



## Research Article

## Gastroprotective effects of the nonsaponin fraction of Korean Red Ginseng through cyclooxygenase-1 upregulation

Jeong-Oog Lee<sup>1</sup>, Ji Hye Kim<sup>2</sup>, Sunggyu Kim<sup>3,4</sup>, Mi-Yeon Kim<sup>5,a</sup>, Yo Han Hong<sup>2,\*\*\*</sup>, Han Gyung Kim<sup>2,\*\*</sup>, Jae Youl Cho<sup>2,3,\*</sup><sup>1</sup> Department of Aerospace Information Engineering, Bio-Inspired Aerospace Information Laboratory, Konkuk University, Seoul, Republic of Korea<sup>2</sup> Department of Integrative Biotechnology, Sungkyunkwan University, Suwon, Republic of Korea<sup>3</sup> Research and Business Foundation, Sungkyunkwan University, Suwon, Republic of Korea<sup>4</sup> Department of Biocosmetics, Sungkyunkwan University, Suwon, Republic of Korea<sup>5</sup> School of Systems Biomedical Science, Soongsil University, Seoul, Republic of Korea

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## ABSTRACT

**Background:** Korean Red Ginseng is known to exhibit immune-enhancing and anti-inflammatory properties. The immune-enhancing effects of the nonsaponin fraction (NSF) of Korean Red Ginseng have been studied in many reports. However, the gastroprotective effect of this fraction is not fully understood. In this study, we demonstrate the activities of NSF for gastrointestinal protection and its related critical factor.

**Methods:** The *in vitro* and *in vivo* regulatory functions of NSF on cyclooxygenase-1 (COX-1) messenger RNA and protein levels were examined by reverse transcription polymerase chain reaction and immunoblotting analyses. Gastroprotective effects of NSF were investigated by histological score, gastric juice pH, and myeloperoxidase activity on indomethacin-induced, cold stress-induced, and acetylsalicylic acid-induced gastritis and dextran sulfate sodium-induced colitis in *in vivo* mouse models.

**Results:** NSF did not show cytotoxicity, and it increased COX-1 messenger RNA expression and protein levels in RAW264.7 cells. This upregulation was also observed in colitis and gastritis *in vivo* models. In addition, NSF treatment in mice ameliorated the symptoms of gastrointestinal inflammation, including histological score, colon length, gastric juice pH, gastric wall thickness, and myeloperoxidase activity.

**Conclusion:** These results suggest that NSF has gastroprotective effects on gastritis and colitis in *in vivo* mouse models through COX-1 upregulation.

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## 1. Introduction

Innate immunity is an essential system for protecting hosts from abnormal stimuli [1]. Danger stimuli are recognized by toll-like receptors, which induce inflammatory responses when triggered [2]. Acute inflammation proceeds in the short term and induces body defense mechanisms [3]. However, chronic inflammation lasts for a long time and often causes serious diseases such as arthritis and inflammatory bowel disease [4–7]. Therefore, studies on inflammatory responses are essential, and many researchers aim to develop anti-inflammatory drugs [8–10].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a popular treatment used for analgesic, antipyretic, and anti-inflammatory effects [11]. Representative NSAIDs include aspirin-based ibuprofen, naproxen, and indomethacin. Most NSAIDs inhibit cyclooxygenase (COX)-1 and -2, leading to a reduction in prostaglandin (PG) and thromboxane syntheses [12,13]. NSAIDs cause the most common drug toxicity in the United States; 8% of patients complain of dyspepsia, and continual use can lead to digestive ulcers [14,15]. In addition, alcohol, smoking, high-dose or multiple NSAIDs, anticoagulants, and corticosteroids increase the risk of gastrointestinal bleeding [16,17], and long-term use of NSAIDs is known to

\* Corresponding author: Department of Integrative Biotechnology, Sungkyunkwan University, 2066 Seobu-ro, Suwon, 16419, Republic of Korea.

\*\* Corresponding author: Department of Integrative Biotechnology, Sungkyunkwan University, 2066 Seobu-ro, Suwon 16419, Republic of Korea.

\*\*\* Corresponding author: Department of Integrative Biotechnology, Sungkyunkwan University, 2066 Seobu-ro, Suwon, 16419, Republic of Korea.

E-mail addresses: [ghddyks13@naver.com](mailto:ghddyks13@naver.com) (Y.H. Hong), [hanks523@skku.edu](mailto:hanks523@skku.edu) (H.G. Kim), [jaecho@skku.edu](mailto:jaecho@skku.edu) (J.Y. Cho).<sup>a</sup> The author contributed equally to this work.

increase the risk of death from cardiovascular diseases such as heart attack, stroke, and myocardial infarction [18]. NSAIDs are absorbed well in acidic environments because of their structural properties, so they are highly absorbed in the gastrointestinal tract [19]. NSAIDs then migrate through the mucosal layer into the epithelium and become neutral, causing epithelial tissue damage. The most important mechanism of gastrointestinal damage is to block production of PG, which inhibits COX-1 and protects the gastric mucosa. Anti-inflammatory analgesics such as aspirin reduce the gastro-protective effect by blocking COX-1 or COX-2 nonselectively. There are two ways to disrupt gastrointestinal protection: accelerating ulceration by inhibiting PG, which plays a critical role in maintaining gastric mucosal defense system and trapping and accumulation of NSAIDs in cells due to reionization by neutral pH [20].

Korean Red Ginseng (KRG) consists of a saponin fraction (SF), ginsenoside, and a nonsaponin fraction (NSF) composed of polyacetylene, a phenolic compound, and an acidic polysaccharide [21,22]. Through modern analytical technology, 32 kinds of saponins have been identified from red ginseng [23]. Some saponins derived from red ginseng have been reported to have antithrombosis, antifatigue, and anticancer effects [24–26]. Nonsaponin components, including polyacetylene-based compounds, phenol-based compounds, and acid polysaccharides, have also been reported to enhance the immune system, inhibit oxidation, provide resistance to aging, and inhibit inflammation [27,28]. Recently, it has been confirmed that acid polysaccharides induce macrophage activation and thus exhibit an immunity-enhancing effect [29,30]. In addition, polyacetylenes in NSF inhibit *Helicobacter pylori* infection [31]. To date, there are not many studies on the pharmacological efficacies of nonsaponin components in view of gastroprotective effects, and the components protecting against gastric damage are not yet known. In this study, we evaluated the gastroprotective effects of NSF and compared these effects to those of other ginseng components such as KRG water extract and SF.

## 2. Materials and methods

### 2.1. Materials

KRG, the NSF of KRG, and the SF of KRG were provided by the Korea Ginseng Cooperation (Daejeon, Korea). Detailed phytochemical profiles of these fractions are given in the Supplementary Information as previously reported [32]. Murine bone marrow-derived macrophage RAW264.7 cells were obtained from the American Type Culture Collection (Rockville, MD). Fetal bovine serum, Roswell Park Memorial Institute (RPMI) 1640, and antibiotics (penicillin and streptomycin) were purchased from Gibco (Grand Island, NY), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sigma Chemical Co. (St. Louis, MO). TRIzol reagent was purchased from Molecular Research Center (Montgomery, OH). A complementary DNA synthesis kit was purchased from Thermo Fisher Scientific (Waltham, MA). The forward and reverse primers used for reverse transcription polymerase chain reaction (RT-PCR) were synthesized by Macrogen (Seoul, Korea). PCR premix was purchased from Bio-D Inc. (Seoul, Korea). COX-1 and  $\beta$ -actin antibodies were purchased from Cell Signaling Technology (Beverly, MA). Male ICR mice (age: 6–8 weeks; weight: 17–21 g) and male Sprague Dawley (SD) rats (age: 6 weeks; weight: 150–160 g) were purchased from Orient Bio (Gyeonggi, Korea). All other chemicals were obtained from Sigma Chemical Co.

