



Research Article

Restorative effects of Rg3-enriched Korean Red Ginseng and *Persicaria tinctoria* extract on oxazolone-induced ulcerative colitis in miceH.M. Arif Ullah^{a,1}, Evelyn Saba^{b,1}, Yuan Yee Lee^a, Seung-Bok Hong^c, Sun-Hee Hyun^d, Yi-Seong Kwak^d, Chae-Kyu Park^d, Sung Dae Kim^a, Man Hee Rhee^{a,*}^a Department of Veterinary Medicine, College of Veterinary Medicine, Kyungpook National University, Daegu, Republic of Korea^b Department of Veterinary Biomedical Sciences, Faculty of Veterinary and Animal Sciences, Pir Mehr Ali Shah-Arid Agriculture University, Pakistan^c Department of Clinical Laboratory Science, Chungbuk Health & Science University, Chungbuk, Republic of Korea^d R&D Headquarters, Korean Ginseng cooperation, Daejeon, Republic of Korea

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ABSTRACT

Background: Ulcerative colitis (UC) is the large intestine disease that results in chronic inflammation and ulcers in the colon. Rg3-enriched Korean Red Ginseng extract (Rg3-RGE) is known for its pharmacological activities. *Persicaria tinctoria* (PT) is also used in the treatment of various inflammatory diseases. The aim of this study is to investigate the attenuating effects of Rg3-RGE with PT on oxazolone (OXA)-induced UC in mice.

Methods: A total of six groups of mice including control group, OXA (as model group, 1.5%) group, sulfasalazine (75 mg/kg) group, Rg3-RGE (20 mg/kg) group, PT (300 mg/kg) group, and Rg3-RGE (10 mg/kg) with PT (150 mg/kg) group. Data on the colon length, body weight, disease activity index (DAI), histological changes, nitric oxide (NO) assay, Real-time PCR of inflammatory factors, ELISA of inflammatory factors, Western blot, and flow cytometry analysis were obtained.

Results: Overall, the combination treatment of Rg3-RGE and PT significantly improved the colon length and body weight and decreased the DAI in mice compared with the treatment with OXA. Additionally, the histological injury was also reduced by the combination treatment. Moreover, the NO production level and inflammatory mediators and cytokines were significantly downregulated in the Rg3-RGE with the PT group compared with the model group. Also, NLR family pyrin domain containing 3 (NLRP3) inflammasome and nuclear factor kappa B (NF-κB) were suppressed in the combination treatment group compared with the OXA group. Furthermore, the number of immune cell subtypes of CD4⁺ T-helper cells, CD19⁺ B-cells, and CD4⁺ and CD25⁺ regulatory T-cells (Tregs) was improved in the Rg3-RGE with the PT group compared with the OXA group.

Conclusion: Overall, the mixture of Rg3-RGE and PT is an effective therapeutic treatment for UC.

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1. Introduction

Ulcerative colitis (UC) is a type of chronic inflammatory disease that results in intestinal abnormalities, such as inflammation of the colonic mucosa, abdominal pain, and bloody diarrhea [1–3]. Loss of body weight and anemia are also common symptoms of UC [4,5]. Although the etiology of UC is unknown, it has been reported that the commonly considered risk factors for UC are the dysfunction of

the immune system, environmental factors, lifestyle, Western diet, and family health history [5]. Without proper treatment, complications may occur, including colon dilation and colon cancer. Steroidal, non-steroidal, anti-inflammatory, and immune-suppressive drugs are commonly used in the treatment of UC [1,6]. However, prolonged treatment with these drugs results in complications and side effects. Hence, studies have focused on the use of herbal products as an alternative therapeutic method in addition to the modern treatment for UC.

Panax ginseng (family: *Araliaceae*) is an important alternative medicine. Since it induces numerous pharmacological activities, it is one of the widely used medicinal herbs worldwide, especially in

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Asia [2,5]. Over many years, studies have been conducted to identify various medicinal properties and active phytochemicals in ginseng. *Panax ginseng* is widely reported to possess analgesic, anti-inflammatory, anti-oxidant, anti-ulcer, anti-cancer, immunosuppressive, anti-fungal, anti-hypertensive, anti-platelet, anti-thrombotic, anti-diabetic properties [7–9]. Ginsenosides are the active biological components of ginseng, and Rg3 is one of the important ginsenosides that have anti-inflammatory and anticancer properties [2]. *Persicaria tinctoria* (PT) (family: *Polygonaceae*) is also an important natural medicine and is traditionally used in the treatment of inflammation, fever, and edema [10]. It has also been reported that PT induces anti-oxidant and anti-inflammatory activities [2].

Previous studies have shown that an imbalance of immune cells, such as T-helper (Th) cells and regulatory T cells (Tregs), occurs during the pathogenesis of UC [2,6]. Oxazolone (OXA) was used as the colitis model inducer agent due to the similarities between human UC and OXA-mediated UC [6,11]. OXA induces several intracellular signaling pathways [6]. NLR family pyrin domain containing 3 (NLRP3) inflammasomes, and nuclear factor-kappa B (NF- κ B) are responsible for the overproduction of inflammatory mediators and cytokines, such as cyclooxygenase (COX-2), inducible nitric oxide synthase (iNOS), interleukin-1 β (IL-1 β), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-13 (IL-13) and tumor necrosis factor- α (TNF- α) [12–14].

