The *Sasa quelpaertensis* Leaf Extract Inhibits the Dextran Sulfate Sodium-induced Mouse Colitis Through Modulation of Antioxidant Enzyme Expression

Original Article

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Background: Oxidative stress plays an important role in the pathogenesis of inflammatory bowel disease. The objective of this study is to investigate the protective effect of *Sasa quelpaertensis* leaf extract (SQE) against oxidative stress in mice with dextran sulfate sodium (DSS)-induced colitis.

Methods: Mice were treated with SQE (100 mg/kg or 300 mg/kg body weight) by gavage in advance two weeks before inflammation was induced. Then, the mice were administered with 2.5% DSS in drinking water for 7 days and normal drinking water for 7 days between two DSS treatment. Disease activity index values, gut motility, and severity of the resulting oxidative DNA damage were analyzed. The antioxidant effect of SQE was evaluated by measuring malondialdehyde (MDA) and superoxide dismutase (SOD) activity in plasma samples. Catalase activity and expressions levels of glutathione peroxidase 1 (Gpx1), SOD1, and SOD2 were also detected in colon tissues.

Results: Administration of SQE significantly reduced the severity of DSS-induced colitis compared to the control (Ctrl) group. Levels of 8-oxo-dG, an oxidative DNA damage marker, were significantly lower in the SQE group compared to the untreated DSS Ctrl group. In the SQE (300 mg/kg) group, MDA levels were significantly lower, while SOD and catalase activity levels in the plasma samples were significantly higher compared with the DSS Ctrl group. The expression levels of the antioxidant enzymes, SOD2 and Gpx1, were significantly higher, while the levels of SOD 1 expression were lower, in the colon tissues of the DSS Ctrl group compared with those of the Ctrl group. In contrast, administration of SQE significantly down-regulated SOD2 and Gpx1 expressions and up-regulated SOD1 expression.

Conclusions: These results indicate that SQE efficiently suppresses oxidative stress in DSS-induced colitis in mice, and its action is associated with the regulation of antioxidant enzymes.
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Key Words: *Sasa quelpaertensis* leaf, Dextran sulfate, Colitis, Antioxidant

INTRODUCTION

Inflammatory bowel diseases (IBD), as well as Crohn's disease and ulcerative colitis (UC), are intestinal inflammatory disorders that lead to mucosal disruption and ulceration characterized by abdominal pain and diarrhea.[1] The incidence rates and prevalence of IBD are high in Western countries, although the rates are beginning to rise in developing countries as well.[2] Currently, the etiologies of IBD remain unclear, although it is known that they are commonly caused by a combination of factors, including genetic predisposition and environmental factors.[1] Among the various causes of IBD, oxidative stress due to reactive oxygen species (ROS) is one of the most important factors in the genesis and progression of IBD.[3] ROS also plays an important role in the malignant progression of tumor cells and carcinogenesis.[4] During the progression of IBD, activated inflammatory cells produce large amounts of ROS.[5] Several studies have reported that epithelial cell injury leading to the production of

ROS has been detected in IBD patients and in an animal model of dextran sulfate sodium (DSS)-induced inflammation.[6-8] The initial oxidative stress activates a defense mechanism that leads to detoxification of the ROS. However, the accumulation of oxidative stress can induce membrane damage and DNA adducts, and eventually can cause the degeneration of tissues and cellular transformation.[9]

Inflammatory injury to tissues can also induce excessive production of ROS. To counteract the harmful effects of ROS, the intestinal mucosa possesses an efficient antioxidant system. In this system, superoxide dismutase (SOD), catalase, and glutathione peroxidase (Gpx) play important roles. SOD converts the highly reactive superoxide anion, $O_2{}^-$, to the less reactive species, H_2O_2 and O_2.[10] Gpx and catalase normally remove H_2O_2 produced by SOD enzyme.[11] In the intestinal mucosa of IBD patients, the balance of antioxidant enzyme levels has been found to be seriously impaired.[12,13]

To study IBD, a DSS-induced colitis model is generally used[14] since it reflects many characteristics of IBD, including elevated levels of ROS and their metabolites.[15] It is hypothesized that the study of ROS events may facilitate our understanding of the primary and downstream secondary pathophysiological mechanisms that mediate intestinal inflammation.[16]