### 2.2. Cell culture

RAW264.7 cells were cultured in RPMI medium with 10% fetal bovine serum and 1% antibiotics in a CO<sub>2</sub> incubator at 37°C.

### 2.3. In vitro drug treatment

A stock solution of NSF was prepared in dimethyl sulfoxide at a concentration of 100 mg/ml. Target concentrations (50, 100, and 200  $\mu$ g/ml) were achieved by dilution with culture medium.

### 2.4. Cell viability assay

RAW264.7 cells were seeded in 96-well plates at a density of  $1.0 \times 10^5$  cells/well with fresh complete RPMI medium. To test the cytotoxicity of NSF alone, RAW264.7 cells were treated with NSF (50, 100, and 200  $\mu$ g/ml) for 24 h. Cell viability was determined by MTT assay.

### 2.5. In vivo drug treatment

Stock solutions of KRG, NSF, and SF were prepared in 0.5% carboxy-methyl-cellulose (CMC) at a concentration of 500 mg/kg. Target concentrations (100 and 200 mg/kg) were achieved by dilution with 0.5% CMC. Control compounds also were prepared in 0.5% CMC at the target concentration.

### 2.6. Dextran sulfate sodium colitis mouse model

Colitis was induced in seven 6-week-old male ICR mice via oral administration of 3% dextran sulfate sodium (DSS) (w/v) in fresh tap water for seven days. KRG (200 mg/kg), NSF (200 mg/kg), or SF (200 mg/kg) was orally administered every morning for seven days. Consumption of the DSS solution was controlled for all groups, and no major differences were detected. The anti-inflammatory effects of KRG, NSF, or SF in colitis were examined by measurement of colon length on day 7.

### 2.7. Indomethacin-induced gastritis in rat model

Gastric mucosal injury was induced in 6-week-old male SD rats by oral administration of indomethacin (40 mg/kg) suspended in 0.5% CMC. NSF (100 or 200 mg/kg) or omeprazole (10 mg/kg) were orally administered every morning for five days along with indomethacin (40 mg/kg). The anti-inflammatory effects of NSF in gastritis were examined by observing gastric mucosal lesions. The areas (mm<sup>2</sup>) of mucosal erosive lesions were measured under a dissecting microscope with a pixel counter on day 5.

### 2.8. Cold-stress gastritis model

Gastric mucosal injury was induced in 6-week-old male ICR mice by cold stress at 4°C for two hours. NSF (100 and 200 mg/kg) or ranitidine (20 mg/kg) was orally administered three times at intervals of 12 h. Cold stress was induced after the third oral administration. The gastroprotective effects of NSF in cold stress-induced gastritis were examined by observing gastric mucosal lesions.

### 2.9. Acetylsalicylic acid gastritis model

Gastric mucosal injury was induced in 6-week-old male ICR mice by acetylsalicylic acid (aspirin). NSF and ranitidine (20 mg/kg) were orally administered three times at intervals of 12 h. Acetylsalicylic acid (900 mg/kg) was given after the third oral administration of NSF or ranitidine. The protective effects of NSF in acetylsalicylic acid-induced gastritis were examined in gastric mucosal lesions.

**Table 1**  
Primer Sequences for Reverse Transcription Polymerase Chain Reaction

Name		Sequence (5' to 3')
Mouse COX-1	F	ATGAGTCGAAGGAGTCTCTCGC
	R	CAGGGATGGTACAGTTGGGG
Mouse GAPDH	F	ACCACAGTCCATGCCATCAC
	R	CCACCACCCTGTTGCTGTAG
Rat COX-1	F	GCCCTCCAACCTACAACACA
	R	TCCCTTCTCAGCAGCAATCG
Rat GAPDH	F	ACCACAGTCCATGCCATCAC
	R	TCCACCACCCTGTTGCTGT

COX-1, cyclooxygenase-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

### 2.10. Analysis of messenger RNA levels by RT-PCR

To quantify COX-1 messenger RNA (mRNA) expression levels, total RNAs from *in vivo* colitis and gastritis and *in vitro* RAW264.7 samples were isolated with TRIzol reagent according to the manufacturer's instructions. RT-PCR was performed as described previously [9]. The primers used in this study are listed in Table 1.

### 2.11. Immunoblotting

The total lysates prepared from *in vivo* colitis and gastritis and *in vitro* RAW264.7 samples were subjected to Western blot analysis for COX-1 and  $\beta$ -actin. The immunoreactive bands were visualized as described previously [33].

### 2.12. Histological analysis

Tissue samples from the stomachs of various gastritis mouse models were fixed with 10% formalin in phosphate buffered saline and then embedded in paraffin. Approximately 4- $\mu$ m-thick tissue sections were stained with hematoxylin and eosin for histopathological examination as reported previously [34].

### 2.13. Myeloperoxidase assay

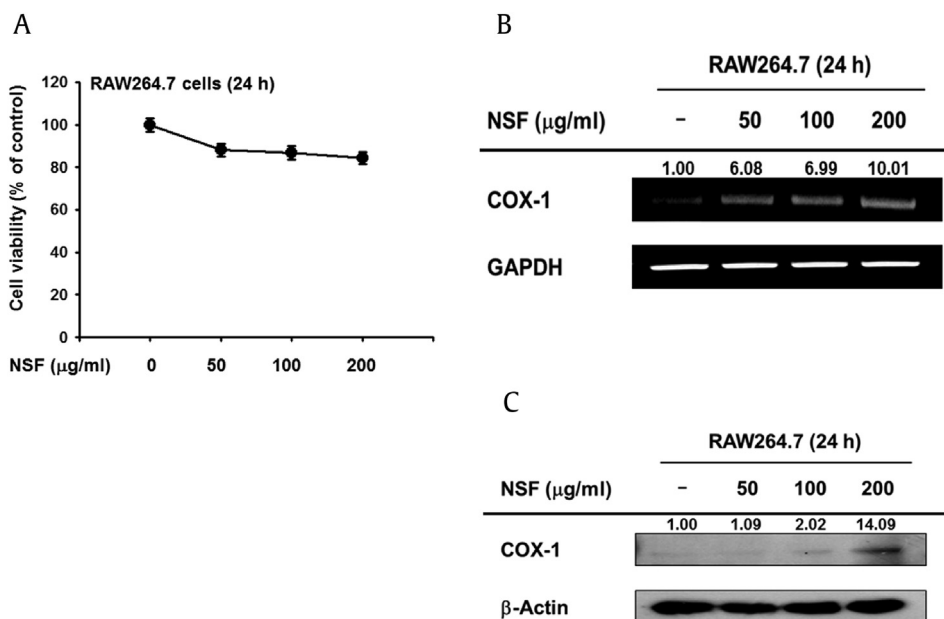
Myeloperoxidase (MPO) activity was measured using an MPO activity colorimetric assay kit from BioVision (Milpitas, CA) [35]. Stomachs were homogenized in four volumes of phosphate buffered saline containing 0.1% NP-40. After lysis, samples were centrifuged at  $13000 \times g$  for 10 min to remove insoluble material. Soluble materials were diluted in MPO assay buffer. MPO substrate was added, samples were incubated at 25°C for 60 min, and then stop mix was added. Incubation was continued for an additional 10 min to stop the reaction. Trinitrobenzene sulfonic acid reagent containing the 5,5'-dithiobis-(2-nitrobenzoic acid) probe and tris(2-carboxyethyl)phosphine hydrochloride was added. The presence of MPO was measured at 412 nm, and MPO activity was expressed as U/g tissue. One unit of MPO activity was defined as the amount of MPO that generates sufficient taurine chloramines to consume 1  $\mu$ M of trinitrobenzene sulfonic acid/min at 25°C.