There is no study to reveal restorative properties of the mixture of Korean Red Ginseng extract (Rg3-RGE) with PT on OXA-induced UC in mice. Therefore, this study investigated the inhibiting properties of Rg3-RGE and PT extracts in OXA-induced UC in mice. Herein, we hypothesized that Rg3-RGE and PT potentially suppressed OXA-induced UC, suggesting that the mixture treatment of Rg3-RGE with PT can be used in addition to the modern treatment for UC.

2. Materials and methods

2.1. Chemicals and reagents

The oxazolone and sulfasalazine were purchased Sigma-Aldrich (St. Louis, MA, USA). Primary antibodies for western blot including phosphorylated (p)-NF- κ B (Catalog No. 3033), and NLRP3 (Catalog No. 15101), and horseradish peroxidase-linked secondary antibody (Catalog No. 7074) were purchased Cell Signaling Technology (Danvers, MA, USA). The conjugated fluorescence antibodies for flow cytometry analysis (FACS) including PE-Cy5 anti-CD3, PE anti-CD4, fluorescein isothiocyanate (FITC) anti-CD8, FITC anti-CD19, and FITC anti-CD25, were obtained BD Biosciences (San Diego, CA, USA). All other chemicals and reagents were obtained from Sigma Aldrich.

2.2. Sample preparation

Red ginseng was obtained from Korea Ginseng Corporation (Daejeon, Republic of Korea). Briefly, the Rg3-RGE used in this study was made by extracting 25:75 red ginseng root/stem with distilled water and then adding 55% ethanol. The extract was then subjected to a high-performance liquid chromatography system (HPLC) according to our previous studies [15,16]. The resulting constituent profile is shown in Table S1. In addition, *Persicaria tinctorial* (PT) was made by boiling the plant's leaves in 70% ethanol, condensing the extract, and lyophilizing the powdered form.

2.3. Animals and treatment

6–8-week-old and C57BL/6 male mice (18–23 g) were used in this study. Animals were kept in a pathogen-free environment and Food and water were given *ad libitum*. They were divided into six groups (n = 6/group) according to treatment: (1) control; (2) 1.5% OXA; (3) sulfasalazine (75 mg/kg) + OXA (positive control); (4) Rg3-RGE (20 mg/kg) + OXA; (5) PT (300 mg/kg) + OXA; and (6) combination of Rg3-RGE (10 mg/kg) and PT (150 mg/kg) + OXA. All non-control groups were orally administered OXA (1.5%) with drinking water for 7 days. Oral administration of Rg3-RGE, PT, combination of Rg3-RGE with PT and sulfasalazine were performed together on the same day as the administration of OXA. Animal were anesthetized, blood, and tissues (colon and spleen) were collected for the further experiment on the 7th day. All animal experiment procedures were followed the Institutional Animal Care and Use Committee's guidelines (IACUC). The animal experiment protocol was approved by Kyungpook National University's Institutional Animal Use and Care Committee in Daegu, Republic of Korea. (approval number-KNU2018-002).

2.4. Assessment of colon tissues and disease activity index (DAI)

Animal were sacrificed, and their colon tissues were removed. The length of colon tissues was measured by the scale. Body weight was measured daily for 7 days of the experiment period. The mice were observed general disposition, stool consistency, and presence of blood in stools. Using the disease activity index (DAI) scoring system, combined body score, stool consistency, and bloody stools were determined according to the previous study [2,6].

2.5. Histological analysis

The colon tissues of mice were removed and harvested in the 10% formalin and routinely processed in a graded ethanol series and toluene. For histological analysis, the colon tissues were embedded in paraffin and then 5- μ m-thick slices were cut. The sections of tissues were stained with hematoxylin and eosin (H&E) according to previous method [17,18]. The stained samples were observed using a light microscope.

2.6. Nitric oxide (NO) assay

Nitric oxide was determined using the Griess reaction assay method as described previously [19]. Briefly, the supernatant was collected and added with the equal volume of Griess reagent (reagent A and reagent B) for the determination of nitric oxide (NO). Samples were measured using a microplate reader at 540 nm absorbance (Molecular Devices, San Jose, CA, USA).

2.7. Quantitative real-time polymerase chain reaction (qRT-PCR)

The colon tissues were used and qRT-PCR reactions were carried out using CFX96 (Bio-Rad, Hercules, CA, USA). Briefly, the total RNA was extracted from colon tissues using Trizol reagent and reverse transcribed using a cDNA kit (Bioneer, Daejeon, Republic of Korea) as previously described [20]. The cDNA underwent a qRT-PCR. GAPDH was used as a loading control gene. The qRT-PCR primer sequences are given to Table 1.

2.8. Enzyme-linked immunosorbent assay (ELISA)

TNF- α , IL-1 β , IL-5, and IL-13 levels in the plasma of oxazolone-induced colitis mice were measured as described previously [21] using mouse TNF- α , mouse IL-1 β , mouse IL-5, and mouse IL-13.

