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ORIGINAL ARTICLE

Improvement Effect of Evodiae Fructus Water Extract against DSS-induced Ulcerative Colitis

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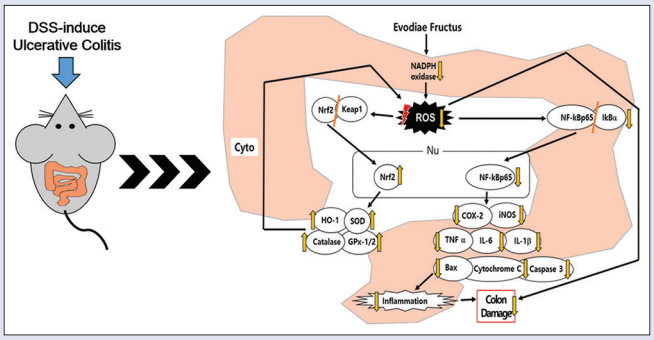
ABSTRACT

Background: Evodiae fructus is a natural plant that is used as a medicinal material in many Asian countries such as Korea and China. However, little is known about its effect on ulcerative colitis. **Objectives:** Thus, the purpose of this study was to find the improvement effect of Evodiae fructus in mice model with dextran sulfate sodium (DSS)-induced colitis. **Materials and Methods:** The animal experiments were conducted using ulcerative colitis animals induced by DSS. To induce ulcerative colitis in BALB/c mice used in the experiment, 5% DSS was supplied as drinking water for 7 days. BALB/c mice were divided into five groups (n = 8): Group 1 (normal group), Group 2 (5% DSS control group), Group 3 (5% DSS + sulfasalazine 60 mg/kg treated), Group 4 (5% DSS + Evodiae fructus water extract 50 mg/kg treated), Group 5 (5% DSS + Evodiae fructus water extract 100 mg/kg treated). After the animal experiment, the expression of antioxidant, oxidative stress, inflammatory, and necrosis-related factors in colonic tissues was analyzed by western blotting. **Results:** As a result, the Evodiae fructus treatment group reduced oxidative stress by increasing the expression of Nrf2, an antioxidant-related transcription factor. Also, the expression of NF-κB, an inflammatory transcription factor, was reduced, thereby reducing the expression of pro-inflammatory proteins and inflammatory cytokines. **Conclusion:** These results suggest that Evodiae fructus treatment has an inhibitory effect on inflammation against DSS-induced colitis. Therefore, Evodiae fructus has the potential to be used as a natural medicine for ulcerative colitis.

Key words: Antioxidant, anti-inflammatory, dextran sulfate sodium, Evodiae fructus, sulfasalazine, ulcerative colitis

SUMMARY

- In this study, the improvement effect of Evodiae fructus water extract on 5% dextran sulfate sodium-induced ulcerative colitis was investigated. As a result, Evodiae fructus water extract showed the effect of suppressing oxidative stress, inflammation, and necrosis by increasing the expression of Nrf2, an antioxidant transcription factor, in the colon tissue.



Abbreviations used:
DSS: Dextran sulfate sodium; UC: Ulcerative colitis; EF: Evodiae fructus water extract; Nrf2: NF-E2–related factor 2; NOX4: NADPH oxidase 4; NF-κB: Nuclear factor-kappa B; IκBα: inhibitor of nuclear factor-kappa B alpha.

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INTRODUCTION

Inflammatory bowel disease (IBD) refers to an inflammatory condition of a multifactorial etiology characterized by increased inflammation, which can exacerbate and cause serious complications such as colon cancer.[1] The main symptoms are diarrhea with mucus and blood.[2] IBD is divided into ulcerative colitis (UC), which damages only the rectum and colon and causes lesions, and Crohn's disease (CD), which uniformly and continuously damages all parts of the gastrointestinal tract.[3,4] Treatments for IBD include aminosalicylates-based drugs (5-ASA), sulfasalazine, corticosteroids, adrenocorticotropic hormone (ACTH), immunomodulatory (thiopurines, azathioprine, biologic agents (anti-TNF agents), antibiotics (metronidazole, and ciprofloxacin), and immunomodulatory agents (6-mercaptopurine, methotrexate, and cyclosporine) are commonly used.[5] However, these treatments have limited effectiveness and may adversely affect the body due to side effects.[6] Many plants are known to have high antioxidant and anti-inflammatory effects by inhibiting oxidative stress.[7] In addition, many natural substances with a few side effects even when taken for a long time have been studied. Accordingly, several researchers are conducting a lot of research to develop IBD therapeutics using natural products with a few side effects.