### 2.14. Measurement of murine intragastric pH

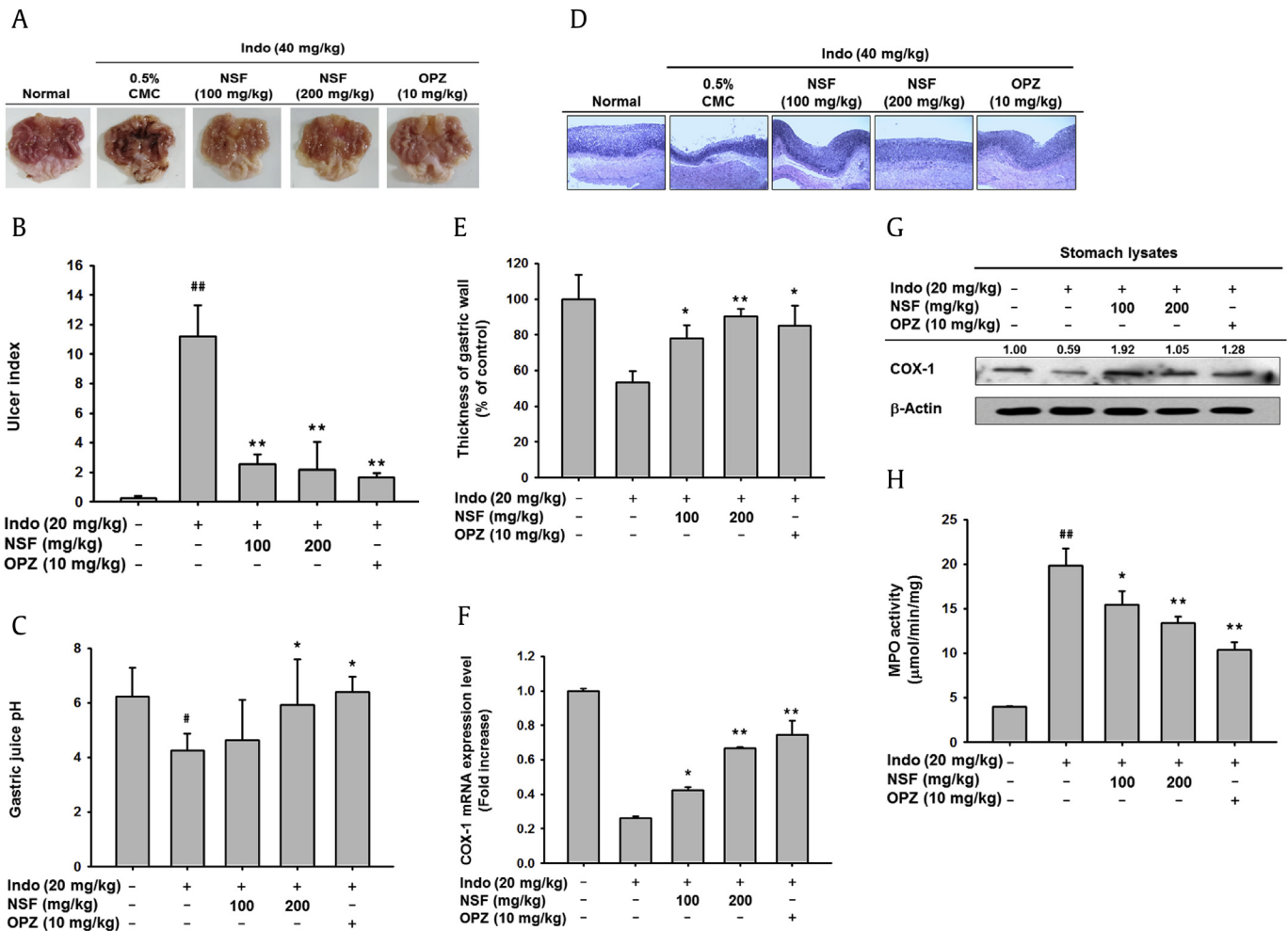
For intragastric pH measurements, mice were sacrificed with pentobarbital (50 mg/kg intraperitoneally), stomachs were excised, and the pH of the gastric contents was immediately measured using a pH meter.

### 2.15. Statistical analysis

All data are presented as mean  $\pm$  standard deviation, and each experiment consisted of three replications. The Mann-Whitney U test was used to analyze the statistical difference between groups. A *p* value less than 0.05 was regarded as statistically significant. All statistical tests were performed using SPSS software (IBM Corp., Armonk, NY).



**Fig. 1.** Effects of NSF on cell viability and COX-1 expression in RAW264.7 cells. (A) RAW264.7 cells were treated with NSF (0–200  $\mu$ g/ml) for 24 h, and cell viability was determined by MTT assay. (B) RAW264.7 cells were treated with NSF (0–200  $\mu$ g/ml) for 24 h, and the mRNA levels of COX-1 and GAPDH were determined by immunoblotting. Band intensity was measured by ImageJ. COX-1, cyclooxygenase-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NSF, nonsaponin fraction; RT-PCR, reverse transcription polymerase chain reaction.



**Fig. 2.** Effects of NSF in an indomethacin-induced gastritis rat model. (A, B) SD rats were orally treated with NSF (100 or 200 mg/kg) or omeprazole (10 mg/kg) once a day along with indomethacin for five days. After sacrifice of the rats, gastric lesions were imaged using an optical digital camera. Formation of stomach lesions was evaluated using a pixel counter. (C) The pH of collected gastric juice was measured by a pH meter. (D, E) Histological examination of sections of gastric tissue stained with hematoxylin and eosin. Images were captured using an optical digital camera. Thicknesses of gastric walls were measured using ImageJ. (F) COX-1 mRNA level was determined by real-time PCR. (G) Protein levels of COX-1 and  $\beta$ -actin were determined by immunoblotting. (H) MPO activity as an indicator of neutrophil infiltration in the stomach was analyzed in total lysates of stomach.  $^{\#}p < 0.05$  and  $^{\#\#}p < 0.01$  compared with the normal group.  $^*p < 0.05$  and  $^{**}p < 0.01$  compared with the control group. Band intensity was measured using ImageJ. COX-1, cyclooxygenase-1; MPO, myeloperoxidase; NSF, nonsaponin fraction; PCR, polymerase chain reaction; SD, Sprague Dawley.