Several antiinflammatory drugs including sulfasalazine (SSZ) are commonly colon specific drugs used for treatment IBD.[17] However, these drugs had limitations and showed associated side effects.[18,19] Therefore, recently plant extracts or blend tea from plant leaves for IBD management have been growing interest by their therapeutic effects.[20-22] Natural herbal medicines have shown to be beneficial for the treatment of IBD and they also help to maintain healthy intestinal functions.[23] In particular, a bamboo grass that is widely grown in Korea, China, and Russia, named Sasa leaves, has exhibited antiinflammatory, antidiabetic, and antipyretic properties.[24] Among the known types of Sasa leaves, *Sasa quelpaertensis* Nakai is endemic to the area surrounding Mt. Halla on Jeju Island, Korea has exhibited antiinflammatory, antidiabetic, and antipyretic properties. Recent studies have reported that *S. quelpaertensis* Nakai have antidiabetic, antiobesity, antioxidative, and anticancer effects.[25-27] *S. quelpaertensis* leaf extract (SQE) contains amino acids and polyphenols, including *p*-coumaric acid and tricin. *p*-coumaric acid has been shown to exert an antiinflammatory effect, while tricin has suppressed inflammatory-related carcinogenesis in experimental animals.[28,29]

Considering the results of these previous studies, we hypothesize that SQE exerts an antioxidant effect in a mouse model of DSS-induced colitis by protecting intestinal tissues and cells from oxidative stress. SQE may also help restore the balance among relevant antioxidant enzymes.

MATERIALS AND METHODS

1. Extraction of *Sasa quelpaertensis* Nakai leaves

S. quelpaertensis Nakai leaves were collected from Mt. Halla on Jeju Island in South Korea. Extractions were performed as previously described.[30] Briefly, leaves (1 kg) were dried and washed in running water, were cleaned twice with deionized water, and then were dried and extracted with 70% ethanol for 48 hours at room temperature (RT). The SQE extract was subsequently filtered and dried on a rotary evaporator under reduced pressure. Following lyophilization, the extraction was crushed into a powder and was stored at -20°C until needed.

2. Animal groups and induction of dextran sulfate sodium-induced colitis

Five-week-old male C57BL/6 mice (Central Lab. Animal Inc., Seoul, Korea) were maintained under standard laboratory conditions: 22°C $\pm$ 2°C, 50% $\pm$ 5% humidity, and 12 hours/12 hours light/dark cycles. Animals received a modified American Institute of Nutrition (AIN)-93G pellet diet (Unifaith Inc., Seoul, Korea). All mice were allowed to adapt to their new husbandry conditions for 1 week. The mice were randomized into five groups with 10 animals per group.

The five experimental groups included: i) control (Ctrl), ii) DSS-induced colitis (DSS Ctrl), iii) DSS + 100 mg/kg body weight (bw)/d SSZ (SSZ), iv) DSS + 100 mg/kg bw/d SQE (SQE 100), v) DSS + 300 mg/kg bw/d SQE (SQE 300). The Ctrl group received a standard diet and normal drinking water. To induce colitis, mice were administered with 2.5% DSS (molecular weight: 36-50 KD; MP Biomedicals, Costa Mesa, CA, USA) in drinking water for 7 days, followed by 7 days of untreated drinking water, and another 7 days of 2.5% DSS drinking water. The SSZ group was orally administered SSZ dissolved in distilled water for 21 days. Both SQE groups were pretreated with SQE for 14 days prior to DSS treatment. During the experimental period, bw and diet intake were recorded twice a week. After five weeks, all of the mice were sacrificed and plasma and tissue samples were collected and stored at -80°C. Animal care and experimental protocols for this study were approved by the Animal Care and Use Committee of Ewha Womans University (IACUC approval no: IACUC 14-070).

3. Disease activity index

Disease activity index (DAI) score were calculated by adding combined scores for weight loss, stool consistency, and fecal bleeding, and dividing this sum by three. Mean values were assigned for stool consistency according to the presence of loose feces and watery diarrhea. For fecal bleeding was scored as normal, slightly bloody, and blood in whole colon compared to the Ctrl group.[30] DAI scoring was performed from the start of DSS administration until the end of the experimental period.