Table 1
Real-Time Polymerase Chain Reaction Primer Used in This Study

| Primer | Forward primer sequences (5'-3') | Reverse primer sequences (5'-3') |
|--------|----------------------------------|----------------------------------|
| iNOS | GGCAGCCTGTGAGACCTTTG | GCATTGGAAGTGAAGCGTTTC |
| COX-2 | GGCAGCCTGTGAGACCTTTG | GCATTGGAAGTGAAGCGTTTC |
| IL-1β | CAACCAACAAGTGATATTCTCCATG | GATCCACACTCTCCAGCTGCA |
| IL-5 | GAAGTGTGGCGAGGAGAGAC | GCACAGTTTTGTGGGGTTTT |
| IL-6 | TCCAGTTGCCTTCTTGGGAC | GTGTAATTAAGCCTCCGACTTG |
| IL-13 | AGCATGGTATGGAGTGTGGA | TTGCAATTGGAGATGTTGGT |
| TNF-α | TGCTATGTCTCAGCCTCTTC | GAGGCCATTTGGGAAGCTCT |
| NLRP3 | TGCTCTTCACTGCTATCAAGCCCT | ACAAGCCTTTGCTCCAGACCTAT |
| GAPDH | CCTCACGGCAAATCAACGGCAC | GACTCCAGCATACTCAGCAC |

ELISA kits were used according to the manufacturer's protocols (R&D Systems, Minneapolis, MN, USA). Samples were analyzed with three independent experiments.

2.9. Western blot analysis

Western blot was performed with modifications, as described previously [19,20]. Briefly, the proteins were extracted from the colon tissues, concentrations were measured, and samples were prepared in sodium dodecyl sulfate and boiled for 5 min. The samples were separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins were transferred to the poly vinylidene fluoride membranes, blocked with skim milk (5%) for 1 h at room temperature. Membranes were washed with tris-buffered saline with tween (TBST) (washing buffer) three times/each 10 min. Membranes were incubated with the p-NF-κB

(Catalog No. 3033) and NLRP3 (Catalog No. 15101) primary antibodies (1:1000) overnight at 4°C. Furthermore, the membranes were washed with TBST (washing buffer) three times/each 10 min. and were incubated with HRP-labeled secondary antibodies (1:3000) for 1 h and again washed with TBST three times/each 10 min. The protein bands were detected using enhanced chemiluminescence solution (1/1 ratio) in Imager ALS 500 (General Electric, Boston, MA, USA).

2.10. Fluorescence-activated cell sorting (FACS) analysis

Briefly, mice were sacrificed and the spleen tissues were isolated and pulverized lightly using a syringe plunger. Then spleen tissues were passed through a cell strainer (70 μm) into the phosphate-buffered saline (PBS) and centrifuged at 245 g, 5 min at 25 °C. In addition, red blood cells (RBCs) were lysed and then centrifuged to get splenocytes. The splenocytes (1 × 10⁵) were stained with specific antibodies. Cells were resuspended in 2% FBS in phosphate-buffered saline (FACS buffer). Finally, samples were analyzed using BD FACSAria III™ (BD Biosciences, San Jose, CA, USA).

2.11. Statistical analysis

Data are represented as mean and standard error of mean (SEM). The statistical significance was analyzed by One-way analysis of variance (ANOVA). The statistical analyses with a ***p of <0.001, **p of <0.05, and *p of <0.01 were considered significant when compared to the OXA group, and ###p of <0.001 when compared to the control group.