### 3. Results

#### 3.1. Noncytotoxicity and COX-1 upregulation effects of NSF in RAW264.7 cells

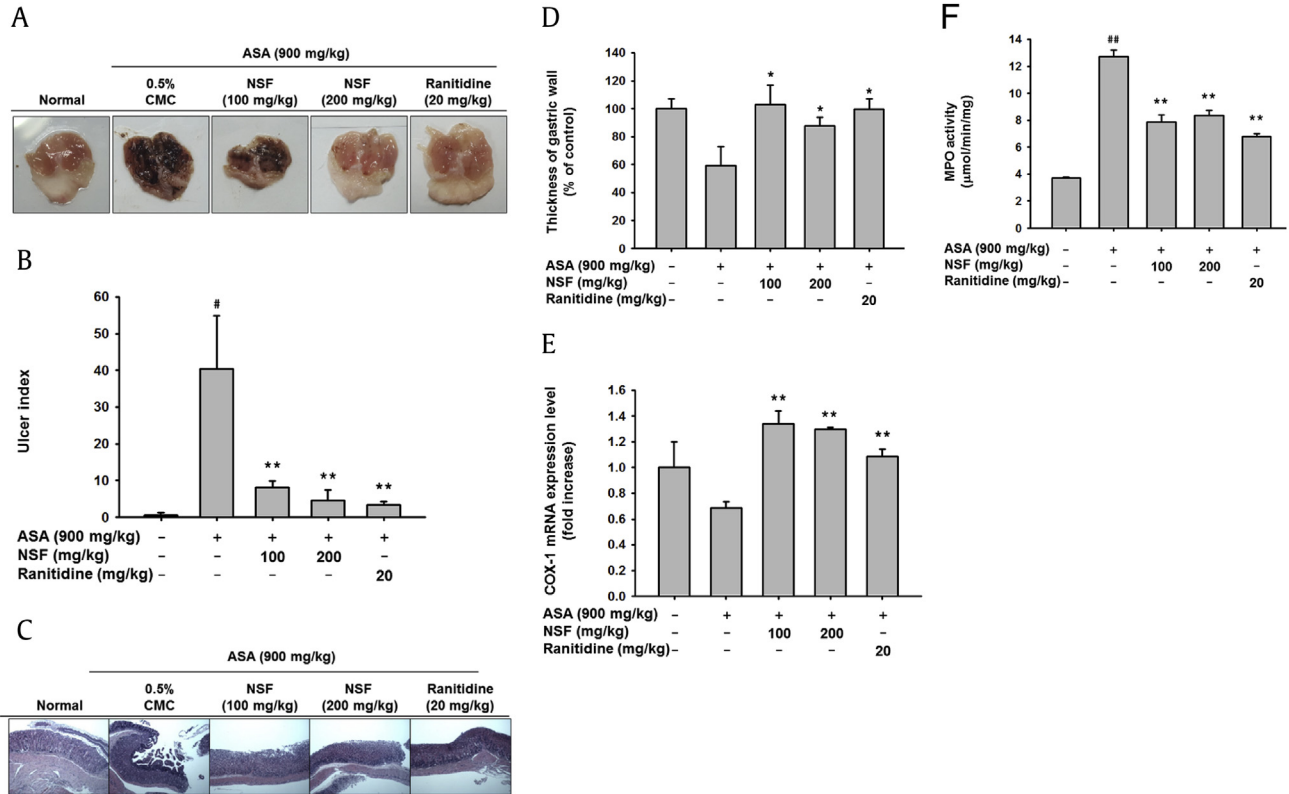
To test its effect on cell viability, NSF was administered at concentrations of 0 to 200 mg/ml in RAW264.7 cells. As shown in Fig. 1A, treatment with NSF had no effect on RAW264.7 cells at concentrations up to 200  $\mu$ g/ml. This implies that 200  $\mu$ g/ml of NSF has little cytotoxic effect up to 10%, but NO production was inhibited by the treatment of NSF. Then, we examined whether NSF regulated COX-1 expression. NSF increased the mRNA and protein levels of COX-1 in RAW264.7 cells (Fig. 1B and C). In line with previous reports, these results suggest that NSF has gastro-protective effects through regulation of COX-1 [36–38].

#### 3.2. Gastroprotective effects of NSF in the indomethacin-induced gastritis rat model

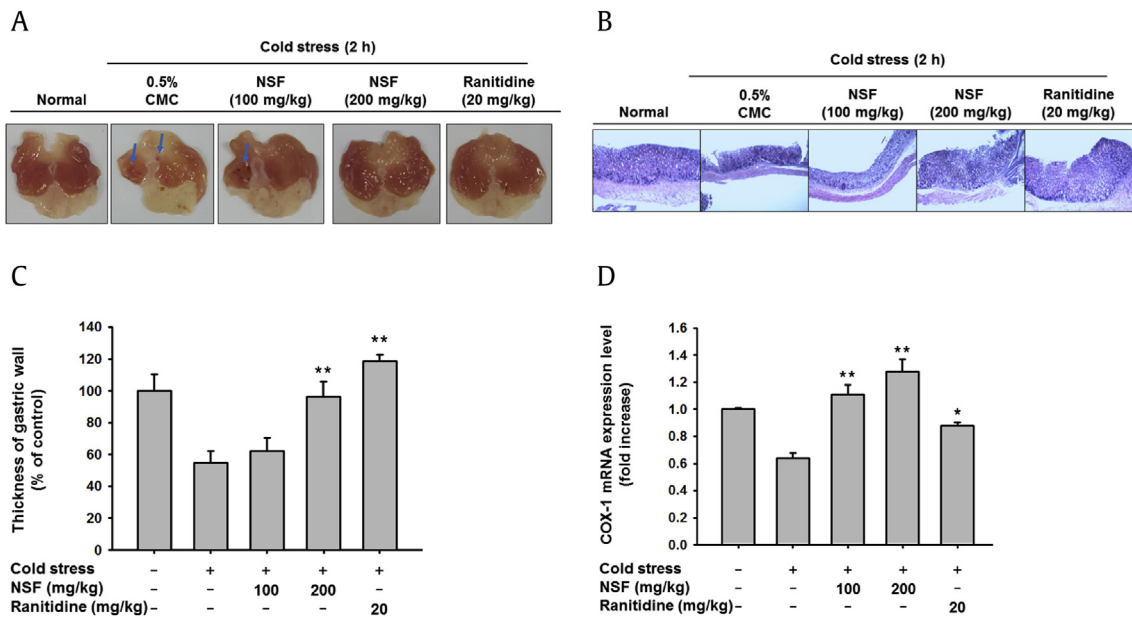
In previous reports, overdoses of NSAIDs caused gastritis [37,39]. To examine the gastroprotective effects of NSF in indomethacin-

induced gastritis in rats, SD rats were treated with NSF (100 and 200 mg/kg) or omeprazole (10 mg/kg) as a control drug along with indomethacin (40 mg/kg) for five days. Orally administered NSF (100 mg/kg) ameliorated the ulcerative lesions in stomachs of rats (Fig. 2A and B). We then measured the pH of gastric juices in the indomethacin-induced gastritis rat model. When gastritis occurs, the pH of gastric juice decreases with a large amount of gastric acid secretion, worsening the condition. Normally, gastric juice is maintained at pH 6 but decreases to pH 4 under inflammatory conditions. However, the pH of gastric juice reduced by indomethacin recovered to normal level upon treatment with NSF (200 mg/kg) (Fig. 2C). Histological evaluations of the gastric mucosal tissue in the indomethacin-induced gastritis model are demonstrated in Fig. 2D and E. Indomethacin induced severe damage to the gastric epithelium with inflammatory infiltrates. In the histological observation of gastric lesions induced by indomethacin, rats treated with NSF (100 and 200 mg/kg) exhibited ameliorated mucosal damage compared with the indomethacin group. Furthermore, the thickness of the gastric wall in rats treated with NSF (200 mg/kg) or omeprazole (10 mg/kg) as a control drug appeared comparable to that in normal rats (Fig. 2D and E). Then,