4. Histopathologic analysis and immunohistochemical detection of 8-oxo-dG

For each collected colon tissue, fecal residue was removed, the tissue was gently rinsed in saline solution, and the tissue was fixed in 10% neutral-buffered formaldehyde. After 24 hours, each tissue was embedded in paraffin. Cut sections (4 μm) were stained with H&E according to standard procedures.[31]

For immunohistochemical staining of 8-oxo-dG, sections were deparaffinized, rehydrated, and incubated overnight at 4°C with a primary anti-8 hydroxyguanosine antibody (Abcam, Cambridge, UK). After incubating the section with an appropriate secondary antibody at RT for 1 hour, the sections were washed, stained with 3,-3'-diaminobenzidine, and counterstained with hematoxylin for 1 minute. Slides were mounted using Permount mounting medium (Fisher Scientific, Pittsburgh, PA, USA). The percentage of positively stained cells among the total number of cells was evaluated in three randomly chosen fields and the mean value was recorded.[30]

5. Measurement of gut transit time and gut motility

To study gut motility, whole gut transit time was measured.[32] Briefly, 300 μL of a carmine red solution (3 g carmine in 50 mL of 0.5% methylcellulose; Sigma Aldrich, St. Louis, MO, USA) was administered to each mouse by gavage. These mice were then placed in individual cages contained a white sheet on the bottom of the cage in order to distinguish the red stools from the normal stools. Total whole gut transit time was recorded following the first appearance of a red stool pellet. Gut motility was subsequently calculated as gut transit time divided by colon length.

6. Measurement of plasma malondialdehyde, plasma superoxide dismutase activity, and colonic catalase activity

To detect malondialdehyde (MDA), lipid peroxidation in plasma was measured with a TBARS Assay Kit (ZeptoMetrix,

Buffalo, NY, USA), according to the manufacturer's instructions. Plasma SOD activity was measured with a SOD Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA) according to the manufacturer's protocol.

Colon rectum tissues were collected and homogenized in 500 μL cold reaction buffer supplemented with a protease inhibitor cocktail (Sigma Aldrich). After centrifugation (4°C, 15 minutes, $16,000 \times g$), supernatants were stored at $-80°C$. Catalase activity was detected by using an Amplex Red[®] Catalase Assay Kit (Invitrogen, Carlsbad, CA, USA.) according to the manufacturer's instructions.

7. Western blot analysis

Frozen whole colon tissues were homogenized in ice-cold PRO-PREP protein extraction solution (Intron Biotechnology, Seoul, Korea). Homogenates were centrifuged (4°C, $12,000 \times g$, 15 minutes) and supernatants were stored at $-80°C$. Heat-denatured tissue homogenates were separated using gradient (12%-15%) sodium dodecyl sulfate polyacrylamide gel electrophoresis. For western blot analysis, proteins were transferred to polyvinylidene difluoride membranes and were blocked with 5% skim milk or 3% bovine serum albumin + 2% skim milk in tris-buffered saline containing Tween-20 (TBST). Immunoblot analysis was performed using the following primary antibodies: SOD1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), SOD2 (Santa Cruz Biotechnology), Gpx1 (Abfrontier, Seoul, Korea), and α-tubulin (Sigma Aldrich). After an overnight incubation at 4°C, the membranes were washed three times with TBST and then were incubated with the appropriate secondary rabbit or mouse immunoglobulin G-conjugated horseradish peroxidase antibodies (Santa Cruz Biotechnology) at RT for 1 hour. After the membranes were washed three times, bound antibodies were detected using an enhanced chemiluminescence reagent (Animal Genetics Inc., Suwon, Korea).

8. Statistical analysis

Data shown are the mean ± SEM for each group. Statistical analyses were performed using GraphPad PRISM software (GraphPad Software, San Diego, CA, USA). For multiple comparisons, one-way ANOVA with Tukey's post-hoc test was used. A P-value less than 0.05 was considered statistically significant.

RESULTS

1. Administration of *Sasa quelpaertensis* leaf extract suppresses the development of dextran sulfate sodium-induced colitis in mice

