# Amelioration of alcohol-induced gastric mucosa damage by oral administration of food-polydeoxyribonucleotides

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**Abstract.** Gastritis refers to inflammation caused by injury to the gastric epithelium, which is usually due to excessive alcohol consumption and prolonged use of nonsteroidal anti-inflammatory drugs. Millions of individuals worldwide suffer from this disease. However, the lack of safe and promising treatments makes it urgent to explore and develop leads from natural resources. Therefore, food as medicine may be the best approach for the treatment of these disorders. The present study described the protective effects of food-polydeoxyribonucleotides (f-PDRNs) in a rat model of gastric mucosal injury induced by HCl-EtOH. Administration of f-PDRN was performed with low-PRF002 (26 mg/kg/day), medium-PRF002 (52 mg/kg/day) and high-PRF002 (78 mg/kg/day) on the day of autopsy. The site of damage to the mucous membrane was also analysed. In addition, an increase in gastric juice pH, total acidity of gastric juice and decrease in gastric juice secretion were confirmed, and gastric juice secretion-related factors corresponding to the administration of f-PDRN were analysed. Administration of f-PDRN reduced the mRNA expression of histamine H2 receptor, muscarinic acetylcholine receptor M3, cholecystokinin 2 receptor and $H^+/K^+$ ATPase related to gastric acid secretion and downregulation of histamine, myeloperoxidase and cyclic adenosine monophosphate. In addition, it was histologically confirmed that the loss of epithelial cells and the distortion of the mucosa were recovered in the group in which f-PDRN was administered compared to the model group with gastric mucosa damage. In summary, the present study suggested that f-PDRN has therapeutic potential and may have beneficial effects if taken regularly as a food supplement.

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## Introduction

Gastritis refers to inflammation caused by damage to the gastric epithelium (1-3). The symptoms of gastritis include epigastric pain, nausea, vomiting, belching and weight loss (4). Acute gastritis is usually caused by excessive alcohol consumption, long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) and stress (5,6). This stimulation increases gastric acid secretion and damages the gastric mucosa (7,8). The secretory pathways of gastric acid include activation of histamine-induced histamine H2 receptor (H2R), cholinergic receptor muscarinic 3 (CHRM3) and cholecystokinin 2 receptor (CCK2R) activation by gastrin (9-13). Thus, activated H2R, M3R and CCK2R increase the concentration of cyclic adenosine monophosphate (cAMP) in parietal cells and activate $H^+/K^+$ ATPase. This increases the concentration of gastric acid, which damages the gastric mucosa (14,15).

Gastric juice contains hydrochloric acid, a highly corrosive acid (16), and the high concentration of acid secreted from the gastric mucosa is fatal to cells (17). However, it usually has a useful action in the stomach, killing the ingested bacteria and promoting the formation of pepsin (18). Furthermore, it prevents damage to and contact with the stomach wall through a series of complex and complementary physical and chemical processes (19).

Collectively, this process of structural and functional protection of the stomach against the destruction of autologous acids and pepsin, reflux bile and pancreatic enzymes, as well as ingested abrasives or toxic substances, is called the gastric mucosal barrier (20,21). On the gastric mucosa, epithelial cells form a strong barrier against penetration by the lumen content, including hydrogen ions (22). Cells have tight junctions, hydrophobic and abundant lipids, and a mucosal surface that removes acids and secretes bicarbonate and mucus (23). When acid and pepsin diffuse back into the tissues, the secretion of acid and pepsin is further stimulated, leading to decreased mucosal blood flow and gastric motility (24,25). Subsequently, as the acid and pepsin diffuse back into the tissues, the secretion of acid and pepsin is further stimulated and mucosal blood flow and gastric motility decrease (26,27). Acid also damages connective tissue and submucosal capillaries, causing local mucosal bleeding and microulcers (28). Severe and long-term exposure to acids may lead to significant

gastric ulceration (29). However, elimination of the causative agent or adequate and immediate treatment may quickly restore mucosal integrity (30,31).

The treatment of gastritis and gastric ulcers involves inhibition of the offensive factors through the use of antacids, anticholinergic drugs, H2R antagonists, anti-gastrin drugs, hydrogen pump inhibitors, mucosal protective agents and inhibitors of gastric secretions, such as prostaglandin-related drugs and anti-pepsins (32,33). Drugs that induce defensive factors and suppress the central nervous system to reduce anxiety and stress have also been used (34). However, these drugs to treat gastritis and gastric ulcer have various side effects, including arrhythmia, erectile dysfunction, gynecomastia and recurrence, and it is necessary to develop novel drugs or health supplements that are safer and more effective in the treatment of gastritis (35,36).

Polydeoxyribonucleotides (PDRNs) extracted from salmon sperm are known to help in the regeneration of tissues in burns and wounds (37). A mixture of DNA fragments with a composition that is the most similar to human DNA promotes tissue regeneration and repair after surgery (38-42). PDRN is an adenosine A2A receptor agonist that enhances the expression of vascular endothelial growth factor and promotes wound healing through angiogenesis in diabetic foot ulcers (43,44). It also stimulates the conversion of growth factors and extracellular matrix in damaged cells or tissues through the purine adenosine A2A receptor (42). PDRN activates A2A receptors that promote wound healing by preventing pro-inflammatory cytokines and releasing pro-fibrotic cytokines (45,46). It exerts anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines such as interleukin-6 and tumour necrosis factor-α and upregulating the production of anti-inflammatory cytokines (45,47,48).