**Fig. 3.** Effects of NSF in an acetylsalicylic acid–induced gastritis mouse model. (A, B) Mice were orally treated with NSF (100 or 200 mg/kg) or ranitidine (20 mg/kg) once a day for two days before induction of gastritis with acetylsalicylic acid. After sacrifice of the mice, gastric lesions were imaged using an optical digital camera. Formation of stomach lesions was evaluated using a pixel counter. (C, D) Histological examination of sections of gastric tissue stained with hematoxylin and eosin. Images were captured using an optical digital camera. Thicknesses of gastric walls were measured using ImageJ. (E) COX-1 mRNA level was determined by real-time PCR. (F) MPO activity as an indicator of neutrophil infiltration in the stomach was analyzed in total lysates of stomach. <sup>#</sup>*p* < 0.05 and <sup>##</sup>*p* < 0.01 compared with the normal group. <sup>\*</sup>*p* < 0.05 and <sup>\*\*</sup>*p* < 0.01 compared with the control group. COX-1, cyclooxygenase-1; MPO, myeloperoxidase; NSF, nonsaponin fraction; PCR, polymerase chain reaction.



**Fig. 4.** Effects of NSF in a cold stress–induced gastritis mouse model. (A) Mice were orally treated with NSF (100 or 200 mg/kg) or ranitidine (20 mg/kg) once a day for two days before induction of cold stress. After sacrifice of the mice, gastric lesions were imaged using an optical digital camera. (B, C) Histological examination of sections of gastric tissue stained with hematoxylin and eosin. Images were captured using an optical digital camera. Thicknesses of gastric walls were measured using ImageJ. (D) COX-1 mRNA level was determined by real-time PCR. <sup>#</sup>*p* < 0.05 and <sup>##</sup>*p* < 0.01 compared with the normal group. <sup>\*</sup>*p* < 0.05 and <sup>\*\*</sup>*p* < 0.01 compared with the control group. COX-1, cyclooxygenase-1; NSF, nonsaponin fraction; PCR, polymerase chain reaction.

we tested the mRNA and protein expression levels of COX-1 in the indomethacin-induced gastritis *in vivo* model. Treatment with NSF (100 and 200 mg/kg) increased the mRNA and protein levels of COX-1 that had been reduced by indomethacin (Fig. 2F and G). We investigated whether NSF regulates MPO activity as an indicator of neutrophil infiltration into damaged gastric mucosal tissue. Enhancement of MPO activity is related to severity of gastric mucosal injury [38,40]. MPO activity was upregulated by indomethacin. However, NSF (100 and 200 mg/kg) significantly reduced indomethacin-mediated MPO activity in a dose-dependent manner (Fig. 2H) [35].

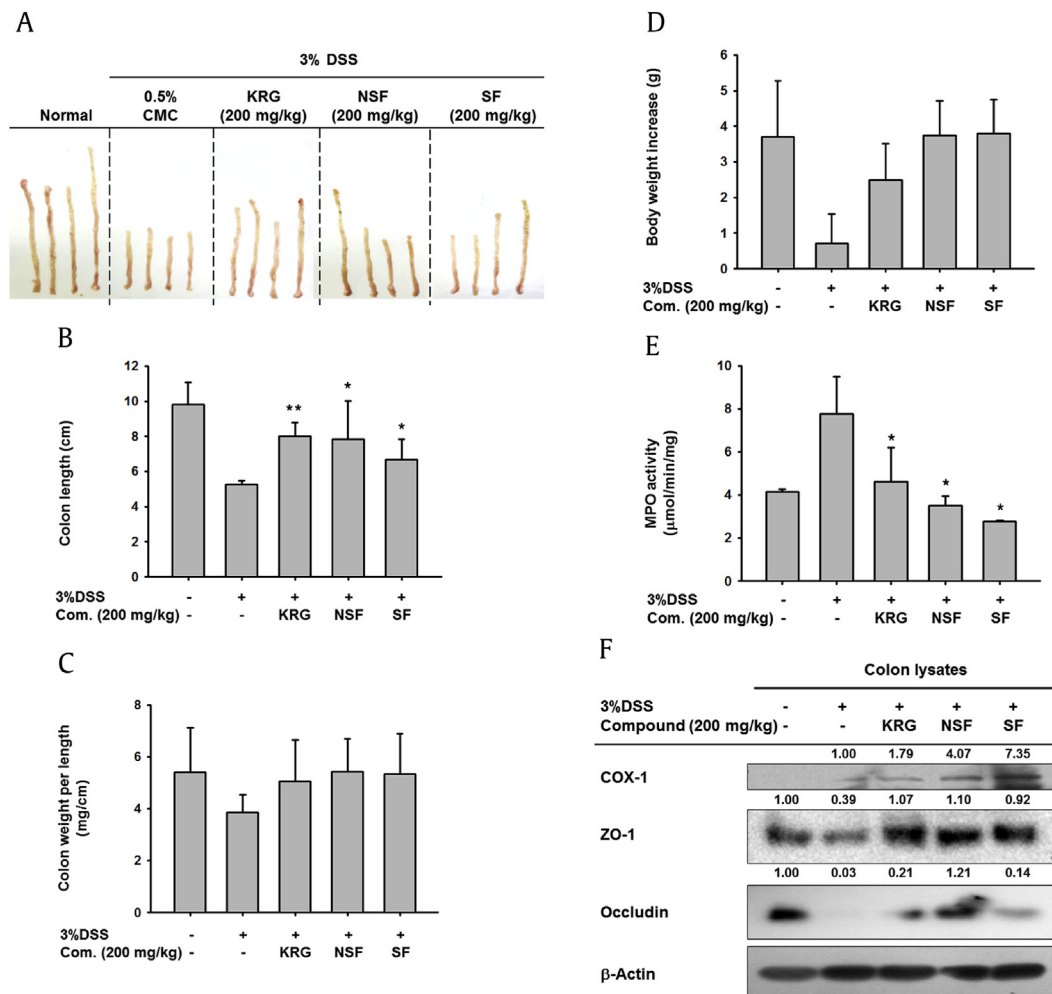
### 3.3. Gastroprotective effects of NSF in the acetylsalicylic acid–induced gastritis mouse model

To study the gastroprotective effects of NSF under conditions of acetylsalicylic acid abuse, we orally administered NSF (100 and 200 mg/kg) or ranitidine (20 mg/kg) as a control drug to ICR mice. After that, mice were orally administered acetylsalicylic acid (900 mg/kg) for two hours to induce gastritis. In this acetylsalicylic acid–induced gastritis *in vivo* model, treatment with NSF dose-

dependently suppressed the gastric ulcerative lesions (Fig. 3A and B). According to histological analysis, gastric mucosal tissues were impaired by acetylsalicylic acid. However, this gastric mucosal tissue damage was ameliorated by NSF (100 and 200 mg/kg) treatment (Fig. 3C and D). Then, we analyzed mRNA expression level of COX-1 in the acetylsalicylic acid–induced gastritis model. Treatment with NSF (100 and 200 mg/kg) significantly increased the COX-1 mRNA level that had been reduced by acetylsalicylic acid (Fig. 3E). In addition, acetylsalicylic acid enhanced MPO activity, but this upregulation was significantly inhibited by NSF (100 and 200 mg/kg) treatment (Fig. 3F).