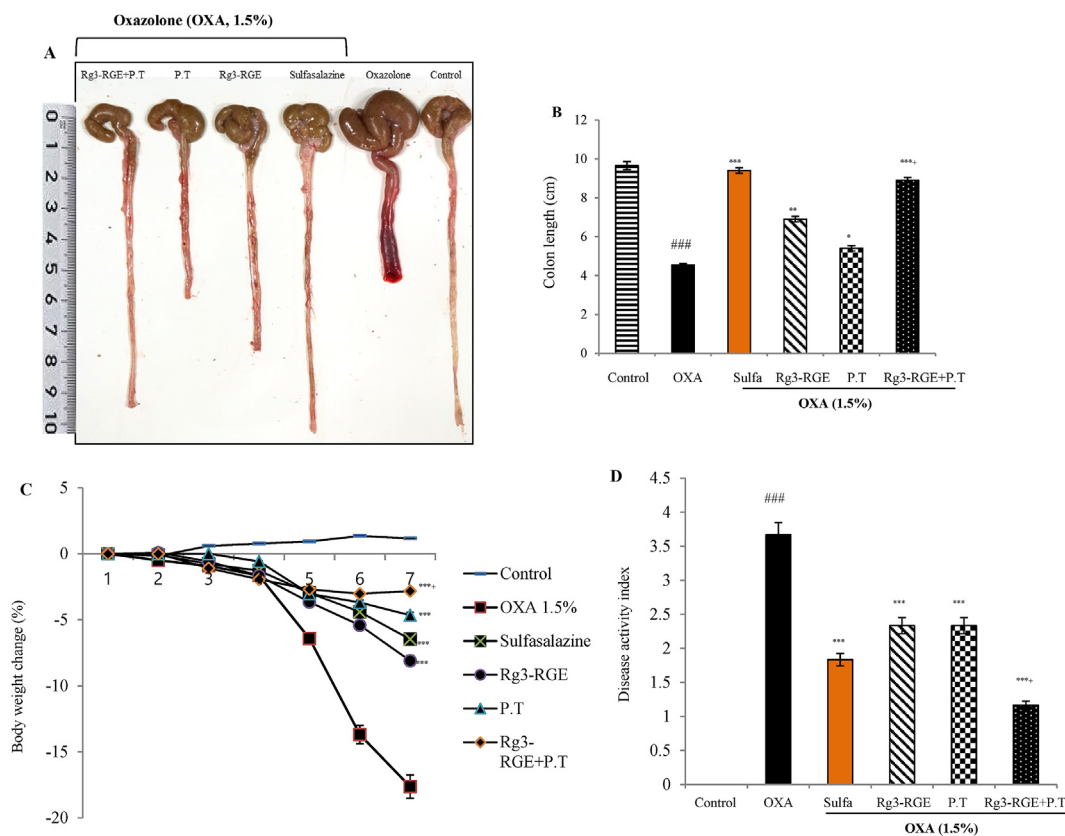


Fig. 1. Effects of Rg3-enriched Korean Red Ginseng extract and *Persicaria tinctoria* on mice with oxazolone (OXA)-induced ulcerative colitis. (A) Gross observation of the colon tissues in different groups. (B) Measurement of colon length. (C) Mean body weight over 7 days. (D) Disease activity index of experimental mice. Data are presented as mean ± standard error of the mean (SEM) of n = 6 mice/each group. ###p < 0.001, compared with the control group; *p < 0.05, **p < 0.01, and ***p < 0.001, compared with the OXA group; ***+p < 0.001, compared with the Rg3-RGE and P.T independently.

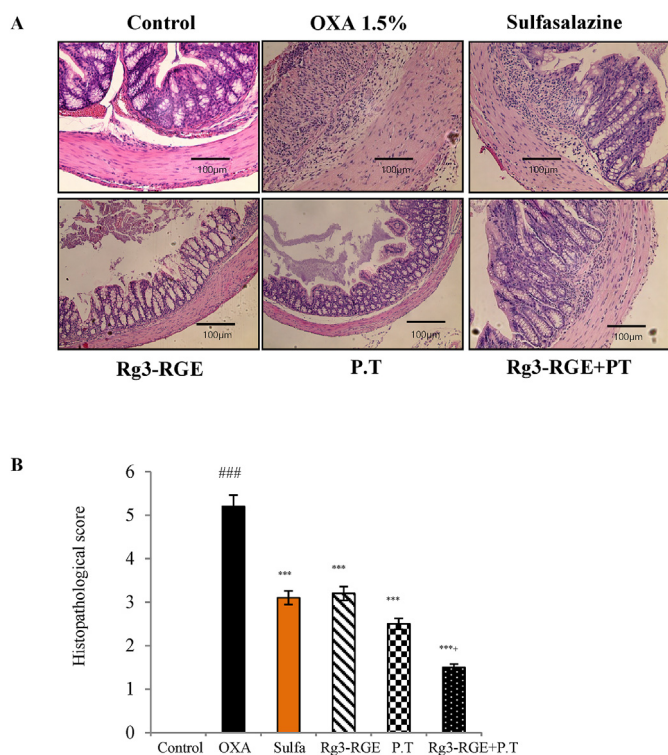


Fig. 2. Rg3-enriched Korean Red Ginseng extract and *Pericaria tinctoria* prevented oxazolone (OXA)-induced ulcerative colitis. (A) Colon tissues were stained with hematoxylin and eosin to detect morphological changes. (B) Pathological score for colon damage. Data are presented as mean ± standard error of the mean (SEM) of n = 6 mice/each group. ###p < 0.001, compared with the control group; *p < 0.05, **p < 0.01, and ***p < 0.001, compared with the OXA group; ****p < 0.001, compared with the Rg3-RGE and P.T independently.

3. Results

3.1. Effects of Rg3-enriched Korean Red Ginseng extract with *Pericaria tinctoria* on oxazolone-induced ulcerative colitis

The aim of this study is to investigate the preventive effects of Rg3-RGE and PT on OXA-induced UC using a mice model. The mice were treated for 7 days with the extracts of Rg3-RGE and PT alone as well as their combination treatment with the sulfasalazine being a positive control. Compared to the control, the OXA group had a remarkably lower colon length, which showed the establishment of colitis. In the Rg3-RGE with PT group, the colon length was retained similar to the sulfasalazine group (Fig. 1A and B). As expected, the body weight was markedly decreased in the OXA group compared to control group, and the body weight significantly recovered with the treatment of Rg3-RGE with PT (Fig. 1C).

Moreover, the DAI was markedly higher in the OXA-induced UC group compared to the control, and the DAI was significantly lesser in the Rg3-RGE with PT group than that in the alone treatment (Rg3-RGE or PT) (Fig. 1D). These results suggesting that the combine treatment of Rg3-RGE with PT surprisingly reduced the degree of UC in mice.

3.2. Rg3-enriched Korean Red Ginseng extract and *Pericaria tinctoria* prevented oxazolone-induced ulcerative colitis

To further investigate the histological changes, H&E staining was analyzed (Fig. 2A and B), and the pathological scores were calculated in accordance with the study of Geboes et al [22]. In

control group, the basic colon structure was normal, and the mucosa, submucosa, serosa, muscle, and crypt of the colon tissues were intact. Moreover, no infiltrated inflammatory cells were observed in the layers of the colon tissues. However, damage in the structures of the colon tissues, complete loss of crypt and epithelial cell integrity, and inflammatory cell infiltration were clearly observed in the OXA group. After the treatment with the Rg3-RGE and PT alone and their combined treatment, the OXA-induced injury was significantly relieved. Interestingly, a better therapeutic effect was observed in the combination treatment of Rg3-RGE and PT. Hence, the combination treatment of Rg3-RGE (10 mg/kg) and PT (150 mg/kg) was better for the prevention of OXA-induced injuries.