The present study investigated the protective effects of f-PDRN extracted from salmon milt against HCl-EtOH-induced gastric mucosal damage in rats. In addition, the regulatory effects of f-PDRN on the expression of molecules involved in the gastric acid secretion and inflammation pathways were investigated in gastric parietal cells.

## Materials and methods

*Materials*. The f-PDRN PRF002 (PharmaResearch) used in the present study is a fragmented DNA polymer extracted from the testes of adult salmon (*Oncorhynchus keta*, Salmonidae) that returned to their breeding grounds in Namdaecheon (Gangwon, Korea) (49). PRF002, with a molecular weight of 50-1,500 kDa, was dissolved in 0.9% NaCl solution, resulting in a DNA concentration of 75% or higher. Stillen Tab (Dong-A ST Co.) is a nonsteroidal anti-inflammatory analgesic that improves gastric mucosal lesions (haemorrhage, erythema and oedema) and acute and chronic gastritis (50).

*Construction of rat models of gastric mucosal damage*. A total of 60 male Hsd:Sprague Dawley® (SD) rats (age, 7 weeks; bodyweight, 200±10 g) were purchased from Koatech (49). The animals were housed one per cage and allowed free access to tap water and food that contained 0.44% sodium and 22.5% protein. Acclimatized to a colony room with controlled ambient temperature (24±1˚C), humidity (50±10%) and 12-h light-dark cycles. All experiments involving laboratory animals were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of the KNOTUS (Incheon, South Korea). The rats were anaesthetized with a mixture of Zoletil (30 mg/kg) and Rompun (10 mg/kg) solution (3:1 ratio, 1 ml/kg, i.p.). Euthanasia was performed by introducing a flow rate of 5 l/min of 100% carbon dioxide into a bedding-free cage initially containing room air with the lid closed at a rate sufficient to induce rapid anaesthesia for a 10 l volumetric chamber, with death occurring within 2.5 min. The experimental design was approved by the KNOTUS Management and Use Committee (no. 19-KE-262). The SD rats were divided into six groups consisting of 10 rats/group as follows: A normal control group and alcoholic gastric irritation test control groups. The alcoholic gastric irritation test control groups included the vehicle group, low-dose PRF002 concentration (26 mg/kg/day) group, medium-dose PRF002 concentration (52 mg/kg/day) group, high-dose PRF002 concentration (78 mg/kg/day) group and a positive control group (Stillen, a non-steroidal anti-inflammatory drug; 150 mg/kg/day) (51). The f-PDRN dose selection in this study was based on a dose of 52 mg/kg/day, which was previously used for the treatment of arthritis (52). The vehicle group was treated with 0.9% NaCl and the alcoholic gastric irritation model group was treated with 0.9% NaCl and 40% ethanol. The low-, medium-, high-PRF002 and Stillen groups were administered the indicated doses of PRF002 and Stillen by oral gavage and 40% ethanol for 7 days to irritate the stomach. All dietary intake was stopped 24 h prior to the experiment to empty the stomach (53).

*Analysis of the damaged area of the gastric mucosa*. HCl (150 mM) was added to 70% ethanol and 1 ml of the mixture was orally administered to each of the rats by oral gavage. The rats were sacrificed 1 h later, blood was collected and the stomach was excised. The excised stomach was incised along the greater curvature and washed in saline, and images were acquired using a digital camera (Coolpix P5100; Nikon Corporation). Areas of damage to the gastric mucosa were quantitated using ImageJ software (version 1.4.3; National Institutes of Health). Measurements of the damaged area and the total area of the stomach were used to calculate the damaged area (%) as follows: Damaged area (%)=(damaged area/total area) x100.

*Measurement of the volume of gastric juice secretion*. The excised gastric pylorus was slightly incised and gastric juice was collected in a 15-ml tube. The collected gastric juice was centrifuged at 2,400 x g at 4˚C for 20 min and the supernatant constituted the total amount of gastric fluid (ml).

*Analysis of the acidity and pH of the gastric juice*. To the supernatant (1 ml) of the separated gastric juice, 50 µl of 0.5% dimethylaminobenzene alcohol and 50 µl 1% phenolphthalein alcohol solution was added. When the gastric juice turned red, a 0.1N NaOH solution was added. The total acidity was calculated as follows and expressed in mEq/l: Acidity=(volume of NaOH x normality of NaOH x100)/0.1 (mEq-1/100 g).

*Separation and culture of gastric parietal cells*. After sacrificing the rats by $CO_2$ gas asphyxiation, the gastric tissue was separated and washed with PBS. HEPES (20 mM) and cimetidine (5M) were added to DMEM (HyClone; Cytiva) containing 1 mg/ml type 4 collagenase (Worthington Biochemical Corporation) and 1 mg/ml BSA (Thermo Fisher Scientific, Inc.) and stored at 37˚C for 30 min. The cell suspension was filtered through a nylon mesh (0.2 mm) and centrifuged at 1,000 x g at 4˚C for 15 min. The separated cells were filtered through a 40-$\mu$m cell strainer (BD Biosciences) and centrifuged for 10 min at 1,350 x g at 4˚C. To the separated cells, complete DMEM/F-12 (Gibco-BRL; Thermo Fisher Scientific, Inc.), 20 mM HEPES, 0.2% BSA, 10 mM glucose, 1 insulin-transferrin-selenium-A (Gibco; Thermo Fisher Scientific, Inc.), 1 mM glutamine, 100 U/ml penicillin/streptomycin, 400 $\mu$g/ml gentamicin sulfate and 15 mg/ml geneticin (pH 7.4) were added, followed by centrifugation for 10 min at 1,350 x g at 4˚C. The collagenase-isolated cells were placed in a Matrigel® Matrix plate (Corning, Inc.) and incubated at 37˚C with 5% $CO_2$.