Evodiae fructus is the unripe fruit of *Evodia rutaecarpa* (Juss.) Benth., a member of the Rutaceae family. Dried Evodiae fructus has been widely

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used to treat diseases such as headache, vomiting, pain, and inflammation. It is used as an astringent, analgesic, antiemetic, and antihypertensive agent in Korean medicine.[8,9] In Korea, a lot of research has been conducted especially on inflammatory diseases.[10-12] Heretofore volatile oils, a number of alkaloids, and fatty acids have been identified from the Evodiae fructus.[13] The main components of Evodiae fructus include evodiamine, evodene, rutaecarpine, dehydroevodiamine, rutaevin, ocimene, evodol, limonin, and evodinone.[14] Among them, evodiamine, as a reference compound, is known to have anti-inflammatory, antioxidant, and anti-tumor excellent effects.[15,16]

Therefore, in this study, using Evodiae fructus, which has excellent anti-inflammatory and antioxidant effects, the improvement effect was investigated in a mouse model in which UC was induced with dextran sulfate sodium (DSS).[17]

MATERIALS AND METHODS

Materials

Folin–Ciocalteu's phenol reagent, sodium hydroxide, gallic acid, naringin, 2,2-diphenyl-1-picrylhydrazyl, sodium carbonate, 7 mM 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), phenylmethylsulfonyl fluoride (PMSF), potassium phosphate dibasic, potassium phosphate monobasic, Sulfasalazine (purity 98%), and diethylene glycol were purchased from Sigma-Aldrich (St. Louis, MO, USA). For high performance liquid chromatography (HPLC) analysis, evodiamine was purchased from the Natural Product Institute of Science and Technology (Anseong, Korea). DSS was purchased from MP Biologicals (Santa Ana, California, USA). For tissue protein quantification, a bicinchoninic acid (BCA) protein kit was purchased from Thermo Fisher Scientific (Waltham, MA, USA) and used. For western blotting experiments, enhanced chemiluminescence (ECL) reagents and pure nitrocellulose membranes were purchased from GE Healthcare (Chicago, IL, USA). Ethylenediaminetetraacetic acid and protease inhibitor mixture solution were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). NF-E2–related factor 2 (Nrf2), Bax, tumor necrosis factor-alpha (TNF-α), NADPH oxidase 4 (NOX4), home oxygenase–1 (HO-1), cycloxygenase-2 (COX-2), superoxide dismutase (SOD), catalase, Cytochrome C, glutathione peroxidase-1/2 (GPx-1/2), an inhibitor of nuclear factor-kappa B alpha (IκBα), interleukin-6 (IL-6) and IL-1β, p22phox, nuclear factor-kappa B p65 (NF-κB), inducible nitric oxide synthase (iNOS), caspase-3, phospho-IκBα (p-IκBα), β-actin, and histone were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). The secondary antibody used for western blotting was purchased from GeneTex, Inc. (Irvine, LA, USA).

Preparation of the plant material

For use in the experiment, 100 g of dried Evodiae fructus was put in 10 times distilled water and boiled at 100°C for 2 h. Next, the extract was made into a powder form by evaporating moisture with a rotary evaporator at 50°C. The extracted powder was named Evodiae fructus water extract (EF). Then, the EF was kept at −80°C until experimentation.

Evodiamine content

In total, 20 mg of the sample were dissolved in 1 mL of MeOH. A calibration curve was prepared using evodiamine as a standard material. The content of evodiamine was analyzed using an INNO C18 column (25°C). Then, 10 μL of the sample and standard were injected. The detector was set to a UV absorbance of 254 nm. The mobile phase comprised two solvents: 0.5% acetic acid in water (A) and MeOH (B). Gradient elution was consisting of 0.5% acetic acid in water (A) and MeOH (B) with a flow rate of 1 mL/min. The total analysis was carried out for 55 min and the solvent conditions were as follows; the initial condition was set at 95% (A) → after 10 min 85% (A) → after 25 min 70% (A) → after 30 min 20% (A) → after 35 min 0% (A). This condition was maintained for 40 min. It was then increased to 95% at 50 min and held for 5 min.

Total polyphenol and flavonoid content

The total polyphenol content of EF was measured by the Folin–Denis method.[18] Gallic acid was used as the reference material. Next, 50 μL of Folin–Ciocalteu's phenol reagent, 790 μL of distilled water, and 10 μL of the sample were placed in an e-tube and mixed well. Then, 150 μL of a sodium carbonate solution (20%) was added and reacted at room temperature for 2 h, and the value was measured at 765 nm using a Microplate Reader (M200 PRO, Tecan, Switzerland).

The total flavonoid content of the extract was measured as follows.[19] Then, 1 mL of diethylene glycol, 10 μL of 1 N sodium hydroxide solution, and 100 μL of the sample were added to the e-tube, mixed well, and then reacted at 37°C for 1 h. The value was then measured at 420 nm. As standard material naringin was used.

DPPH free and ABTS radical scavenging activity

Antioxidant activity determination of EF was performed using the DPPH radical scavenging method according to the Blois method.[20] In total, 100 μL of 60 μM DPPH solution and 100 μL of the sample were mixed well and reacted in the dark for 30 min. After 30 min of reaction, the absorbance was measured at 540 nm.