3.3. Effects of Rg3-enriched Korean Red Ginseng extract with *Pericaria tinctoria* on inflammatory cytokine production in oxazolone-induced ulcerative colitis

Next, we aimed to investigate the induction of NO and mRNA expression of pro-inflammatory mediators and cytokines (Fig. 3A–C). The NO induction is remarkably higher in the OXA group, in comparing with control. However, sulfasalazine and the combination treatment of Rg3-RGE and PT significantly suppressed the NO production compared with OXA-induced UC.

The mRNA expression levels of inflammatory factors, including inducible COX-2, iNOS, IL-6, TNF- α , IL-1 β , IL-5, IL-13, and NLRP3, were determined. In the sulfasalazine group and the Rg3-RGE (10 mg/kg) and PT (150 mg/kg) group, marked inhibition of COX-2, iNOS, TNF- α , IL-1 β , IL-6, IL-5, IL-13, and NLRP3 was observed (Fig. 3A and B). On the other hand, the OXA-induced UC group showed a significant upregulation of iNOS, COX-2, TNF- α , IL-1 β , IL-5, IL-6, IL-13, and NLRP3 compared with the control group. Furthermore, the results indicated that Rg3-RGE and PT have potent anti-inflammatory effects in the treatment of UC.

3.4. Effects of Rg3-enriched Korean Red Ginseng extract with *Pericaria tinctoria* on signaling pathway in oxazolone-induced ulcerative colitis

To elaborate on the mechanism of signaling pathways and protein expression levels in OXA-induced UC, the ELISA was used to confirm the protein expression levels of pro-inflammatory factors such as TNF- α , IL-1 β , IL-5, and IL-13 (Fig. 4A–D). The pro-inflammatory cytokine levels markedly increased in the OXA group compared with the control group. The contents of TNF- α , IL-1 β , IL-5, and IL-13 were remarkably reduced in the positive control (sulfasalazine) group and Rg3-RGE with the PT group compared with the OXA group.

The signaling pathway of Rg3-RGE and PT in the down-regulation of the OXA-induced UC was investigated using Western blot analysis (Fig. 4E). NF- κ B is the key regulator of pro-inflammatory mediators and cytokine production. Protein expression of NLRP3 and phosphorylated NF- κ B (p-NF- κ B) in the OXA group was significantly overexpressed compared with the control group. However, the sulfasalazine and Rg3-RGE with PT groups significantly suppressed the activation of NLRP3 and NF- κ B, which indicated that the treatment with Rg3-RGE and PT had targeted the NF- κ B signaling pathway reversing the OXA-induced inflammation.

3.5. Effects of Rg3-enriched Korean Red Ginseng extract and *Pericaria tinctoria* on immune cell subtype regulation in spleen

To justify the imbalance in the immune system, we determined the immune cell subtypes using an OXA-induced colitis model (Fig. 5A–C). In the OXA group, CD4⁺ T-cells, CD8 T-cells, CD19 B-