*Measurement of cAMP levels in gastric parietal cells*. After stabilising the cells cultured in the Matrigel® Matrix plate for 24 h, the growth media (DMEM containing 1 mg/ml glucose and 50 $\mu$g/ml gentamycin) was removed and the cells were centrifuged for 10 min at 1,350 x g at 4˚C. The supernatant was used to quantitate cAMP levels using a cAMP direct immunoassay kit (Biovision) according to the manufacturer's protocol. Measurements were performed using an ELISA plate reader (Molecular Devices, LLC).

*Reverse transcription-quantitative PCR (RT-qPCR)*. mRNA from each sample of the stomach wall was extracted using an RNeasy Mini Kit (Qiagen GmbH) and reverse-transcribed using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems; Thermo Fisher Scientific, Inc.; cat. no. 4374966) according to the manufacturer's instructions. The cDNA (1 $\mu$g) was amplified using the following rat primers: H2R sense, 5'-ACCAGCCTGGATGTCATGCT-3' and antisense, 5'-CCTGTCAAGGCTGATCATGAAG-3'; $H^+/K^+$ ATPase sense, 5'-CCTCACACAGAGGAGACTA-3' and antisense, 5'-TGCCCAGTGTCCGGGTTCCA-3'; CCK2R sense, 5'-ACGTGGCGGCTTCCAA-3' and antisense, 5'-CCAGGCCCCAGTGCTCTGATGGTGG-3'; M3R sense, 5'-ACGCTCGCCAGGATGAAGT-3' and antisense, 5'-GGCTTGGCTTCCAGCTCTT-3'; mucin (MUC)5AC sense, 5'-GGCCAATGCGGCACTTGTACCAAT-3' and antisense, 5'-GTCATCTGGACAGAAGCAGCCCTC-3'; matrix metalloproteinase-3 (MMP-3) sense, 5'-CCTGCTTTGTCCTTTGATGC-3' and antisense, 5'-TGAGTCAATCCCTGGAAAGTC-3'; MMP-9 sense, 5'-CATTCGCGTGGATAAGGA-3' and antisense, 5'-ACAAGAAAGGACGGTGGG-3'; and GAPDH sense, 5'-TGATTCTACCCACGGCAAGTT-3' and antisense, 5'-TGATGGGTTTCCCATTGATGA-3'. TOPreal™ qPCR 2X PreMIX (Enzynomics) was used according to the manufacturer's protocol. qPCR was performed using the following thermocycling conditions: Initial denaturation at 95˚C for 30 sec, followed by 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec. Reactions were performed on a CFX96™ Real-Time System (Bio-Rad Laboratories, Inc.) with no template control reactions for each primer set (Fig. S1) (54). The experiments were repeated three times.

*Determination of histamine, cAMP, myeloperoxidase (MPO) and prostaglandin E2 (PGE2) levels*. Whole blood samples were collected in Vacutainers (BD Biosciences) with a clotting activator. After centrifugation at 2,000 x g for 10 min at 17˚C, the supernatants were collected and the histamine concentration in blood was analysed in the plasma. Damaged gastric tissue samples were homogenized in 1 ml phosphate buffer, followed by centrifugation of samples. Subsequently, PGE2 and MPO activity were analysed. Histamine (cat. no. ab213975), MPO (cat. no. ab155458), cAMP (cat. no. ab133051) and prostaglandin E2 (cat. no. ab133021) ELISA kits (Abcam) were used to measure histamine, MPO, cAMP and PGE2 levels, respectively, in the supernatant according to the manufacturer's protocols using a microplate reader.

*Histological analysis*. Paraffin was removed from the paraffin-embedded gastric tissue sections and the tissues were stained according to a previously published protocol (55). Gastric tissue sections were fixed in 10% phosphate-buffered formaldehyde, embedded in paraffin and processed for histological analysis. Sections were cut to 5-$\mu$m thickness, mounted on slides and stained with H&E according to standard procedures.

*Statistical analyses*. All statistical data were derived from five repetitions. The significance of differences among the experimental groups was tested by one-way ANOVA using Prism 7.04 (Graph Pad Software, Inc.) and a post-hoc Tukey's test was performed. $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*Protective effects of f-PDRN against HCl/EtOH-induced gastric mucosa damage in rats*. The protective efficacy of f-PDRN against gastric mucosa damage was investigated using a rat model of HCl/EtOH-induced gastric mucosal injury. The experimental groups were as follows: Normal control, vehicle (negative control), low-PRF002 (26 mg/kg/day) administration group, medium-PRF002 (52 mg/kg/day) administration group, high-PRF002 (78 mg/kg/day) administration group and Stillen (positive control). In the positive control group, Stillen, a novel drug, was used to decrease acute and chronic gastritis and gastric mucosal lesions. Each substance was administered orally at the indicated doses. On the day of the autopsy, the stomach was removed, images of the gastric mucosa from each experimental group were acquired with a digital camera and the damaged area was quantified using Image J software. The results revealed that gastric mucosal injury in the medium- and high-PRF002-administered groups was markedly lower than that in the low-PRF002 group. Comparison and analysis of the sites of gastric mucosal injury revealed that the area of damage in all gastric mucosal injury groups was markedly higher than that in the normal control group. In addition, it was confirmed that in the f-PDRN oral administration groups, the area of gastric mucosal injury decreased in a dose-dependent manner (Fig. 1A and B).