The ABTS radical scavenging activity of EF was measured as follows.[21] First, ABTS (7 mM) and potassium persulfate (2.45 mM) were mixed well to prepare the $ABTS^+$ solution. The $ABTS^+$ solution 95 μL of the prepared $ABTS^+$ solution and 5 μL of the sample were mixed well and allowed to react in a dark place for 15 min. The absorbance was then measured at 415 nm.

The scavenging ability was obtained by substituting the OD value into the following equation. The concentration of the sample at which the DPPH free and ABTS radical scavenging ability are 50% was expressed as the IC_{50} value:

Radical scavenging activity (%)

= (1 −[Sample OD ÷ Blank OD]) × 100

Experimental animals and induction of colitis

This animal experiment was conducted with the approval of the Ethics Committee of Daegu Haany University (Approval No. DHU2020-016). BALB/c mice (7 week-old male) weighing 22–25 g were purchased from Orient (Gyeonggi-do, Korea). BALB/c mice were maintained under a 12 h dark/light cycle, housed at 22°C and humidity of 50%. After adaptation for 1 week in the animal room, drinking water containing 5% DSS was supplied to induce ulcerative colitis. Normal mice received drinking water without DSS throughout the entire experimental period [Figure 1].[22] A total of 35 mice were randomly divided into five groups as follows (n = 8/group).

(A) Normal mice group (Nor)
(B) 5% DSS control mice group (Veh)
(C) Sulfasalazine 60 mg/kg treated mice group (60S, positive control)
(D) Evodiae fructus water extract 50 mg/kg treated mice group (EFL)
(E) Evodiae fructus water extract 100 mg/kg treated mice group (EFH)

The drug was administered orally for 1 week. After that, blood and colon tissue were collected and used for the experiment. Blood was centrifuged at 4000 rpm for 10 min to separate serum. Subsequently, the colon tissue and serum were kept at −80°C until analysis.

Western blotting

Colon tissue samples were prepared according to Komatsu's method.[23] Buffer A containing PMSF, EDTA, $MgCl_2$, HEPES (pH 7.8), KCl, DTT, and protease inhibitor mixture solution was added to colonic tissue and pulverized. Then, the supernatant containing the cytoplasm was obtained by centrifugation at 12,000 rpm at 4°C for 2 min. After removing the supernatant, buffer C containing NaCl, HEPES, DTT, KCl, EDTA, glycerol, and PMSF were added to the remaining tissue and mixed well. Then, the supernatant containing nuclei was obtained by centrifugation at 4°C at 12,000 rpm for 10 min. The 12 μg of protein from each nucleus and cytosols were electrophoresed through 10 to 12% sulfate -polyAcrylamide gel electrophoresis (SDS-PAGE) gel. The proteins separated on the SDS gel were transferred to a nitrocellulose membrane. After attaching the antibody to be analyzed, bands were photographed using ECL reagents and Sensi-Q 2000 Chemidoc (Lugen Sci Co., Ltd., Gyeonggi-do, Korea).[22]

Statistical analysis

Experimental data are expressed as mean ± standard error of the mean (SEM) and mean ± standard deviation (SD). The significance of each data was assessed by the least significant difference (LSD) test of one-way analysis of variance (ANOVA) using the SPSS 25 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Total polyphenol, total flavonoid, and evodiamine contents

As shown in Table 1, the total polyphenol content of EF was 53.74 mg/g and the total flavonoid content of the EF was 45.13 mg/g. Evodiamine, a reference compound of EF, was detected in EF at 35.98 min retention time [Figure 2] and the content was 0.367 mg/g [Table 1].

DPPH free and ABTS radical scavenging activity

As shown in Table 2, the DPPH free radical scavenging activity IC_{50} of EF was 28.13 μg/mL and ABTS radical scavenging activity IC_{50} of EF was 45.22 μg/mL.

Body weight and water intake

To evaluate the effect of EF in the DSS-induced colitis mice model, mice were provided 5% DSS in drinking water for 1 week. As a result, during the study period, the 5% DSS intake groups were observed for symptomatic indicators of colitis, such as weight loss, mucus, watery diarrhea, and rectal bleeding. Figure 3 shows the changes in body weight during the experimental period. Compared to the Nor group, the bodyweight of the Veh group was considerably decreased. In contrast, the EF administration groups showed a significant increase compared to the Veh group.