DAI scores were higher in the DSS-induced group at day 19 compared to the Ctrl group (Fig. 1A). In addition, severe rectal bleeding, loose fecal, and weight loss during the second cycle of DSS were characterized in the DSS Ctrl group. In contrast, SSZ, SQE100, and SQE300 treatments were found to significantly suppress DAI scores by 33.3% ($P < 0.05$), 57.8% ($P < 0.001$), and 72.2% ($P < 0.001$) at day 19 compared to the Ctrl group. In particular, the mean DAI score of the SQE 300 group nearly achieved the mean DAI score of the Ctrl group. Furthermore, colorectal tissues from the Ctrl group exhibited normal histology, whereas colorectal tissues from the DSS Ctrl group exhibited severe inflammation, crypt distortion, and architectural abnormalities (Fig. 1B). However, in the SQE groups, crypt distortion was suppressed and only mild tissue damage was observed.
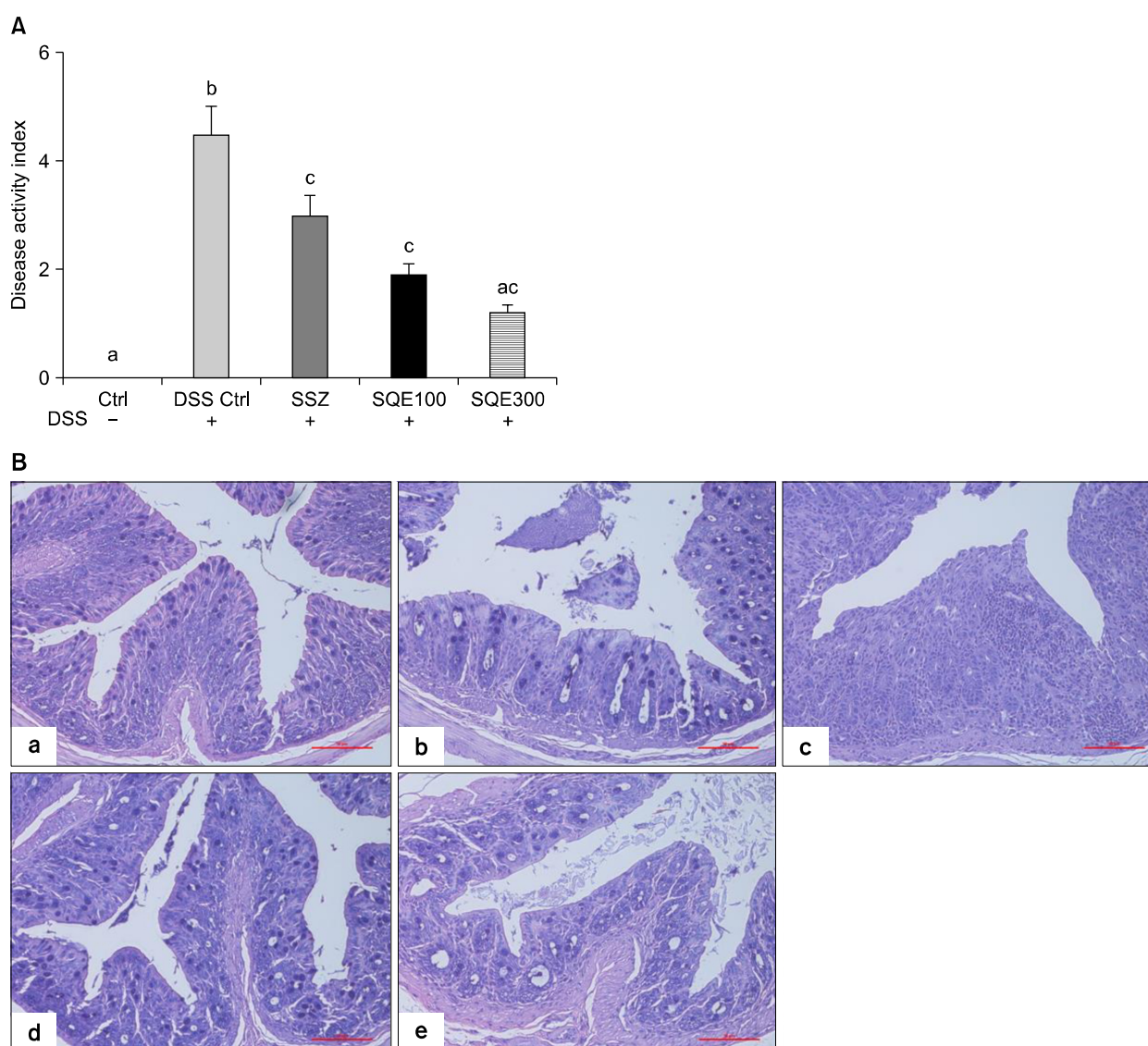

Figure 1. Administration of SQE suppresses the development of DSS-induced colitis in mice. (A) Disease activity index values were evaluated based on scores for body weight loss, stool consistency, and fecal bleeding for each of the groups. (B) Representative histologic damage in colorectal sections obtained from Ctrl (a), DSS Ctrl (b), SSZ (c), SQE 100 (d), and SQE 300 (e) mice. Colorectal tissues sections were stained with H&E (scale bar: 50 μm). Data are shown to as the mean ± SEM and were analyzed using one-way ANOVA and Tukey's post-hoc test ($P < 0.05$); n = 10 mice per group. Different letters are used to indicate significant differences. SQE, *Sasa quelpaertensis* extract; Ctrl, control; DSS, dextran sulfate sodium; SSZ, sulfasalazine.