### 3.4. Gastroprotective effects of NSF in the cold stress–induced gastritis mouse model

We further evaluated the gastroprotective effects of NSF through cold-restrained stress in mice. ICR mice were treated with NSF (100 and 200 mg/kg) or ranitidine (20 mg/kg) as a control drug for two days. After drug administration, mice were exposed to cold stress at 4°C for two hours. NSF (200 mg/kg) reduced the gastric ulcerative lesions in the cold stress–induced gastritis *in vivo* model



**Fig. 5.** Effects of NSF in a DSS-induced colitis mouse model. (A, B) Mice were orally treated with KRG (200 mg/kg), NSF (200 mg/kg), or SF (200 mg/kg) once a day along with 3% DSS in tap water for seven days. After sacrifice of the mice, colon lengths were measured using a ruler. (C) MPO activity as an indicator of neutrophil infiltration in the stomach was analyzed in total lysates of colon. (D) Body weight increase was determined by changes in body weight after oral administration of NSF. (E) Colon weight per length ratio was calculated by measured colon weight and length scales. (F) Protein levels of COX-1, ZO-1, occludin, and  $\beta$ -actin in the colons of DSS-induced colitis mice treated with NSF were determined by immunoblotting.  $^{\#}p < 0.05$  and  $^{\#\#}p < 0.01$  compared with the normal group.  $^*p < 0.05$  and  $^{**}p < 0.01$  compared with the control group. Band intensity was measured using ImageJ.

COX-1, cyclooxygenase-1; DSS, dextran sulfate sodium; KRG, Korean Red Ginseng; MPO, myeloperoxidase; NSF, nonsaponin fraction; SF, saponin fraction.

(Fig. 4A). With respect to histological aspects, cold stress induced gastric mucosal damage and infiltration of immune cells. These histological changes were recovered to normal levels by NSF (200 mg/kg) treatment (Fig. 4B and C). In addition, we investigated COX-1 mRNA expression level in the cold stress–induced gastritis *in vivo* model. Cold stress suppressed the mRNA expression of COX-1; this downregulation was counteracted by NSF (100 and 200 mg/kg) treatment in a dose-dependent manner (Fig. 4D).

### 3.5. Intestinal protective effects of NSF in the DSS-induced colitis mouse model

Then, we determined whether NSF has intestinal protective effects in a DSS-induced colitis *in vivo* model. To induce colitis, a dose of 3% DSS dissolved in water was orally administered to ICR mice. At the same time, KRG, NSF, and SF were also orally administered to the mice at a dose of 200 mg/kg. One of the markers of DSS-induced colitis is a reduction in colon length [41]. The average length of the colon was 9.8 cm in the normal group but decreased to 5.2 cm in the colitis group. When KRG, NSF, and SF were orally administered to the colitis-induced mice, average colon length recovered to 8.0, 7.8, and 6.6 cm, respectively (Fig. 5A and B). To compare the groups, changes in body and colon weights were examined. Colon weight per length and body weight were reduced by 3% DSS-mediated colitis symptoms, but KRG, NSF, and SF administration recovered these measures (Fig. 5C and D). The effect of NSF on MPO activity was determined by an MPO activity assay. DSS-induced colitis strongly enhanced MPO activity, whereas administration of KRG, NSF, and SF significantly reduced this activity (Fig. 5E). In addition, we investigated protein levels of COX-1 and tight junction–related proteins (ZO-1 and occludin) in the DSS-induced colitis *in vivo* model. COX-1 protein expression level increased markedly in the NSF and SF treatment groups, while ZO-1 and occludin were recovered up to normal levels from the downregulation caused by DSS treatment (Fig. 5F).

## 4. Discussion

The aim of this study was to demonstrate the gastroprotective effect of KRG NSF. NSF was administered in experimental animal models of gastrointestinal inflammation, and its role in the treatment of gastrointestinal inflammation was confirmed. The anti-inflammatory effects of red ginseng extract have been shown in previous studies [32], and recent research has been performed to clarify the anti-inflammatory effects of the SF [42]. However, the role of NSF of KRG and its mechanisms are not yet fully understood. Our study identified the specific mechanism of the NSF in red ginseng, suggesting its possible use as a therapy alongside drugs that induce inflammation in the gastrointestinal tract.

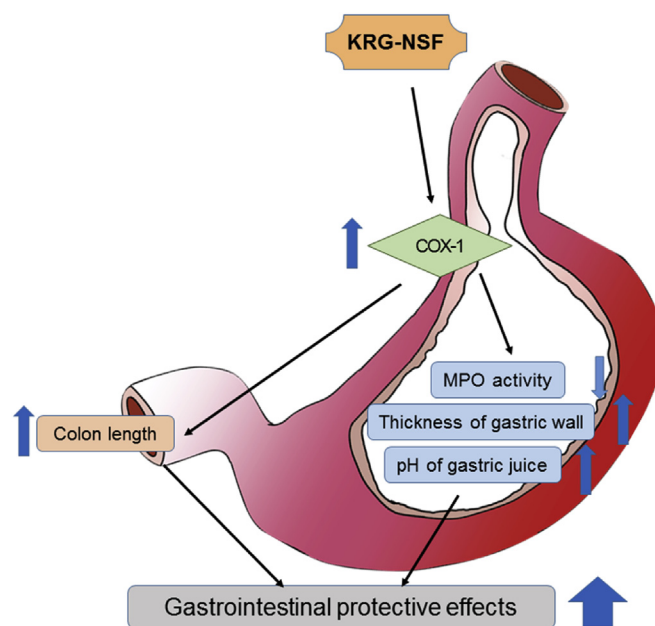
In this study, the effects of NSF on mouse macrophages and the function of NSF were investigated using mouse and rat models with various types of gastrointestinal inflammation. COX-1 expression increased when mouse macrophages were treated with NSF (Fig. 1). Downregulation of COX-1 is a side effect of various anti-inflammatory agents such as indomethacin or acetylsalicylic acid. COX-1 protects the gastrointestinal tract by regulating the expression of PG. When COX-1 is inhibited, it induces damage to the gastric mucosa, leading to gastritis [43,44].

To demonstrate the protective function of the gastrointestinal tract by regulating COX-1 expression with NSF in animal models, we conducted experiments related to ulcers induced by NSAIDs, which are COX-regulating drugs. Indomethacin and acetylsalicylic acid are NSAID drugs that exhibit anti-inflammatory efficacy through a typical COX regulator [45,46]. Both drugs inhibit the synthesis of PG through inhibition of COX, induce gastric lesions at

topical sites, and cause stomach ulcers. PG induced by COX is an essential protein for synthesis of gastric mucus and bicarbonate and induction of secretions to protect the gastric mucosa. PG also increases blood flow in the gastric mucosa and promotes epithelial cell division [47]. Our results show that pretreatment with NSF (200 mg/kg) reduced the severity of gastric lesions induced by indomethacin or acetylsalicylic acid. In addition, NSF treatment recovered the damaged gastric mucosa and increased expression of COX-1 (Figs. 2 and 3).