Macroscopic changes of colons

As shown in Figure 4, the macroscopic viewpoint and length of the colon. Compared to the Nor group, the Veh group was considerably

Table 1: Total Polyphenol, Total Flavonoid, and Evodiamine Contents of EF

Sample	Total polyphenol (mg/g)	Total flavonoid (mg/g)	Evodiamine (mg/g)
EF	53.74±0.45	45.13±0.76	0.367±0.003

EF, Evodiae fructus water extract. All data are expressed mean±SEM

Table 2: Scavenging Activity of Evodiae fructus on DPPH and ABTS free radical

Sample	DPPH (μg/mL)	ABTS (μg/mL)
L-ascorbic acid	1.76±0.01	3.64±0.03
EF	28.13±0.29	45.22±0.76

EF, Evodiae fructus water extract. L-ascorbic acid was used as a standard. All data are expressed mean±SEM

Figure 1: Scheme of the animal experimental design

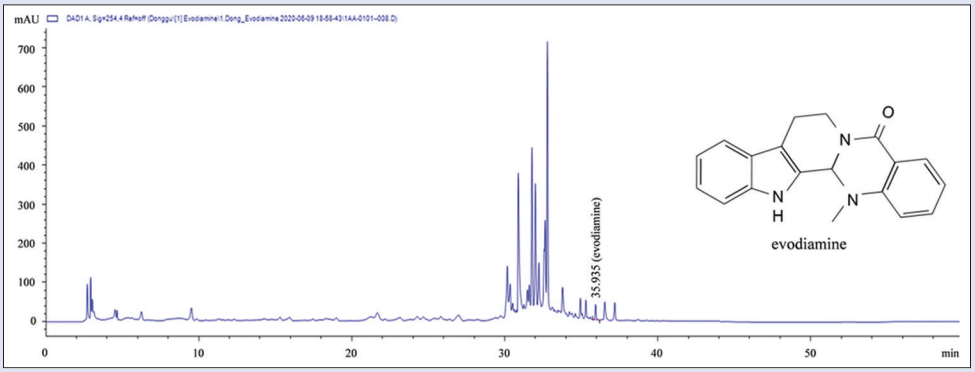

Figure 2: HPLC chromatogram of EF and evodiamine

decreased. The 60S group increased compared with the Veh group. EF treatment also increased in a concentration-dependent compared with the Veh group.

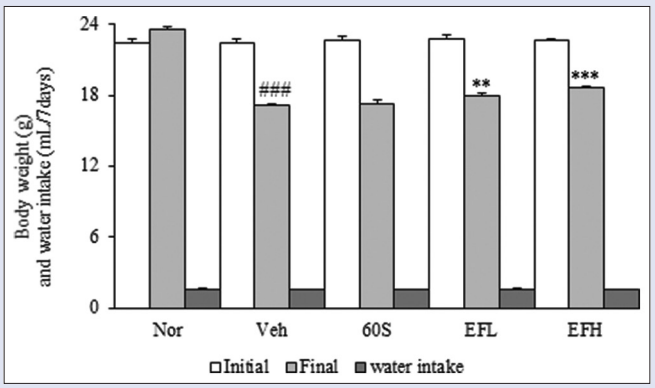

Figure 3: Body weight and water intake in DSS-induced colitis mice. All data are expressed mean ± SD. (n = 8) Significance: ###p < 0.001 compared with normal mice, **p < 0.01, ***p < 0.001 compared with 5% DSS control mice. Nor, normal mice; Veh, 5% DSS control mice; 60S, 5% DSS + sulfasalazine 60 mg/kg-treated mice; EFL, 5% DSS + EF 50 mg/kg-treated mice; EFH, 5% DSS + EF 100 mg/kg-treated mice

Expressions of NADPH oxidases

As shown in Figure 5, the expression of NOX4 and $p22^{phox}$ was significantly elevated in the Veh group compared with the Nor group (NOX4 1.44 fold and $p22^{phox}$ 1.47 fold increased). However, the 60S, EFL, and EFH groups were significantly reduced in comparison with the Veh group.

Expressions of anti-oxidation factors

As shown in Figure 6, the expression of Nrf2, HO-1, SOD, Catalase, and GPx-1/2 was significantly reduced in the Veh group compared with the Nor group (Nrf2 0.73 fold, HO-1 0.60 fold, SOD 0.81 fold, Catalase 0.64 fold, and GPx-1/2 0.66 fold reduced). The 60S, EFL, and EFH groups were elevated in comparison with the Veh group.

Expressions of inflammatory factors

As shown in Figure 7, the expression of NF-κB and p-IκBα was significantly elevated in the Veh group compared with the Nor group (NF-κB 1.45 fold and p-IκBα 1.45 fold increased). The 60S, EFL, and EFH groups were reduced in comparison with the Veh group.