2. *Sasa quelpaertensis* leaf extract administration apparently restored the gut motility in the dextran sulfate sodium-induced colitis mouse model

To determine the effects of SQE on gut motility, whole gut transit time was measured according to the first appearance of a carmine red stained stool (Fig. 2A). There was no significant difference in gut transit time between the groups although the administration of SQE groups tended to have a shorter time than the Ctrl group. When gut motility was determined by dividing the gut transit time by colon length, gut motility was found to be significantly reduced in the DSS Ctrl group compared to the Ctrl group (Fig. 2B), while gut motility for the SQE groups tended to be increased compared to the DSS Ctrl group. However, the differences were not statistically significant.

3. *Sasa quelpaertensis* leaf extract administration alleviates oxidative DNA damage caused by dextran sulfate sodium in the mouse colon

The reaction of an OH radical with a DNA guanosine base generates 8-oxo-dG.[33] Thus, detection of 8-oxo-dG has been used as a marker of DNA damage. The percentage of positively stained epithelial cells with 8-oxo-dG was significantly higher in the colon tissues of the DSS Ctrl group (49.8%) compared with the tissues from the Ctrl group (Fig. 3). SSZ treatment did not affect the number of 8-oxo-dG-positive cells compared to the DSS Ctrl group, while administration of SQE significantly reduced the percentage of 8-oxo-dG- positive cells compared to the DSS Ctrl

group ($P < 0.05$). In particular, the percentage of 8-oxo-dG-positive cells in the SQE 300 tissues was significantly decreased by 36.1% compared to that of the DSS Ctrl group tissues ($P < 0.05$), and this percentage was similar to that of the Ctrl group. Taken together, these results suggest that SQE treatment protects against DNA damage from DSS-induced colitis in mice.

4. *Sasa quelpaertensis* leaf extract administration regulates antioxidant enzyme activity in dextran sulfate sodium-induced colitis in mice

To evaluate the antioxidative capacity of SQE administration, MDA and SOD activity levels in plasma samples, and catalase activity in colon tissues were analyzed. MDA activity in the SQE 300 group was significantly lower by 28.1% compared with that of the DSS Ctrl group (Fig. 4A; $P < 0.05$), and there it was no statistically significant difference between the MDA activity of the SSZ group and the DSS Ctrl group. Expression levels of SOD were lower by 20.7% in the DSS Ctrl group compared with the Ctrl group (Fig. 4B), whereas the SOD levels were increased by 28.1% in the SQE 300 group compared to the DSS Ctrl group ($P < 0.01$). Both of these differences were significant. In combination, these results indicate that SQE protects the levels of SOD activity in the colon tissues of mice with DSS-induced colitis. Regarding the levels of catalase, the lowest levels were detected in the colon tissues of the DSS Ctrl group (Fig. 4C). In contrast, the catalase activity level of the SQE 300 group was significantly increased by 44.7% compared with the DSS Ctrl group ($P < 0.05$). Overall, these results indicate that SQE regulates antioxidant enzyme activity against oxidative stress in DSS-induced colitis in mice.
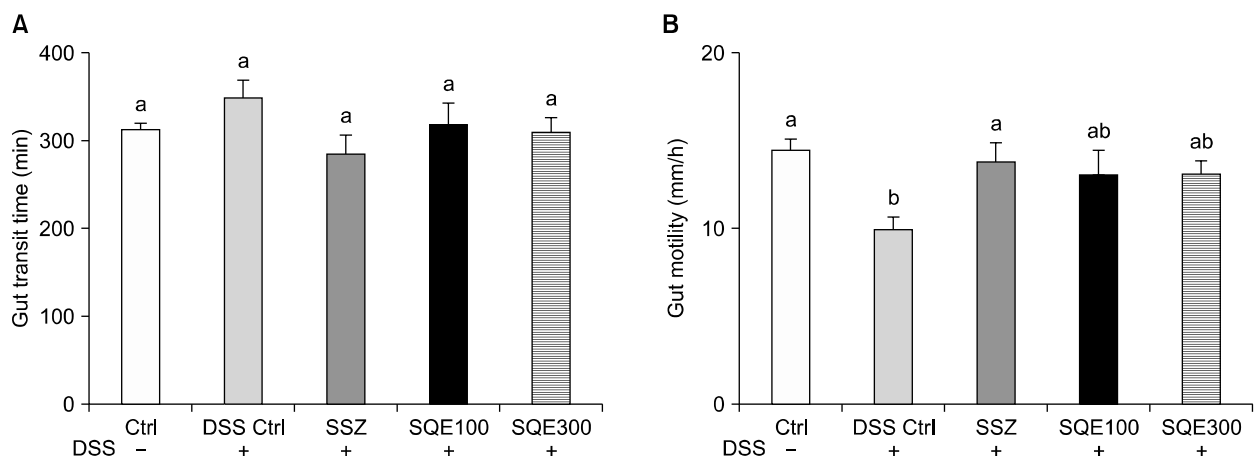