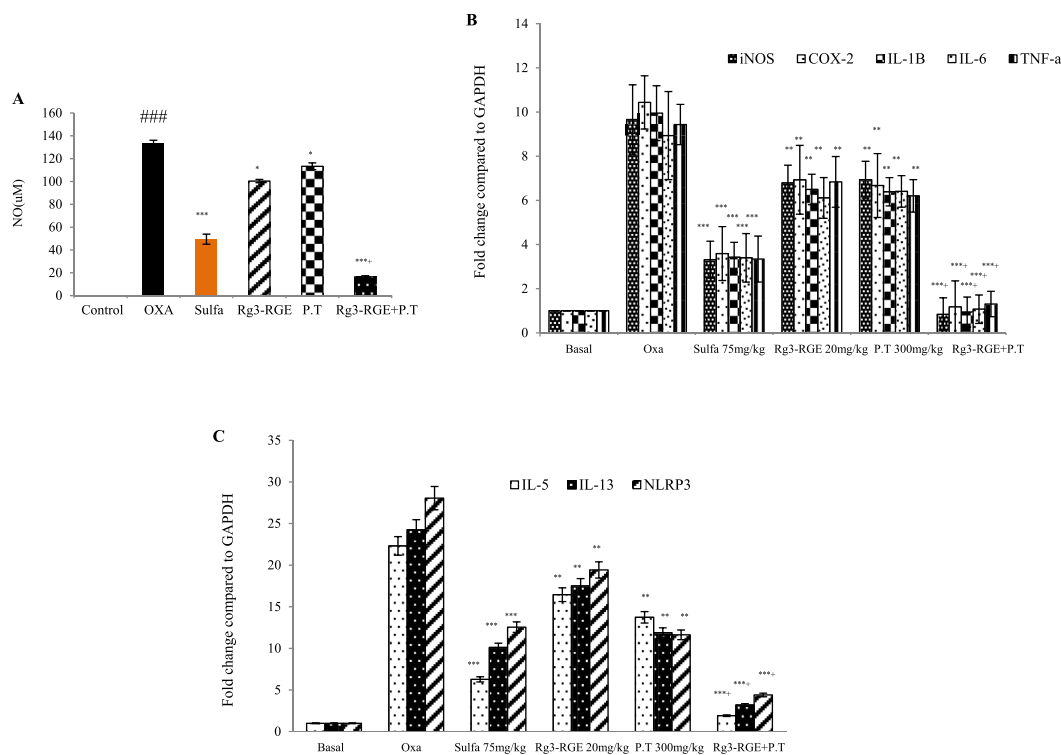


Fig. 3. Effects of Rg3-enriched Korean Red Ginseng extract and *Persicaria tinctoria* on the nitric oxide (NO) and pro-inflammatory cytokines production in oxazolone (OXA)-induced ulcerative colitis in mice. (A) NO production was analyzed in the plasma. (B) Expression of the pro-inflammatory mediators (e.g., iNOS and COX2) and cytokines (e.g., IL-1β, IL-6 and the TNF-α mRNA levels were analyzed. (C) Expression of the pro-inflammatory cytokines (e.g., IL-5 and IL-13) and NLRP3 mRNA levels were analyzed. Data are presented as mean ± standard error of the mean (SEM) of n = 6 mice/each group. ###p < 0.001, compared with the control group; *p < 0.05, **p < 0.01, and ***p < 0.001, compared with the OXA group; ****+p < 0.001, compared with the Rg3-RGE and P.T independently.

cells, and regulatory T-cells (CD4⁺-CD25⁺ Tregs cells) were reduced compared to the control group. Similar results were observed in our previous study [2]. However, treatment with the positive control (sulfasalazine) and the combination treatment of Rg3-RGE with PT significantly increased the T-cells (CD4⁺), B-cells (CD19), and regulatory T-cells. The trend of CD8 cytotoxic T-cells was higher in the Rg3-RGE with the PT group but not markedly compared to the OXA group. The FACS results indicated that combination treatment of Rg3-RGE with PT modulated the immune system in inflammation and UC.

4. Discussion

In the present study, oxazolone (OXA) was used to induce ulcerative colitis (UC) as the symptomatic and histopathological characteristics, which are similar between OXA-induced UC in mice and human UC. Previous studies have shown that the dextran sulfate sodium (DSS)-induced UC model is also extensively used to evaluate the effects of the medicinal plant extract, single compounds, and different drugs on UC [2,23,24]. Our group have shown that protective activity of Rg3-RGE and PT and their mixture on DSS-induced UC mice [2]. Here, we confirmed and demonstrated the alleviating property of Rg3-RGE with PT by using UC inducer such as OXA. Although, DSS-stimulated colitis is the common and extensively used method for colitis study but to elucidate and more confirm the reducing effect of Rg3-RGE with PT in UC, we used OXA to induce colitis model.

In our study, UC induced by the administration of 1.5 % OXA with drinking water in mice for 7 days and observed by the destruction of the mucosa in the colon tissues and inflammatory cells

infiltration in the mucosa and muscle layer. Previous studies also showed that oxazolone-induced damage in the colon structure and inflammatory cell infiltration in the mucosa [6,25]. Although a number of colitis models have been proposed, OXA-induced colitis in mice provides a new approach to assess the potency of the various therapeutic methods for UC.

A common treatment in mild to moderate UC, including steroidal and non-steroidal anti-inflammatory drugs and targeted drugs, depends on the inflammation and severity of the UC [6]. Sulfasalazine as a positive control drug was selected based on the previously reported significant therapeutic effects on UC [6,26,27].

Inflammatory process is the key inhibitory biological response to harmful stimuli, which including infections and tissue injuries [19,20,28]. Previous studies have shown that inflammatory cells are activated in DSS- and OXA-induced UC and lead to the over-production of pro-inflammatory cytokines [6]. Uncontrolled excessive expressions of pro-inflammatory cytokines are responsible for many chronic diseases, such as UC. In this study, we revealed that the oral administration of Rg3-RGE and PT attenuated OXA-induced UC.

It has been stated that IL-1β increases the expression of COX-2 and iNOS, which modulate the synthesis of NO and prostaglandin E2. Nuclear factor-κB (NF-κB) controls the inflammatory response by inducing other cytokines, such as IL-6, to be produced. Moreover, the NLRP3 inflammasome is also a known source of inflammation [2,13,14]. In the present study, Rg3-RGE, PT, and their combined treatment significantly downregulated the NO production and COX-2, iNOS, IL-6, TNF-α, IL-1β, IL-5, IL-13, and NLRP3 mRNA levels in UC mice. Our data are reliable with the previous study where it was indicated that UC was mediated by IL-13 [29].