Expressions of inflammatory enzymes

As shown in Figure 8, the expression of iNOS and COX-2 was significantly elevated in the Veh group compared with the Nor

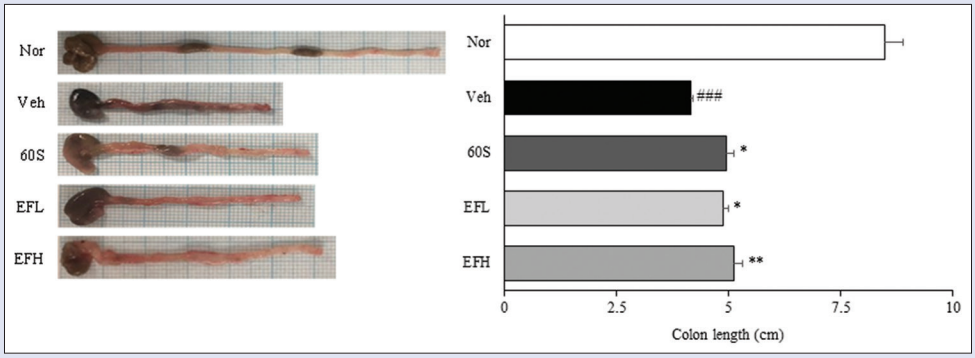

Figure 4: Macroscopic changes of colons and colon length in DSS-induced colitis mice. All data are expressed mean ± SD. (n = 8) Significance: ###p < 0.001 compared with normal mice, *p < 0.05, **p < 0.01 compared with 5% DSS control mice. Nor, normal mice; Veh, 5% DSS control mice; 60S, 5% DSS + sulfasalazine 60 mg/kg-treated mice; EFL, 5% DSS + EF 50 mg/kg-treated mice; EFH, 5% DSS + EF 100 mg/kg-treated mice

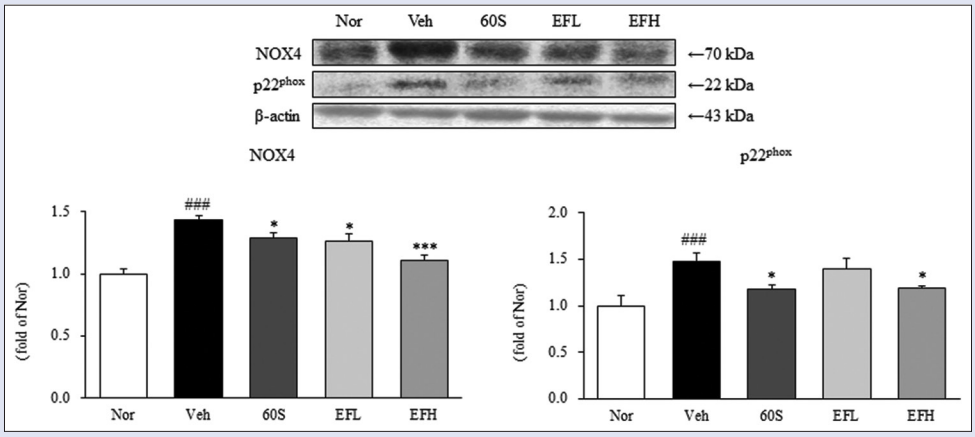

Figure 5: Expressions of NADPH oxidase in DSS-induced colitis mice. All data are expressed mean ± SD. (n = 8) Significance: ###p < 0.001 compared with normal mice, *p < 0.05, **p < 0.01, and ***p < 0.001 compared with 5% DSS control mice. Nor, normal mice; Veh, 5% DSS control mice; 60S, 5% DSS + sulfasalazine 60 mg/kg-treated mice; EFL, 5% DSS + EF 50 mg/kg-treated mice; EFH, 5% DSS + EF 100 mg/kg-treated mice