Figure 2. Administration of SQE alters gut motility in DSS-induced colitis model. (A) Gut transit time for all groups was analyzed by measuring the time of first development of red stools after carmine red administration. (B) Gut motility was estimated by the time and colon length for each group. Data are shown to as the mean ± SEM and were analyzed using one-way ANOVA and Tukey's post-hoc test ($P < 0.05$); n = 10 mice per group. Different letters are used to indicate significant differences. SQE, *Sasa quelpaertensis* extract; DSS, dextran sulfate sodium; Ctrl, control; SSZ, sulfasalazine.
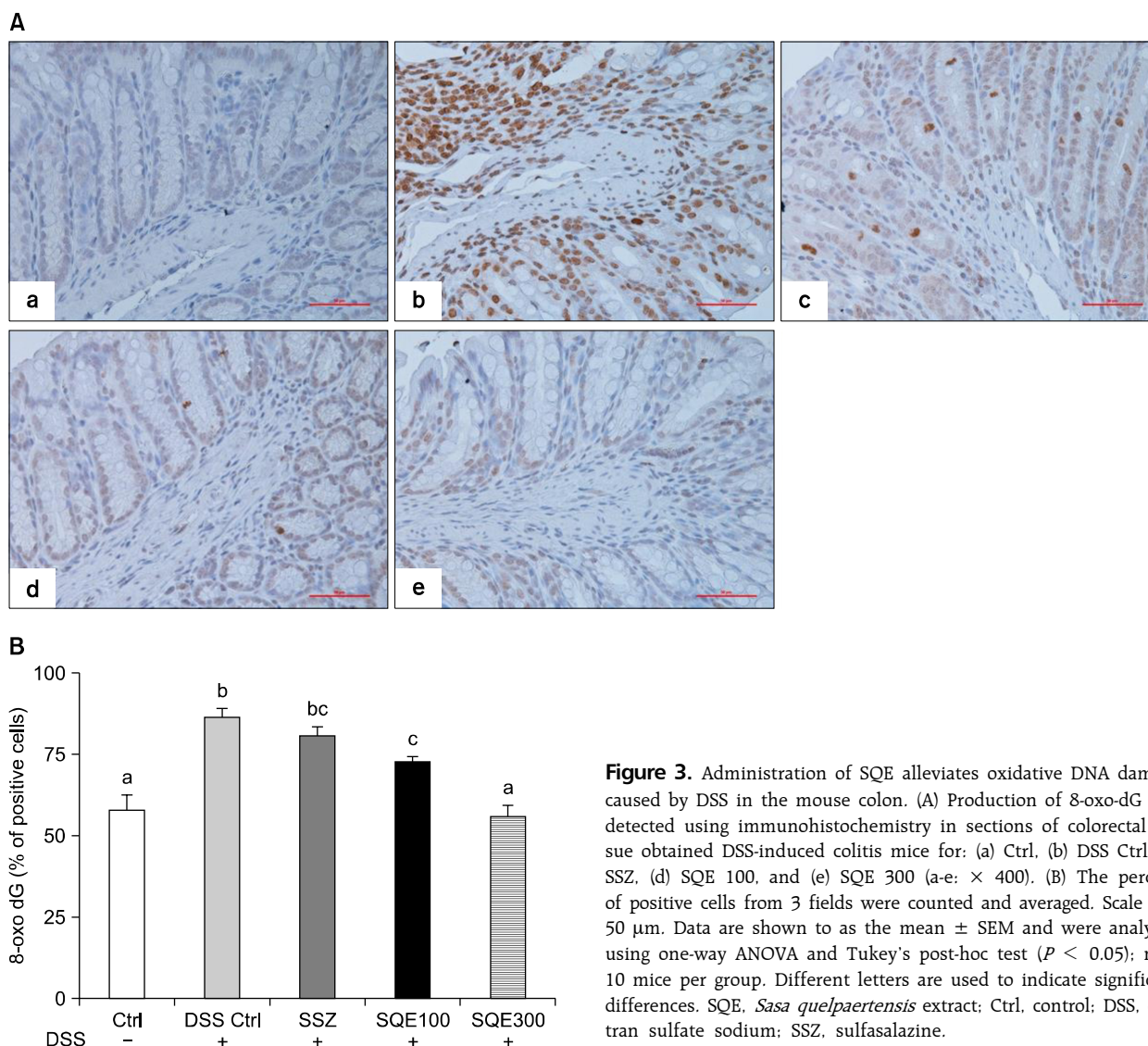
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Figure 3. Administration of SQE alleviates oxidative DNA damage caused by DSS in the mouse colon. (A) Production of 8-oxo-dG was detected using immunohistochemistry in sections of colorectal tissue obtained DSS-induced colitis mice for: (a) Ctrl, (b) DSS Ctrl, (c) SSZ, (d) SQE 100, and (e) SQE 300 (a-e: × 400). (B) The percent of positive cells from 3 fields were counted and averaged. Scale bar: 50 μm. Data are shown to as the mean ± SEM and were analyzed using one-way ANOVA and Tukey's post-hoc test ($P < 0.05$); n = 10 mice per group. Different letters are used to indicate significant differences. SQE, *Sasa quelpaertensis* extract; Ctrl, control; DSS, dextran sulfate sodium; SSZ, sulfasalazine.