*Inhibition of gastric acid secretion and recovery of pH and acidity by f-PDRN*. To confirm the protective effect of f-PDRN

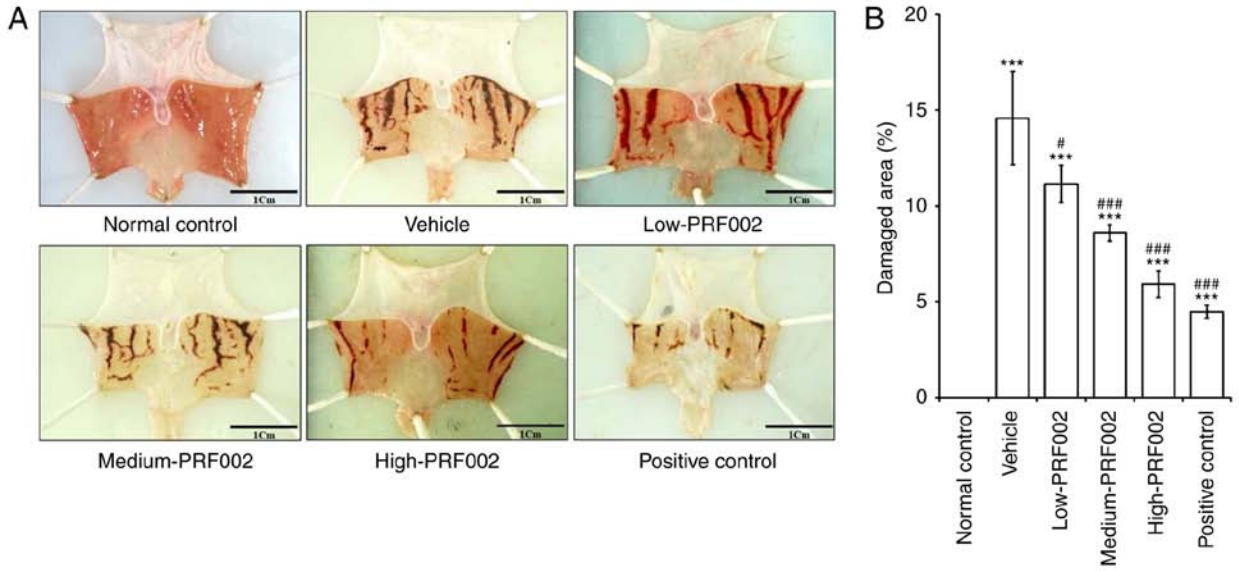


Figure 1. Gastroprotective effect of food-polydeoxyribonucleotides on gastric mucosal damage following oral administration. The groups are normal control, vehicle, low-PRF002, medium-PRF002, high-PRF002, positive control. (A) Comparison of gastric mucosal injury in various treatment groups in a rat model of gastric mucosal damage (scale bar, 1 cm). (B) Measurement of the area of damage in the gastric mucosa in each group. $^{***}$P<0.001 compared to normal control; $^{\#}$P<0.05, $^{\#\#\#}$P<0.001 compared to vehicle.

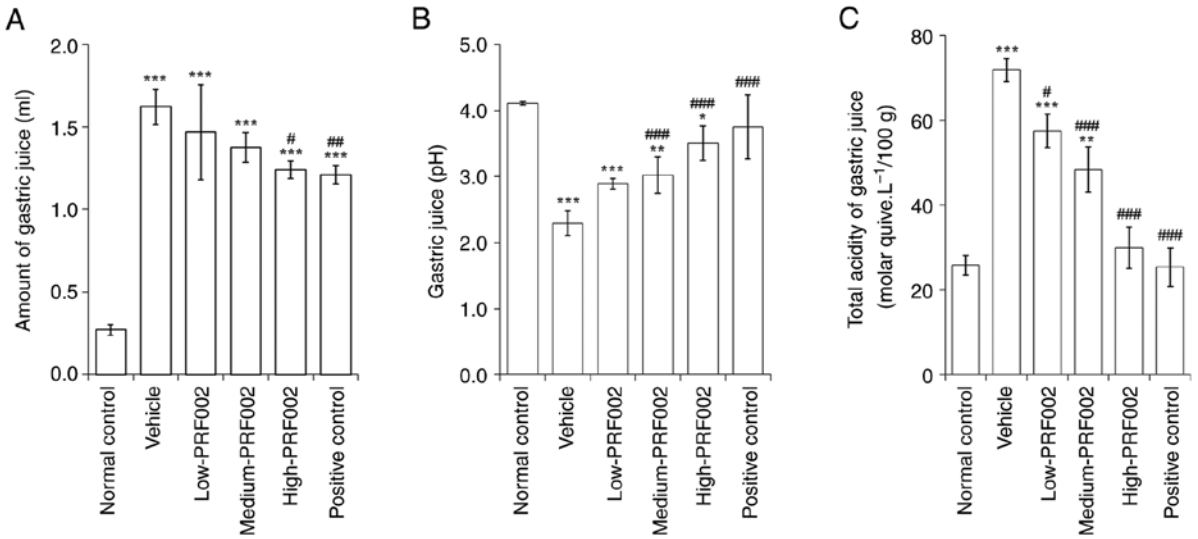


Figure 2. Effect of food-polydeoxyribonucleotides on the volume, pH and total acidity of gastric juice in a rat model of gastric mucosal injury. The groups are the normal control, vehicle, low-PRF002, medium-PRF002, high-PRF002 and positive control. (A) Volume of gastric juice secreted. (B) pH and (C) total acidity of gastric juice. $^{*}$P<0.05, $^{**}$P<0.01, $^{***}$P<0.001 compared to the normal control; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 compared to vehicle.