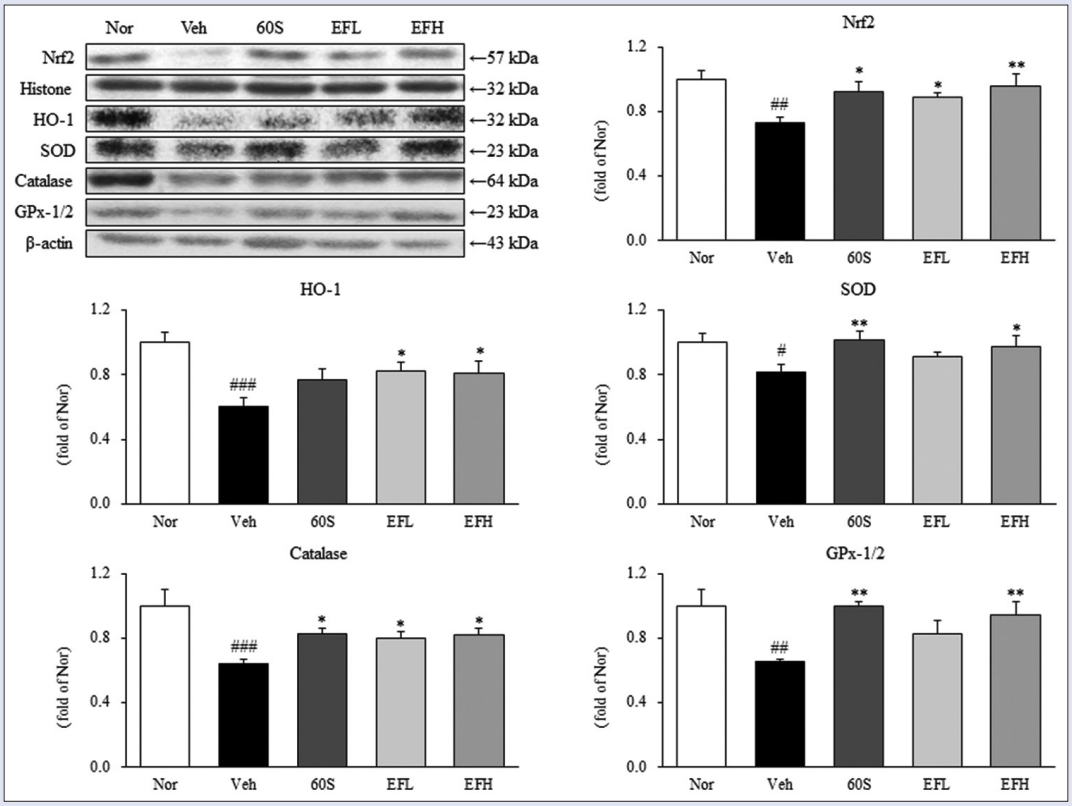

Figure 6: Expressions of anti-oxidation factors in DSS-induced colitis mice. All data are expressed mean ± SD. (n = 8) Significance: ###p < 0.001 compared with normal mice, *p < 0.05, **p < 0.01 compared with 5% DSS control mice. Nor, normal mice; Veh, 5% DSS control mice; 60S, 5% DSS + sulfasalazine 60 mg/kg-treated mice; EFL, 5% DSS + EF 50 mg/kg-treated mice; EFH, 5% DSS + EF 100 mg/kg-treated mice

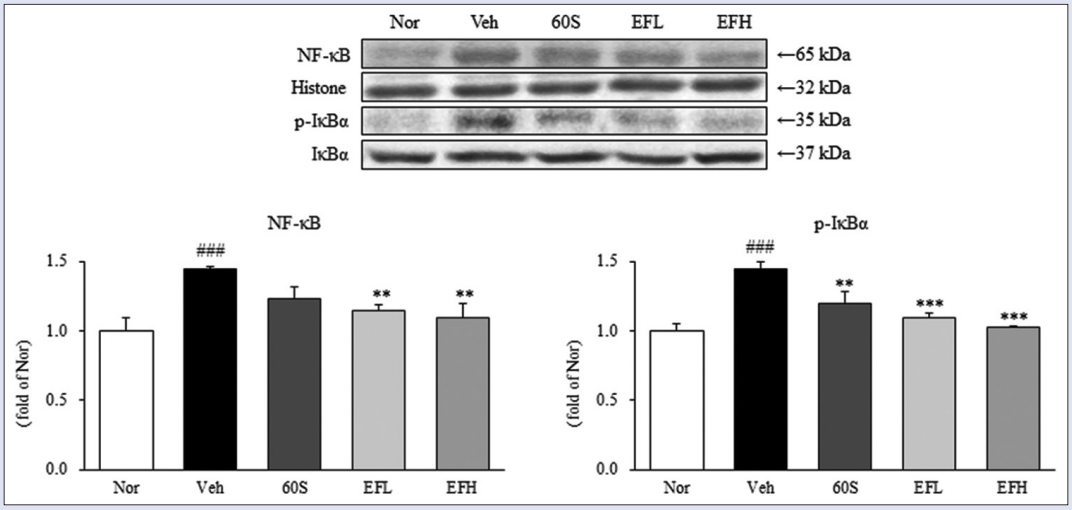

Figure 7: Expressions of inflammatory factors in DSS-induced colitis mice. All data are expressed mean ± SD. (n = 8) Significance: ###p < 0.001 compared with normal mice, **p < 0.01, ***p < 0.001 compared with 5% DSS control mice. Nor, normal mice; Veh, 5% DSS control mice; 60S, 5% DSS + sulfasalazine 60 mg/kg-treated mice; EFL, 5% DSS + EF 50 mg/kg-treated mice; EFH, 5% DSS + EF 100 mg/kg-treated mice

group (iNOS 1.66 fold and COX-2 1.44 fold increased). However, the 60S, EFL, and EFH groups were reduced in comparison with the Veh group.

Expression of inflammatory cytokines

As shown in Figure 9, the expression of IL-6, IL-1β, and TNFα was significantly elevated in the Veh group compared with the Nor group (IL-6 1.70 fold, IL-1β 1.43 fold, and TNFα 1.58 fold increased). However, the 60S, EFL, and EFH groups were reduced in comparison with the Veh group.