5. *Sasa quelpaertensis* leaf extract administration regulates expression of superoxide dismutase and glutathione peroxidase 1 in the colon of mice treated with dextran sulfate sodium

To investigate the role of SQE administration in regulating oxidative stress in a mouse model of DSS-induced colitis, the expression levels of several antioxidant enzymes, SOD1 (Cu-Zn SOD), SOD2 (Mn SOD), and Gpx1, were analyzed. For the DSS group, the levels of SOD1 expression decreased by 27.2% compared with the Ctrl group ($P < 0.01$). However, the SOD1 expression levels were significantly higher in both of the SQE groups compared to the DSS Ctrl group (Fig. 5A). SSZ treatment did not affect SOD expression. In contrast, the enzyme activity levels of SOD2 and Gpx1 in colon tissues from the DSS Ctrl group

were higher than those of the Ctrl group. In the SQE 300 tissues, the enzyme expressions of SOD2 and Gpx1 were significantly lower by 31.9% ($P < 0.001$) and 27.3% ($P < 0.01$), respectively, compared with the DSS Ctrl group (Fig. 5B and 5C). These results suggest that SQE administration regulates the expression levels of certain antioxidant enzymes that are involved in a DSS-induced colitis in mice.

DISCUSSION

In our previous studies, SQE treatment was shown to regulate levels of proinflammatory markers, mitogen-activated protein kinase signaling, and activation of NF-κB in a mouse model of DSS-induced colitis.[30] In the present study, the antioxidative effect of SQE was also demonstrated in a mouse model of
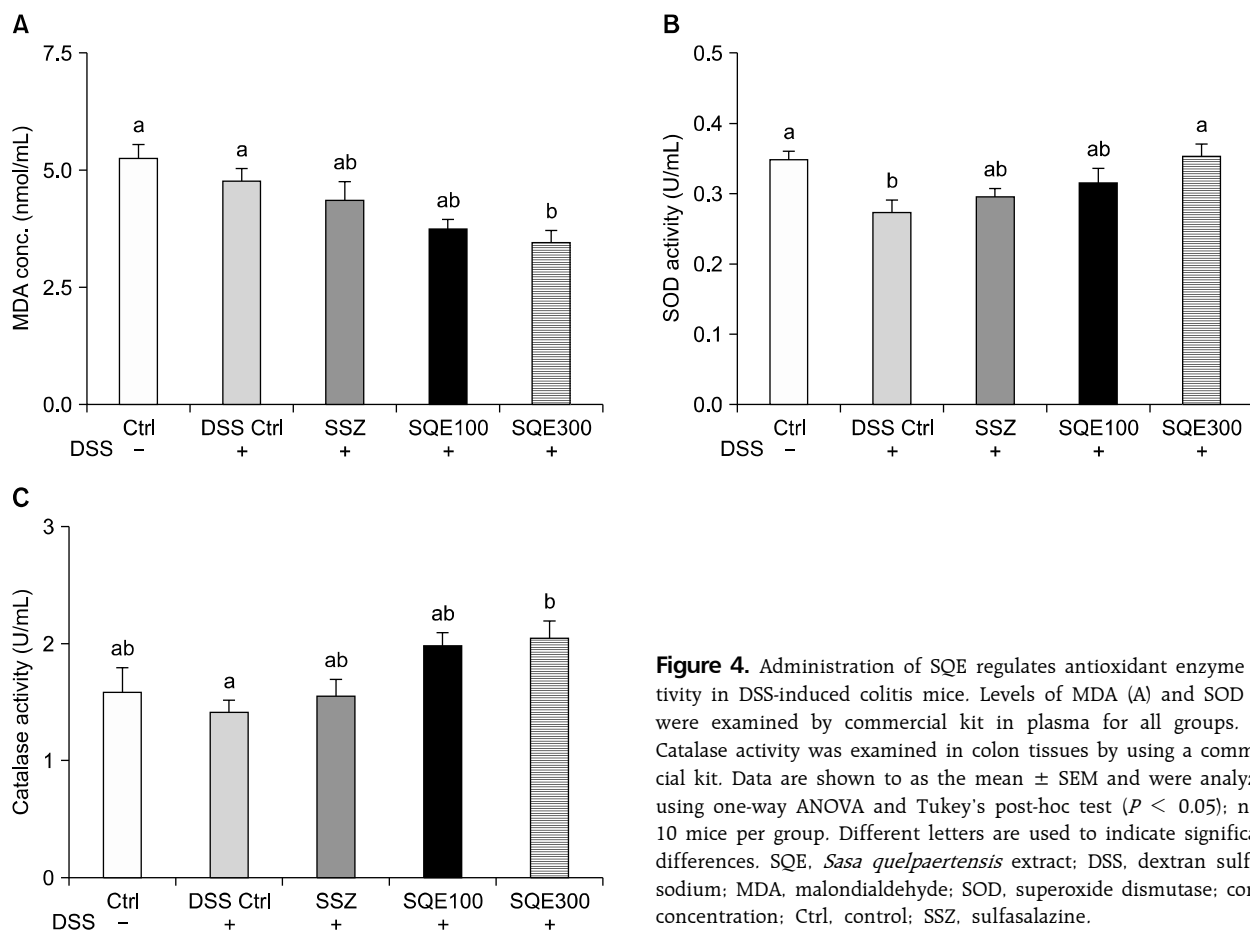
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Figure 4. Administration of SQE regulates antioxidant enzyme activity in DSS-induced colitis mice. Levels of MDA (A) and SOD (B) were examined by commercial kit in plasma for all groups. (C) Catalase activity was examined in colon tissues by using a commercial kit. Data are shown to as the mean ± SEM and were analyzed using one-way ANOVA and Tukey's post-hoc test ($P < 0.05$); n = 10 mice per group. Different letters are used to indicate significant differences. SQE, *Sasa quelpaertensis* extract; DSS, dextran sulfate sodium; MDA, malondialdehyde; SOD, superoxide dismutase; conc., concentration; Ctrl, control; SSZ, sulfasalazine.