To demonstrate the effects of NSF under conditions that induce gastrointestinal injury other than gastrointestinal inflammation by NSAIDs, we used a gastric lesion model based on cold stress–induced gastritis and a DSS-induced colitis model. Stress-based gastric lesions present with serious damage due to extreme stressors including burns, diverse organ failures, major surgery, or sepsis [48]. To identify acute gastric ulcers, an experimental cold-restraint stress model is widely used [49]. COX-1 is a major factor associated with development of gastric stress ulcers during cold-restraint stress [50]. Cold-restraint stress induced gastric mucosa ulceration, while the gastric mucosa was partly recovered by NSF (200 mg/kg). Furthermore, COX-1 was upregulated in the gastric mucosa region (Fig. 4).

DSS is used in most studies to induce colitis in mouse models. Research on the gastrointestinal tract using DSS has been applied to various studies of gastrointestinal inflammation. The specific pathway for the intestinal inflammation induced by DSS is not clear, but DSS stimulates damage to the epithelial monolayer lining in the large intestine, allowing proinflammatory molecules such as bacterial products or cytokines to reach the underlying tissue. DSS is a negatively charged, water-soluble, sulfated polysaccharide with a highly variable molecular weight. DSS dissolved in water was administered to induce intestinal inflammation in mice, producing severe murine colitis after seven days [51]. NSF restored intestinal length and enhanced the expression of COX-1, occludin, and ZO-1 through intestinal mucosal protection in the DSS-induced colitis model (Fig. 5).



**Fig. 6.** Effects of NSF on COX-1–mediated gastrointestinal protection. COX-1, cyclooxygenase-1; KRG, Korean Red Ginseng; MPO, myeloperoxidase; NSF, nonsaponin fraction.

The mechanism by which NSF increases COX-1 level in gastric inflammation conditions is not clear from current data. As NSF was found to modulate COX-1 mRNA expression (Fig. 1B and C), some components in this fraction may be activators of transcription factors that induce expression of the COX-1 gene. Thereafter, increased level of COX-1 seems to be involved in various gastro-protective activities as well as antigastritis effects by suppressing MPO activity and neutralizing gastric juice pH. Previous reports have revealed that MPO activity is regulated by COX-1 and that inhibition of COX-1 increases MPO activity [52,53]. Another study found that gastric acid secretion can be inhibited by COX-1 in rat and mouse [54]. Therefore, we assume that increased transcriptional level of COX-1 by NSF could be a key mechanism to explain the protective role of NSF in gastric damage.

In conclusion, our results show that NSF possesses protective effects against gastric ulcers induced by indomethacin, acetylsalicylic acid, and cold-restraint stress, as well as DSS-induced colitis in mouse models. As summarized in Fig. 6, NSF ameliorates inflammation through regulation of COX-1, which affects colon length, pH of gastric juice, thickness of the gastric wall, MPO activity, and histological factors. Accordingly, we suggest the possibility of NSF as a gastroprotective drug.

### Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

### Acknowledgments

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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.2019.11.001>.

### References

- Medzhitov R, Janeway Jr CA. Decoding the patterns of self and nonself by the innate immune system. *Science* 2002;296:298–300.
- Kärre K, Ljunggren HG, Piontek G, Kiessling RJN. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. *Nature* 1986;319:675.
- Ryan GB, Majno G. Acute inflammation. A review. *Am J Pathol* 1977;86:183–276.
- Podolsky DK. Inflammatory bowel disease. *New England Journal of Medicine* 1991;325:1008–16.
- de Mattos BR, Garcia MP, Nogueira JB, Paiatto LN, Albuquerque CG, Souza CL, Fernandes LG, Tamashiro WM, Simioni PU. Inflammatory bowel disease: an overview of immune mechanisms and biological treatments. *Mediators Inflamm* 2015;2015:493012.
- Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature* 2003;423:356–61.
- Qomaladewi NP, Kim MY, Cho JY. Autophagy and its regulation by ginseng components. *J Ginseng Res* 2019;43:349–53.
- Kim E, Yi YS, Son YJ, Han SY, Kim DH, Nam G, Hossain MA, Kim JH, Park J, Cho JY. BIOGFIK, a compound K-rich fraction of ginseng, plays an anti-inflammatory role by targeting an activator protein-1 signaling pathway in RAW264.7 macrophage-like cells. *J Ginseng Res* 2018;42:233–7.
- Han SY, Kim J, Kim E, Kim SH, Seo DB, Kim JH, Shin SS, Cho JY. AKT-targeted anti-inflammatory activity of Panax ginseng calyx ethanolic extract. *J Ginseng Res* 2018;42:496–503.
- Lee JO, Choi E, Shin KK, Hong YH, Kim HG, Jeong D, Hossain MA, Kim HS, Yi YS, Kim D, et al. Compound K, a ginsenoside metabolite, plays an anti-inflammatory role in macrophages by targeting the AKT1-mediated signaling pathway. *J Ginseng Res* 2019;43:154–60.
- Kulmacz RJ, Lands WE. Stoichiometry and kinetics of the interaction of prostaglandin H synthase with anti-inflammatory agents. *J Biol Chem* 1985;260:12572–8.
- G Perrone M, Scilimati A, Simone L, Vitale P. Selective COX-1 inhibition: a therapeutic target to be reconsidered. *J Current Medicinal Chemistry* 2010;17:3769–805.
- Wongrakpanich S, Wongrakpanich A, Melhado K, Rangaswami J. A comprehensive review of non-steroidal anti-inflammatory drug use in the elderly. *Aging and Disease* 2018;9:143–50.
- Drini M. Peptic ulcer disease and non-steroidal anti-inflammatory drugs. *Aust Prescr* 2017;40:91–3.
- Bhala N, Emberson J, Merhi A, Abramson S, Arber N, Baron J, Bombardier C, Cannon C, Farkouh M, FitzGerald G. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. *Elsevier*; 2013.
- Neutel CI, Appel WC. The effect of alcohol abuse on the risk of NSAID-related gastrointestinal events. *Ann Epidemiol* 2000;10:246–50.
- Koutlas NT, Rusin S, Perjar I, Hollyfield J, Woosley JT, Shaheen NJ, Dellon ES. Impact of smoking, alcohol consumption, and nsaid use on risk for and phenotypes of eosinophilic esophagitis. *Gastroenterology* 2017;152. S857–S857.
- Scott PA, Kingsley GH, Scott DL. Non-steroidal anti-inflammatory drugs and cardiac failure: meta-analyses of observational studies and randomised controlled trials. *Eur J Heart Fail* 2008;10:1102–7.
- Fang WF, Broughton A, Jacobson ED. Indomethacin-induced intestinal inflammation. *Am J Dig Dis* 1977;22:749–60.
- Matsui H, Shimokawa O, Kaneko T, Nagano Y, Rai K, Hyodo I. The pathophysiology of non-steroidal anti-inflammatory drug (NSAID)-induced mucosal injuries in stomach and small intestine. *J Clin Biochem Nutr* 2011;48:107–11.
- Ahn H, Han BC, Kim J, Kang SG, Kim PH, Jang KH, So SH, Lee SH, Lee GS. Nonsaponin fraction of Korean Red Ginseng attenuates cytokine production via inhibition of TLR4 expression. *J Ginseng Res* 2019;43:291–9.
- Okuda H. Biological activities non-saponin fractions in Korean red ginseng. In: *Proc. '99 Korean–Japan ginseng symposium*. Korea: The Korean Society of Ginseng Seoul; 1999. p. 59–69.
- Park K-Y, Kwon DY, Lee KW, Park S. Korean functional foods: composition, processing and health benefits. CRC Press; 2018.
- Lee DH, Cho HJ, Kang HY, Rhee MH, Park HJ. Total saponin from Korean red ginseng inhibits thromboxane A2 production associated microsomal enzyme activity in platelets. *J Ginseng Research* 2012;36:40.
- Oh HA, Kim DE, Choi HJ, Kim NJ, Kim DH. Anti-fatigue effects of 20 (S)-protopanaxadiol and 20 (S)-protopanaxatriol in mice. *Biol Pharm Bull* 2015;38:1415–9.
- Nag SA, Qin J, Wang W, Wang M-H, Wang H, Zhang R. Ginsenosides as anti-cancer agents: in vitro and in vivo activities, structure-activity relationships, and molecular mechanisms of action. *Front Pharmacol* 2012;3:25.
- Han B, Park M, Woo L, Woo W, Han Y. Antioxidant components of Korean ginseng. *Korean Biochem J* 1979;12:33.
- Ahn SI, Kim SK, Yang BW, Lee ES, Kang CS, Hahn YT. Analysis of ginsenosides and non-saponin components of red ginseng from landraces and new varieties. *Kor J Hort Sci Technol* 2016;34:790–8.
- Kang S, Min H. Ginseng, the 'immunity boost': the effects of panax ginseng on immune system. *J Ginseng Res* 2012;36:354–68.
- Byeon SE, Lee J, Kim JH, Yang WS, Kwak Y-S, Kim SY, Choung ES, Rhee MH, Cho JY. Molecular mechanism of macrophage activation by red ginseng acidic polysaccharide from Korean red ginseng. *JMoi* 2012:2012.
- Bae EA, Han MJ, Baek NI, Kim DH. In vitro anti-helicobacterpylori activity of panaxytriol isolated from ginseng. *JAopr* 2001;24:297.
- Baek KS, Yi YS, Son YJ, Yoo S, Sung NY, Kim Y, Hong S, Aravinthan A, Kim JH, Cho JY. In vitro and in vivo anti-inflammatory activities of Korean Red Ginseng-derived components. *J Ginseng Res* 2016;40:437–44.
- Park SH, Choi E, Kim S, Kim DS, Kim JH, Chang S, Choi JS, Park KJ, Roh KB, Lee J, et al. Oxidative stress-protective and anti-melanogenic effects of loliolide and ethanol extract from fresh water green algae, *prasiola japonica*. *Int J Mol Sci* 2018;19:2825.
- Zheng YF, Xie JH, Xu YF, Liang YZ, Mo ZZ, Jiang WW, Chen XY, Liu YH, Yu XD, Huang P, et al. Gastroprotective effect and mechanism of patchouli alcohol against ethanol, indomethacin and stress-induced ulcer in rats. *Chem Biol Interact* 2014;222:27–36.
- Park JG, Kim SC, Kim YH, Yang WS, Kim Y, Hong S, Kim KH, Yoo BC, Kim SH, Kim JH, et al. Anti-inflammatory and antinociceptive activities of anthraquinone-2-carboxylic acid. *Mediators Inflamm* 2016;2016:1903849.
- Baumgartner HK, Starodub OT, Joehl JS, Tackett L, Montrose MH. Cyclooxygenase 1 is required for pH control at the mouse gastric surface. *Gut* 2004;53:1751–7.
- Liu YH, Zhang ZB, Zheng YF, Chen HM, Yu XT, Chen XY, Zhang X, Xie JH, Su ZQ, Feng XX, et al. Gastroprotective effect of andrographolide sodium bisulfite against indomethacin-induced gastric ulceration in rats. *Int Immunopharmacol* 2015;26:384–91.
- Yang Y, Yin B, Lv L, Wang Z, He J, Chen Z, Wen X, Zhang Y, Sun W, Li Y, et al. Gastroprotective effect of aucubin against ethanol-induced gastric mucosal injury in mice. *Life Sci* 2017;189:44–51.
- Hwang HS, Han KJ, Ryu YH, Yang EJ, Kim YS, Jeong SY, Lee YS, Lee MS, Koo ST, Choi SM. Protective effects of electroacupuncture on acetylsalicylic acid-induced acute gastritis in rats. *World J Gastroenterol* 2009;15:973–7.
- Pulli B, Ali M, Forghani R, Schob S, Hsieh KL, Wojtkiewicz G, Linnoila JJ, Chen JW. Measuring myeloperoxidase activity in biological samples. *PLoS One* 2013;8:e67976.
- Yang WS, Lee J, Kim TW, Kim JH, Lee S, Rhee MH, Hong S, Cho JY. Src/NF-κB-targeted inhibition of LPS-induced macrophage activation and dextran