Expression of apoptosis factors

As shown in Figure 10, the expression of Bax, Cytochrome C, and Caspase 3 was significantly elevated in the Veh group compared with the

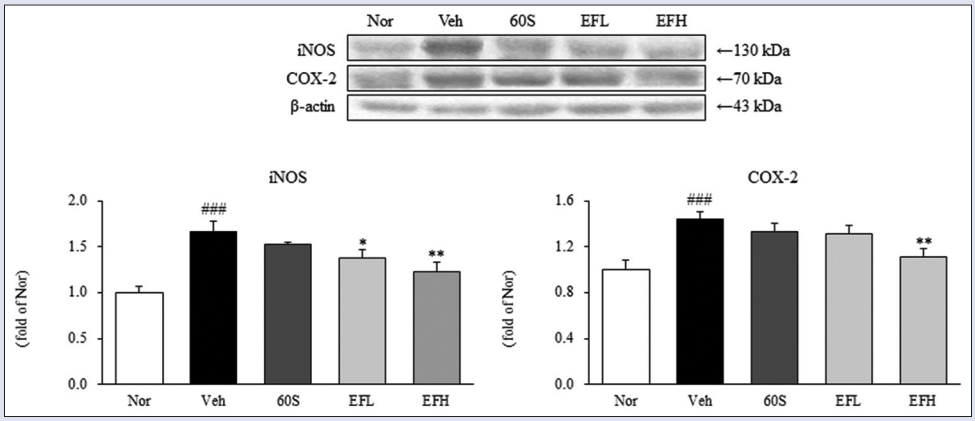

Figure 8: Expressions of inflammatory enzymes in DSS-induced colitis mice. All data are expressed mean ± SD. ($n = 8$) Significance: ###$p < 0.001$ compared with normal mice, *$p < 0.05$, **$p < 0.01$ compared with 5% DSS control mice. Nor, normal mice; Veh, 5% DSS control mice; 60S, 5% DSS + sulfasalazine 60 mg/kg-treated mice; EFL, 5% DSS + EF 50 mg/kg-treated mice; EFH, 5% DSS + EF 100 mg/kg-treated mice

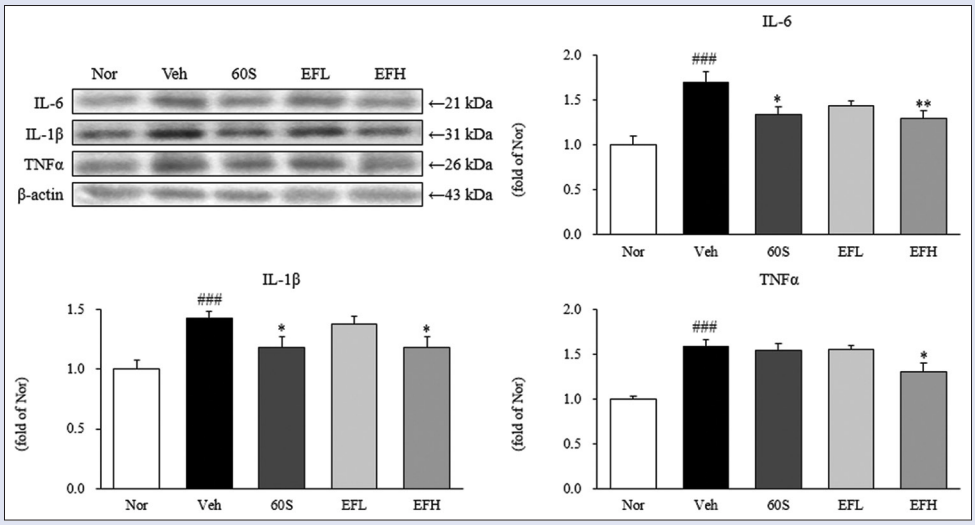

Figure 9: Expressions of inflammatory cytokines in DSS-induced colitis mice. All data are expressed mean ± SD. ($n = 8$) Significance: ###$p < 0.001$ compared with normal mice, *$p < 0.05$, **$p < 0.01$ compared with 5% DSS control mice. Nor, normal mice; Veh, 5% DSS control mice; 60S, 5% DSS + sulfasalazine 60 mg/kg-treated mice; EFL, 5% DSS + EF 50 mg/kg-treated mice; EFH, 5% DSS + EF 100 mg/kg-treated mice

Nor group (Bax 1.43 fold, Cytochrome C 1.42 fold, and Caspase 3 1.49 fold increased). However, the 60S, EFL, and EFH groups were reduced in comparison with the Veh group.

DISCUSSION

Inflammatory bowel disease (IBD) is currently a serious health problem worldwide.[3] Among them, ulcerative colitis, an immune inflammatory disease, causes various dysfunctions due to the excessive production of inflammatory cells and cytokines.[22] This study was performed to evaluate the inflammation of the intestinal mucosa treatment effect of Evodiae fructus water extract (EF), a natural material in the DSS-induced colitis mice model.