on the gastric mucosa, changes in gastric juice secretion, total acidity and decrease in gastric juice pH after oral administration of f-PDRN were analysed. The results indicated that the amount of gastric juice collected from the gastric pyloric area of rats was significantly increased in all gastric mucosal injury-induced groups, compared with that in the normal control (Fig. 2A). In addition, the pH of the gastric juice was significantly decreased in all of the PRF002-administered groups compared with that in the normal control (Fig. 2B). The total acidity was significantly higher in the vehicle, low-PRF002 and medium-PRF002 groups than in the normal controls (Fig. 2C). To summarise the changes in gastric juice secretion, total acidity and pH after f-PDRN administration, it was indicated that the amount of gastric juice secreted was decreased in each administration group compared with that in the vehicle group. In particular, the volumes in the high-PRF002 and positive control groups significantly decreased. The total acidity of gastric juice was significantly decreased in the medium-PRF002, high-PRF002 and positive control groups compared to that in the vehicle group. The total acidity of gastric juice in the high-PRF002 and positive control groups was restored to almost the level of the normal control. In addition, the pH of gastric juice was significantly higher in the medium-PRF002, high-PRF002 and positive control groups than in the vehicle group.

*f-PDRN reduces the mRNA expression of gastric acid secretion-related factors*. The expression levels of the

