agriculture, Food and Rural Affairs Convergence Technologies Program for Educating Creative Global Leader, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA)(grant number:320005-4).
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