- sodium sulphate-induced colitis by Archidendron clypearia methanol extract. *Journal of Ethnopharmacology* 2012;142:287–93.
- [42] Baek KS, Hong YD, Kim Y, Sung NY, Yang S, Lee KM, Park JY, Park JS, Rho HS, Shin SS, et al. Anti-inflammatory activity of AP-SF, a ginsenoside-enriched fraction, from Korean ginseng. *J Ginseng Res* 2015;39:155–61.
- [43] Jackson LM, Wu KC, Mahida YR, Jenkins D, Hawkey CJ. Cyclooxygenase (COX) 1 and 2 in normal, inflamed, and ulcerated human gastric mucosa. *Gut* 2000;47:762–70.
- [44] Suleyman H, Demircan B, Karagoz Y. Anti-inflammatory and side effects of cyclo-oxygenase inhibitors. *Pharmacological Reports* 2007;59:247.
- [45] Lucas S. The pharmacology of indomethacin. *Headache* 2016;56:436–46.
- [46] Vane JR, Botting RM. The mechanism of action of aspirin. *Thromb Res* 2003;110:255–8.
- [47] Malfertheiner P, Chan FK, McColl KE. Peptic ulcer disease. *Lancet* 2009;374:1449–61.
- [48] Kurata JH. Ulcer epidemiology: an overview and proposed research framework. *Gastroenterology* 1989;96:569–80.
- [49] Morsy M, Ashour O, Amin E, Rofaeil R. Gastroprotective effects of telmisartan on experimentally-induced gastric ulcers in rats. *Pharmazie* 2009;64:590–4.
- [50] Tanaka A, Hatazawa R, Takahira Y, Izumi N, Filaretova L, Takeuchi K. Preconditioning stress prevents cold restraint stress-induced gastric lesions in rats: roles of COX-1, COX-2, and PLA(2). *Dig Dis Sci* 2007;52:478–87.
- [51] Chassaing B, Aitken JD, Malleshappa M, Vijay-Kumar M. Dextran sulfate sodium (DSS)-induced colitis in mice. *Curr Protoc Immunol* 2014;104. Unit 15 25.
- [52] Antonisamy P, Kannan P, Aravinthan A, Duraipandiyar V, Arasu MV, Ignacimuthu S, Al-Dhabi NA, Kim JH. Gastroprotective activity of violacein isolated from *Chromobacterium violaceum* on indomethacin-induced gastric lesions in rats: investigation of potential mechanisms of action. *Sci World J* 2014;2014:616432.
- [53] Gretzer B, Maricic N, Respondek M, Schuligoi R, Peskar BM. Effects of specific inhibition of cyclo-oxygenase-1 and cyclo-oxygenase-2 in the rat stomach with normal mucosa and after acid challenge. *Br J Pharmacol* 2001;132:1565–73.
- [54] Wallace JL. Prostaglandins, NSAIDs, and gastric mucosal protection: why doesn't the stomach digest itself? *Physiol Rev* 2008;88:1547–65.