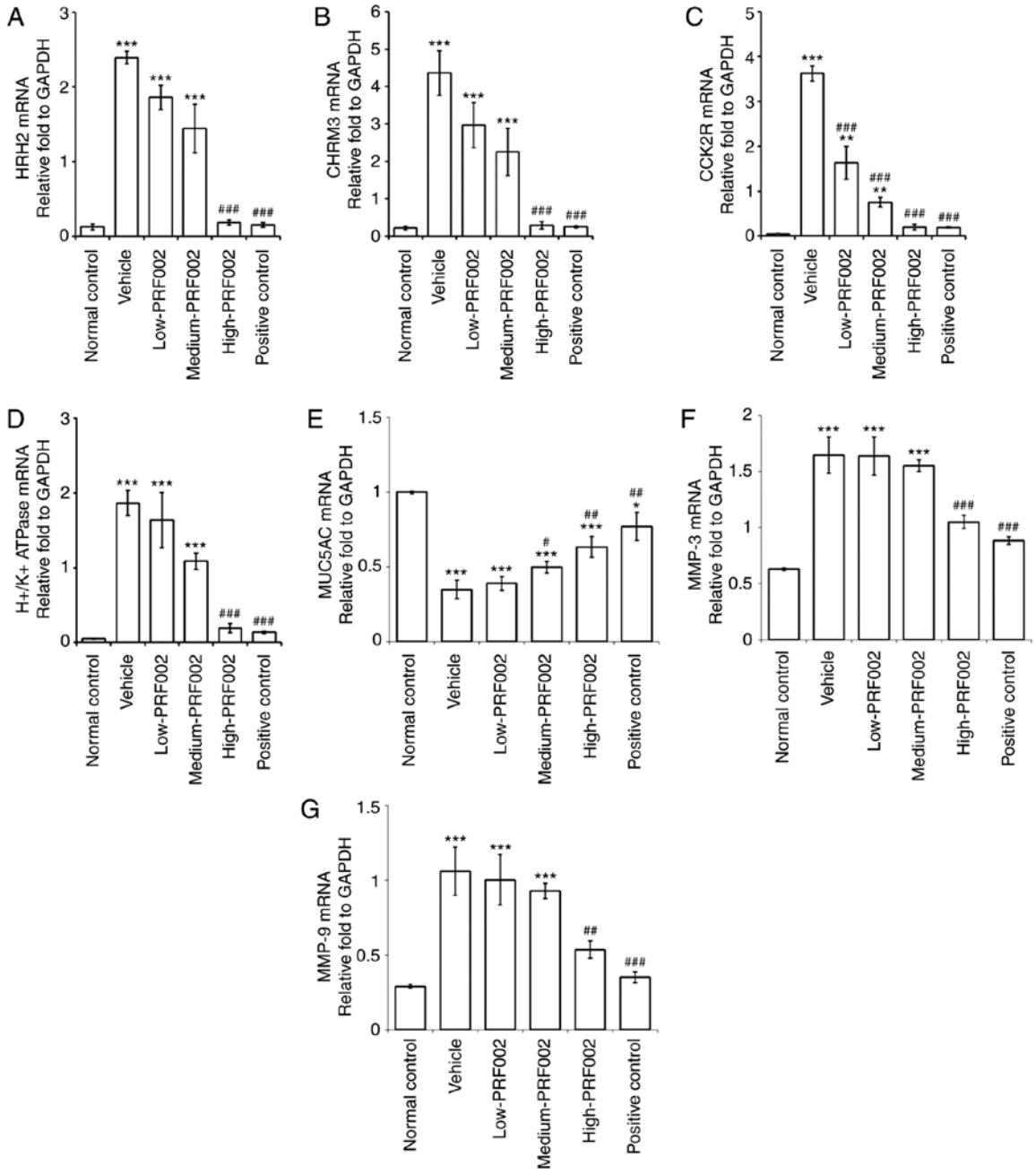


Figure 3. mRNA expression of (A) H2R, (B) CHRM3, (C) CCK2R, (D) H+/K+ ATPase, (E) MUC5AC; (F) MMP-3 and (G) MMP-9 in cells isolated from the gastric wall and gastric mucosa. The groups were the normal control, vehicle, low-PRF002, medium-PRF002, high-PRF002 and positive control. $^{*}$P<0.05, $^{**}$P<0.01, $^{***}$P<0.001 compared to the normal control; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 compared to vehicle. H2R, histamine receptor H2; M3R, muscarinic acetylcholine receptor 3; CCK2R, cholecystokinin 2 receptor; MUC, mucin.

receptors related to gastric acid secretion and inflammation were compared using RT-qPCR (Fig. 3). The results revealed that the mRNA expression of H2R, CHRM3, $H^+/K^+$ ATPase, MMP-3 and MMP-9 was markedly increased in the vehicle group compared with that in the normal control, and significantly reduced in the high-dose PRF002 and positive control groups compared to that in the vehicle group with gastric mucosal damage, and was almost restored to the expression level in the normal control (Fig. 3A, B, D, F and G). The mRNA expression of MUC5AC increased significantly in the medium-PRF002, high-PRF002 and positive control groups compared with that in the vehicle group (Fig. 3E). In addition, the mRNA expression of CCK2R decreased significantly in the low-PRF002, medium-PRF002, high-PRF002 and positive control groups compared with that in the vehicle group (Fig. 3C). These results suggested that f-PDRN decreased the mRNA expression of pre-stage gastric acid secretion factors, such as H2R, CHRM3 and CCK2R (9-13), which led to a decrease in the $H^+/K^+$ ATPase protein transcription factor.

*Regulatory effect of f-PDRN on histamine, MPO, cAMP and PGE2*. To determine the changes in factors related to gastric juice secretion induced by f-PDRN administration, the levels of histamine, MPO, cAMP and PGE2 were compared among the groups of rats with gastrointestinal mucosal damage. The results indicated that the inflammation-related

factors histamine and MPO were markedly upregulated in the vehicle group compared with that in the normal control, and significantly downregulated in the low-, medium- and high-dose PRF002 groups compared with those in the vehicle groups (Fig. 4A and B). Furthermore, cAMP, a precursor of proton pump activation, was also significantly downregulated in the f-PDRN-treated groups low-PRF002, medium-PRF002 and high-PRF002 compared with that in the vehicle group (Fig. 4C). In addition, PGE2, a factor that mediates gastrointestinal protective effects, was upregulated in the low-PRF002, medium-PRF002 and high-PRF002 groups compared with that in the vehicle group with gastric mucosal damage (Fig. 4D). These results suggested that f-PDRN exerted potent gastroprotective effects against gastric mucosal damage.

*Histological effects of oral administration of f-PDRN.* The present results confirmed the prevention of the gastric mucosa damage by f-PDRN administration and it was indicated that the effect of the administration of high concentrations of PRF002 (high-PRF002) was most similar to that in the positive control (Fig. 5). The gastric mucosa in the various groups, including the normal control group with gastric tissue, the vehicle group with damaged gastric tissue and the high-PRF002 group with reduced damage of gastric tissue, was compared using histopathology. The results revealed epithelial cell loss, mucosal or submucosal distortion (blue arrow) and invasive inflammatory cells in the vehicle-administered group (yellow arrow). However, epithelial cell loss and inflammatory cell infiltration were reduced in the high-PRF002-administered group compared with those in the vehicle-administered group. Therefore, these observations confirmed that the gastric mucosa damage was prevented by administration of high-PRF002.

## Discussion

The major objective of the present study was to investigate whether f-PDRN (PRF002) is helpful in gastric mucosal maintenance and protects against HCl/EtOH-induced acute gastric mucosal damage in rats. The present study first investigated the protective effect of f-PDRN on the macroscopic and subtle changes caused by HCl/EtOH in the gastric mucosa of rats. It was demonstrated that administration of f-PDRN significantly prevented the HCl/EtOH-induced morphological and structural changes in the gastric mucosa. Previous data from a rat model suggested that acute ethanol poisoning causes severe lesions and damage through gastric leukocyte infiltration of the submucosal layer (8,16,34). In addition, ethanol-induced gastric mucosal damage is associated with overproduction of gastric juice, resulting from increased expression of receptors such as H2R, CHRM3 and $H^+/K^+$ ATPase, and increased CCK2R at the mRNA level, which are associated with gastric acid secretion (13,48,56). This was further confirmed by changes in histamine, MPO, cAMP and PGE2, which are factors related to gastric juice secretion. Therefore, one of the strategies to inhibit gastric acid secretion is to use receptor antagonists (10,12). Three major receptors have been identified in the parietal cells and antagonists have been developed for each of them (10,12). H2R antagonists and muscarinic receptor antagonists are available for clinical use (10). In addition, E2 type prostaglandins block binding to histamine receptors and cAMP production. Muscarinic receptor antagonists such as atropine and selective M1-antagonists such as pirenzepine are effective inhibitors of acid secretion by dietary stimulation but have certain side effects (57-59). This is owing to the generalised presence of these receptors in numerous organs of the body (60). Furthermore, the degree of inhibition with high doses of these antagonists is insufficient to reduce acid to low-threshold stable levels even in duodenal ulcer disease (61,62). In addition, a close relationship exists between gastric juice production and gastric inflammation. Therefore, it is endeavoured to examine the effect of f-PDRN on pro-inflammatory cytokine production in a future study. Stillen, which was used as a positive control in the present study, has been widely used in the treatment of acute and chronic gastritis (63). It provides anti-gastritis effects following the administration of NSAIDs. More importantly, the present results demonstrated that f-PDRN significantly attenuated HCl-EtOH-induced gastric mucosal damage in a dose-dependent manner. EtOH-induced excessive secretion of gastric juice (associated with the aetiology of gastric damage) reduced the pH and increased the total acidity of gastric acid.