It is known that the length of the colon is related to the severity of colitis.[22] As a result of measuring the length of the colon after the end of the experiment, as shown in Figure 4, the oral administration groups (60S, EFL, and EFH) exhibited significantly decreased increased colon length in comparison with the Veh group.

NADPH oxidases (NOX) produce reactive oxygen species (ROS) in response to bacteria, fungi, and viruses.[24,25] There have been many studies on the importance of oxidative stress in colitis.[26] So, the expression of NADPH oxidases was measured in colon tissues by western blotting. The measurement results [Figure 5] showed that the Veh group showed significantly increased expression of NOX proteins in the colon compared with the Nor group. However, oral administration groups (60S, EFL, and EFH) were significantly downregulated compared with the Veh group.

Activation of Nrf2 signaling induces the enzymic antioxidants, such as HO-1, SOD-1, catalase, and GPx-1/2. As shown in Figure 6, the Veh group showed significantly downregulated expression of Nrf2, HO-1, SOD-1, catalase, and GPx-1/2 in the colon compared with the Nor group. However, oral administration groups (60S, EFL, and EFH) were significantly increased compared with the Veh group. These results support EF playing an important role in the prevention and treatment of colitis via the Nrf2 pathway.

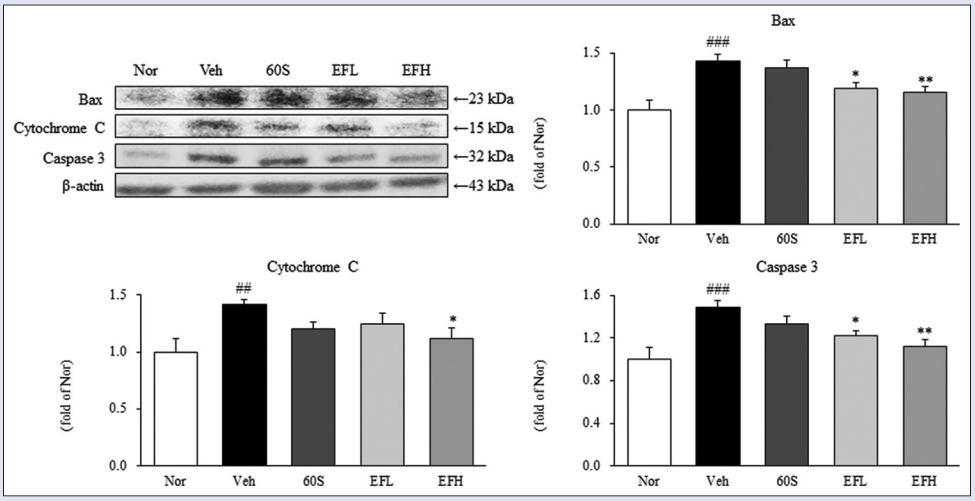

Figure 10: Expressions of apoptosis factors in DSS-induced colitis mice. All data are expressed mean ± SD. ($n = 8$) Significance: ###$p < 0.001$ compared with normal mice, *$p < 0.05$, **$p < 0.01$ compared with 5% DSS control mice. Nor, normal mice; Veh, 5% DSS control mice; 60S, 5% DSS + sulfasalazine 60 mg/kg-treated mice; EFL, 5% DSS + EF 50 mg/kg-treated mice; EFH, 5% DSS + EF 100 mg/kg-treated mice

Moreover, we examined the expression of NF-κB signal transduction pathways. As shown in Figure 7, Figure 8, and Figure 9, the Veh group showed significantly increased expression of inflammatory transcription factor NF-κB, pro-inflammatory proteins, and inflammatory cytokines in the colon compared with the Nor group. In contrast, oral administration groups (60S, EFL, and EFH) were significantly downregulated compared with the Veh group. Therefore, EF could effectively protect the colon tissue by reducing inflammation.

Excessive exposure of the mesothelial membrane to ROS in an inflammatory state increases apoptosis of epithelial cells, which contributes to damage to the colon.[27] As shown in Figure 10, the Veh group showed significantly increased expression of Bax, Cytochrome C, and caspase-3 in the colon compared with the Nor group. However, oral administration groups (60S, EFL, and EFH) were significantly downregulated compared with the Veh group.

Taken together, EF is believed that this will help alleviate bowel disease in patients with IBD disease in future clinical practice, and it is thought that it will be used as a new material for the treatment of colitis. Therefore, it is suggested that more research and evaluation will be needed in the future.

CONCLUSION

In summary, it suggests that Evodiae fructus has a colon protective effect against ulcerative colitis by suppressing oxidative stress, inflammation, and necrosis factors in the colon tissue caused by DSS with its excellent antioxidant activity.

Authors' contributions

Writing - original draft, Writing-review, and editing, M.J. Kim; investigation, J.A. Lee; methodology, M.R. Shin and J.H. Lee; conceptualization, S.H. Lee; funding acquisition and supervision, S.S Roh. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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