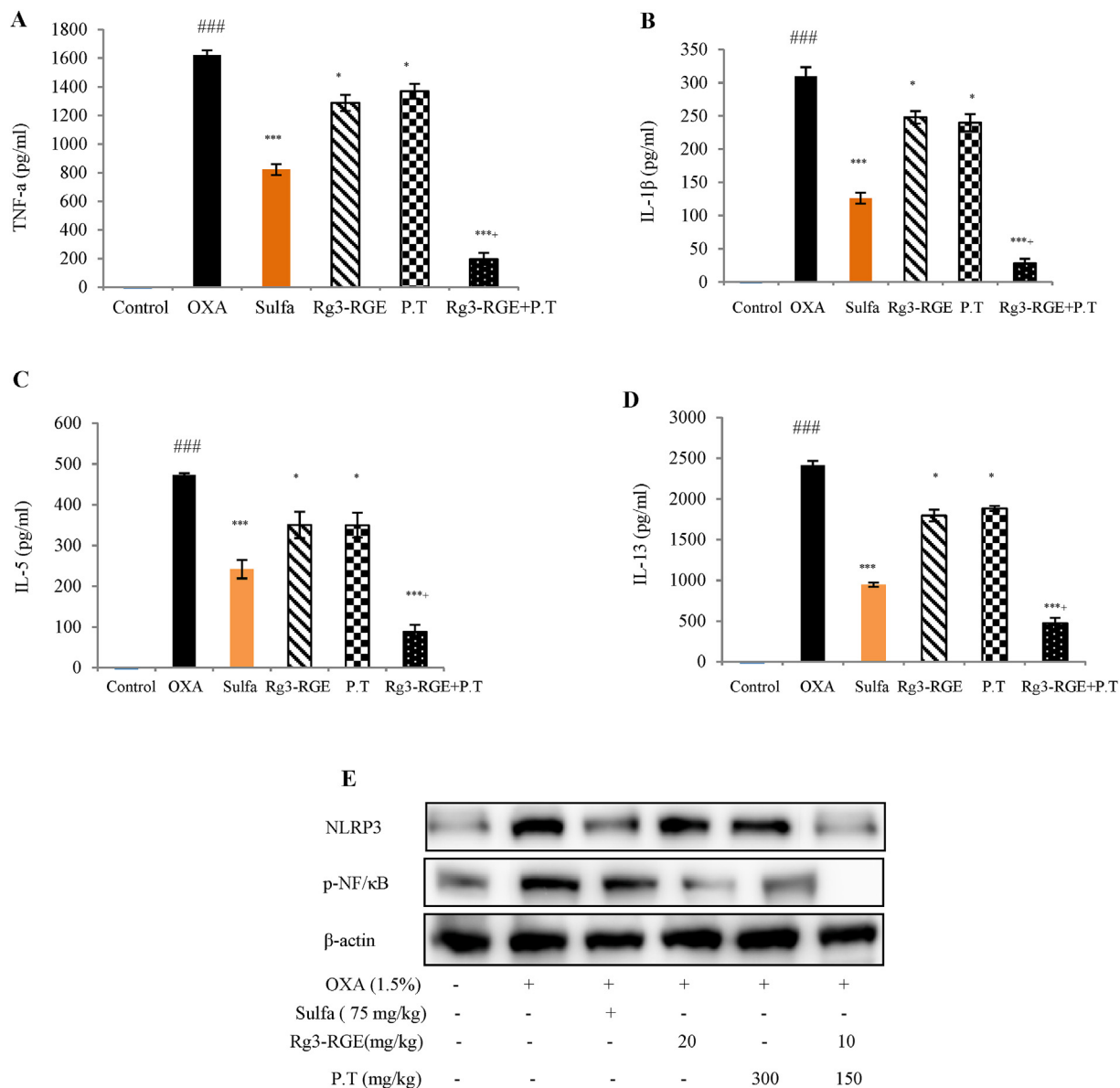


Fig. 4. Effects of Rg3-enriched Korean Red Ginseng extract and *Persicaria tinctoria* on oxazolone (OXA)-induced inflammation in mice. (A–D) Pro-inflammatory cytokines levels determined in the plasma using ELISA. (E) Protein levels of the NLR family pyrin domain containing 3 (NLRP3), nuclear factor-κB and β-actin used as loading control were analyzed in the colon tissues via western blot analysis. Data are presented as mean ± standard error of the mean (SEM) of n = 6 mice/each group. ###p < 0.001, compared with the control group; *p < 0.05, **p < 0.01, and ***p < 0.001, compared with the OXA group; ***+p < 0.001, compared with the Rg3-RGE and P.T independently.

Rg3-RGE and PT markedly reduced the protein levels of TNF-α, IL-1β, IL-5, and IL-13 in plasma. Interestingly, the combination treatment of Rg3-RGE and PT exhibited significant inhibitory effects compared with the individual treatment of Rg3-RGE or PT. OXA-induced UC promotes the overexpression of pro-inflammatory cytokines [30,31]. However, the treatment with Rg3-RGE and PT suppressed the OXA-induced pro-inflammatory cytokines. These results suggest that Rg3-RGE and PT have potent anti-inflammatory properties.

This study showed that Rg3-RGE has significantly reduced the pro-inflammatory cytokines and inhibited the NF-κB signaling pathway involved in inflammatory responses [15,16]. Various chemical agents activate the NF-κB signaling pathway and promote the phosphorylation of NF-κB, resulting in the nuclear translocation of active NF-κB [32–34]. This phenomenon induces the transcription of pro-inflammatory factors.

The results indicate that Rg3-RGE and PT inhibited the OXA-induced phosphorylation of NF-κB in UC mice. It has been reported that DSS-induced UC was suppressed by NF-κB and NLRP3 inflammasome activation [35,36]. The results of the western blot analysis indicated that Rg3-RGE and PT reversed the OXA-induced upregulation of NLRP3. Our data confirm that the suppression of the NF-κB pathway and inhibition of NLRP3 is involved in the anti-inflammatory properties of Rg3-RGE and PT in the UC model.