The upregulation of histamine, acetylcholine and gastrin is known to increase gastric acid secretion (64-66). Gastric acid secretion is also further increased following the upregulation of histamine, acetylcholine, gastrin-associated receptors, H2R, M3R and CCK2R (67,68). The present results confirmed the effect of increased mRNA expression of HRH2, CHRM3 and CCK2R (factors related to gastric acid secretion) and their decreased expression following administration of PRF002 in rat models of HCl-EtOH-induced gastric mucosa damage. In addition, the administration of PRF002 increased the expression of MUC5AC and decreased the expression of MMP-3 and MMP-9 by administration of PRF002. In addition, PRF002 treatment decreased plasma histamine activity and MPO and cAMP levels compared with those in the vehicle group and significantly increased PGE2 production. Therefore, the preventive effect of PRF002 on EtOH-induced gastric mucosal injury may involve two mechanistic components: Inhibition of inflammation and protection of the gastric mucosa.

Reducing neutrophil infiltration into ulcerated gastric tissues prevents damage to the gastric mucosa in rats (69,70). Since neutrophils contain the blood cell protein MPO, the accumulation of neutrophils in gastric mucosal tissue may be measured by MPO activity. In the present study, high PRF002 (78 mg/kg) protected the histological structure of the gastric mucosa and prevented the infiltration of inflammatory cells (neutrophils). Compared to the ethanol group, PRF002 administration clearly inhibited the production of gastric secretory mediators (H2R, CHRM3, CCK2R and proton pumps) during ethanol-induced gastric mucosal damage. Thus, PRF002 reduces gastric mucosal damage caused by gastric juice secretion and protects the stomach.

As observed previously, PDRN interacts with adenosine $A_{2A}$ receptors, stimulates VEGF expression, promotes wound healing and contributes to gastric ulcer healing, probably through the inhibition of inflammation and apoptosis (71). The f-PDRN used in the present study is similar to that used in conventional medicine; however, the extraction, manufacturing method and formulation are different. The mechanism of f-PDRN in gastric mucosal protection is related

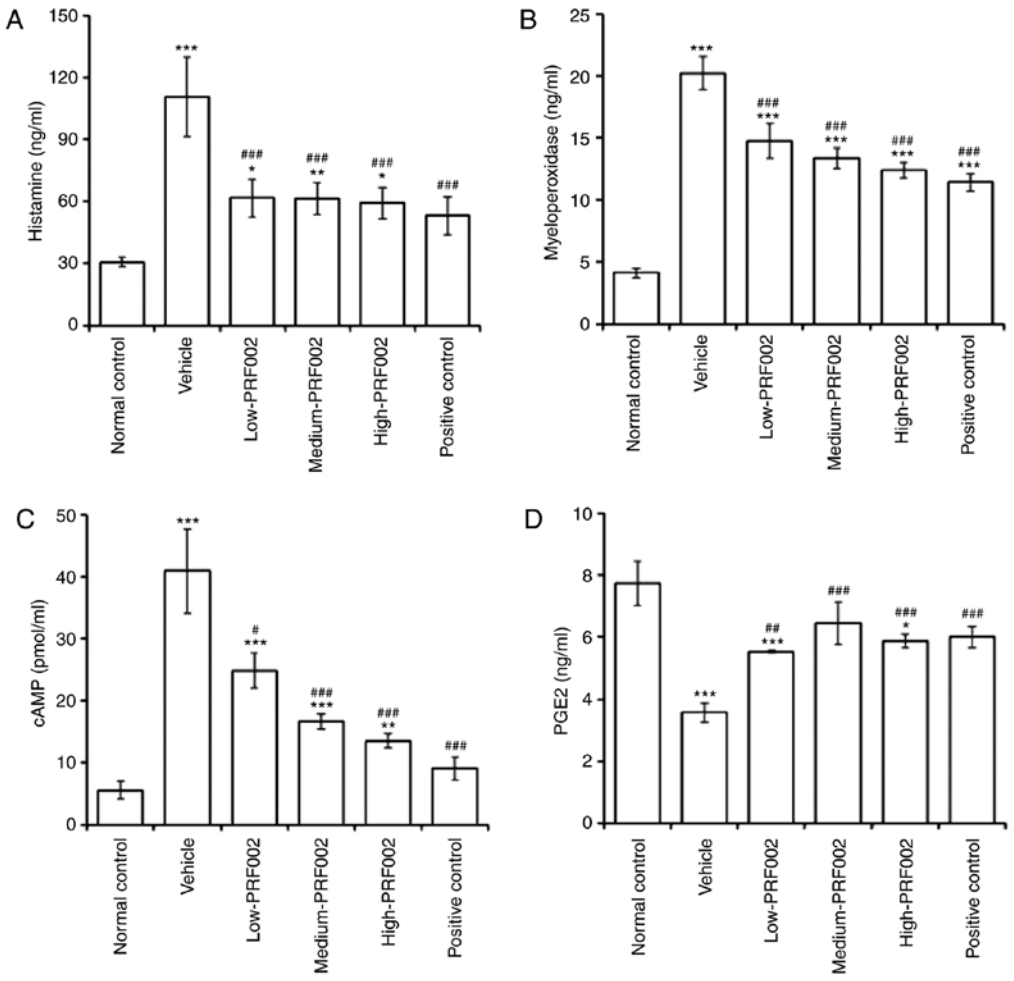


Figure 4. (A) Concentration of histamine, (B) MPO activity, (C) level of cAMP and (D) PGE2 in damaged gastric mucosal tissues. The groups are the normal control, vehicle, low-PRF002, medium-PRF002, high-PRF002 and positive control. $^{*}$P<0.05, $^{**}$P<0.01, $^{***}$P<0.001 compared to the normal control, $^{\#}$P<0.05, $^{\#\#\#}$P<0.001 compared to vehicle. MPO, myeloperoxidase; cAMP, cyclic adenosine monophosphate; PGE2, prostaglandin E2.

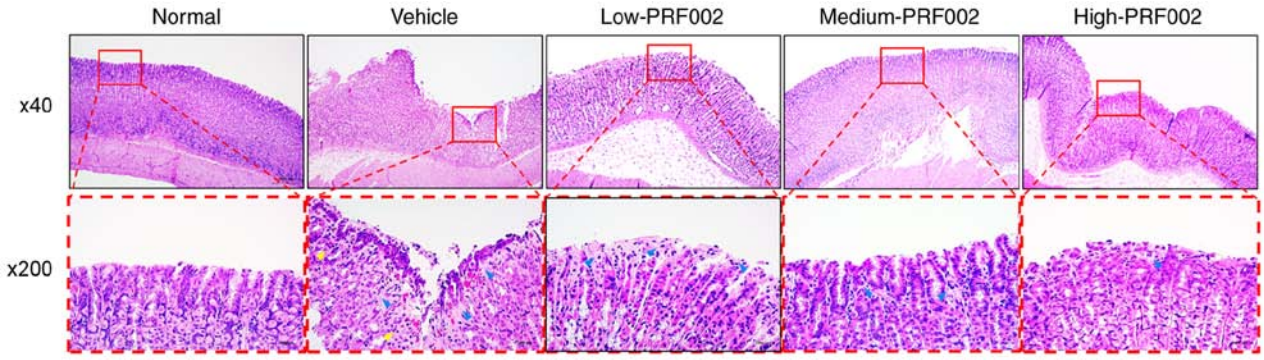


Figure 5. Histological changes in the gastric mucosa following treatment with food-polydeoxyribonucleotides. Blue arrowheads indicate the loss of epithelial cells and distortion of the mucosa or submucosa and yellow arrowheads indicate invasive inflammatory cells in the vehicle-administered group (H&E staining; magnification: Top panel, x40; bottom panel, x200; scale bar, 1,000 $\mu$m).

to cell migration, proliferation, re-epithelialization, gland reconstruction and angiogenesis-bone marrow-derived (stem cell-derived) blood vessel formation. It is also expected to be a complex tissue regeneration process involving the formation of new blood vessels from the newly formed progenitor cells. All of these processes are regulated by growth factors, cytokines, hormones and transcription factors (72-76). In addition, it may be identified by genes encoding early growth factors or growth factors (e.g. EGF, bone-derived fibroblast growth factor, hepatocyte growth factor, VEGF and trefoil peptides) derived from platelets, macrophages and damaged tissues (72,77,78). The possibility of using f-PDRN for mucosal healing was thus confirmed.

Overall, the present results indicated that oral administration of f-PDRN is helpful to gastric mucosal maintenance and protects against alcohol-induced gastric mucosal injury. In

addition, the results of the present study are consistent with the confirmation of gastroprotective effects of f-PDRN administration in HCl/EtOH-induced gastric mucosal injury in a rat model. Therefore, the administration of f-PDRN represents a potentially useful treatment option to prevent the progression of alcohol-induced gastric mucosal damage.

In conclusion, the results of the present *in vivo* f-PDRN administration experiments demonstrated that f-PDRN prevents the progression of alcohol-induced gastric mucosal damage by modulating factors associated with gastric acid secretion. f-PDRN reduced the expression of HRH2, CHRM3, CCK2R and $H^+/K^+$ ATPase related to gastric acid secretion and exerted the protective effects against the gastric mucosa damage through downregulation of histamine, MPO, cAMP, MMP-3 and MMP-9. Therefore, given the therapeutic potential of f-PDRN, future studies are required to further explore the detailed mechanisms of action of f-PDRN.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Authors' contributions**

JoK, SC, SOO and SL performed the experiments and analysed the data. SaK and JuK extracted f-PDRN from salmon milt and characterized its properties. JoK, HK and SuK interpreted the data and drafted the manuscript. JoK and SuK confirm the authenticity of all the raw data. All authors contributed to editing and revising the manuscript. All authors read and approved the final version of the manuscript.

**Ethics approval and consent to participate**

The present study was approved by the Animal Ethics Committee of KNOTUS IACUC (approval no. 19-KE-262).

**Patient consent for publication**

Not applicable.

**Competing interests**

All authors were employees of PharmaResearch Co., the supplier of PRF002. The authors have no other competing interests to declare.

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