Previously, our group has shown that the number of subtypes of immune cells was modulated in the DSS-induced UC mice [2]. The results indicated that the treatment with Rg3-RGE and PT increased the immune cell subtypes, including CD4⁺ Th cells, CD19⁺ B-cells, and CD4⁺ and CD25⁺ regulatory T cells in the spleen tissues (Fig. 5A–C). It was reported that OXA-induced UC was alleviated through Th2/Th17 suppression and Treg induction [37]. Furthermore, the current study showed that Rg3-RGE and PT inhibited the

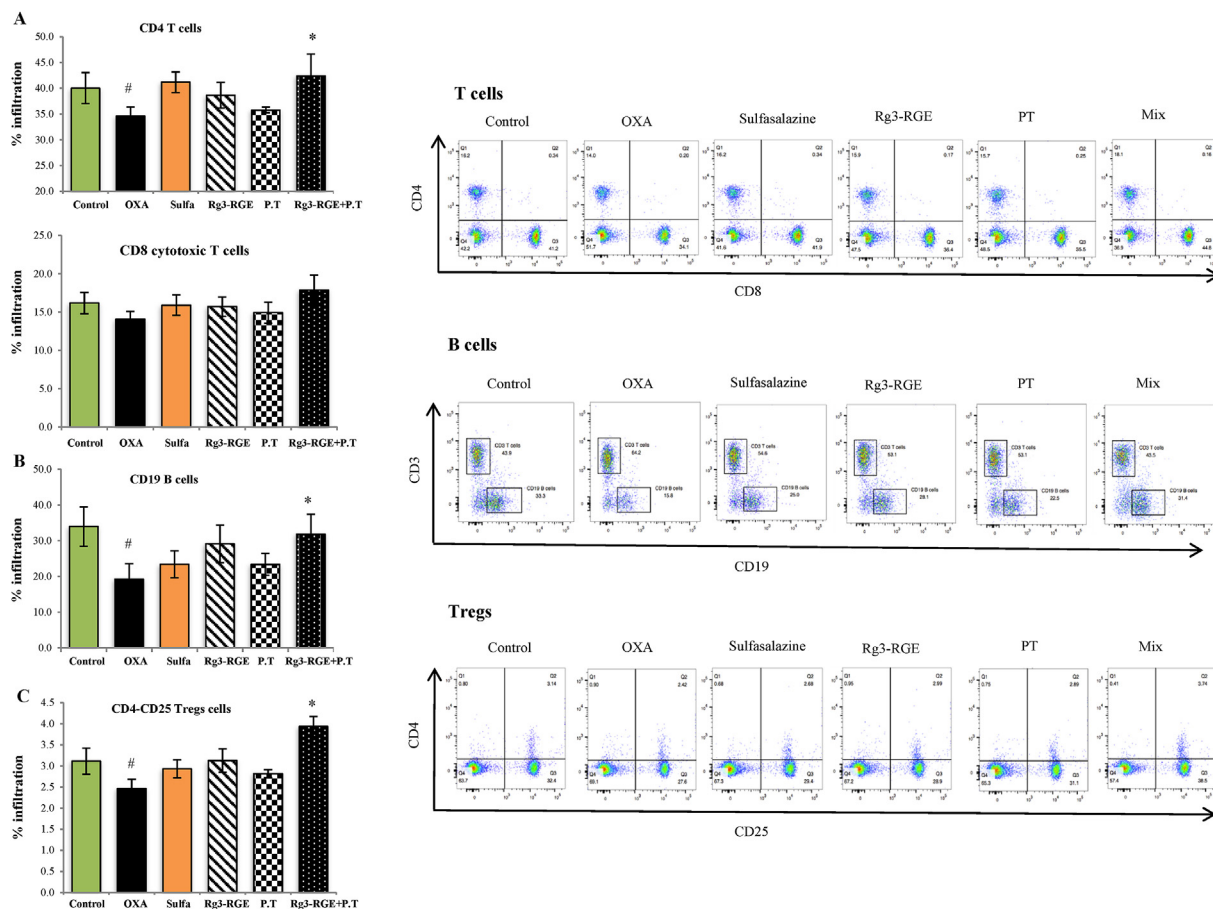


Fig. 5. Effects of Rg3-enriched Korean Red Ginseng extract and *Persicaria tinctoria* on the immune cells in the mice. (A) Percentage of the CD4+ T-cells and CD8+ cytotoxic T-cells were analyzed via fluorescence-activated cell sorting in the spleen tissues. (B) Percentage of the CD19+ B-cells. (C) CD4+ and CD25+ regulatory T-cells (Tregs) in the spleen. Data are presented as mean ± standard error of the mean (SEM) of n = 6 mice/each group. #p < 0.05, compared with the control group; *p < 0.05, compared with the OXA group.

pro-inflammatory cytokines production and upregulated the number of immune cell subtypes in OXA-induced UC.

5. Conclusion

Overall, Rg3-RGE, PT, and their combined treatment protected the mice against OXA-induced inflammation and colon injuries. The preventive effects may be mediated through the inhibition of the NF-κB pathway and reduced by NLRP3. The Rg3-RGE with PT inhibited the production of pro-inflammatory factors in the OXA-induced UC mice. Moreover, the combined treatment with Rg3-RGE and PT improved the OXA-induced macroscopic parameters, such as colon tissue length, loss of body weight, and DAI, and reduced histological colon tissue injuries. The treatment with Rg3-RGE and PT also modulated the CD4+ T-cells, CD19+ B-cells, and CD25+ regulatory T-cells in OXA-induced UC mice. However, it is important to investigate the therapeutic potency of Rg3-RGE and PT in a clinical trial.

Data availability

All data are analysed during this study and added with this manuscript.

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Declaration of competing interest

All authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jgr.2021.07.001>.

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