DSS-induced colitis. DSS administration effectively induces clinical features in mice that are similar to those of patients with IBD, including compromise of the integrity of the mucosal barrier that leads to intestinal epithelial cell inflammation and oxidative stress as a result of ROS generation.[34] ROS can either positively or negatively modulate an inflammatory response, and it plays an important role in the pathophysiology of IBD.[35] Oxidative stress is considered to be critical in the tissue destruction in IBD and it affects carcinogenesis.[36] Many studies have reported that animals subjected to DSS-induced colitis show increased level of oxidized protein in their plasma, as well as increased oxidative injury due to the inflammatory response.[37,38] In the present study, SSZ was used as a drug control. SSZ has previously been characterized as a therapeutic agent for IBD, albeit with side effects that include ulcerogenic potential and additional oxidative stress.[19,39] The dose of SSZ that was used in the present study represents a commonly used dose in animal studies. In the present study, SQE exhibited better protection from DNA damage and oxidative stress compared with SSZ, which indicated the therapeutic potential of SQE for inflammatory IBD.

The observed increase in DAI values for the DSS Ctrl group is similar to that commonly observed for IBD patients.[40] In addition, massive crypt distortion and architectural abnormalities that characterized the colon tissues of the DSS Ctrl group are consistent with those of a previous study, which indicated a severe inflammation response had developed.[30] However, in the present study, SQE treatment reduced the severity of colitis as evidenced by the suppressed DAI scores in the SQE group.

Gut transit time is a parameter that can be used to evaluate the functions of a gastrointestinal system. In the present study, gut transit time was evaluated in relation to inflammation. In the SQE groups, gut transit time was reduced, thereby implying that administration of SQE improves gut motility. However, since gut transit time can be affected by various factors, including nutrient absorption, physical activity, digestive ability, and eating time, additional studies are needed to confirm the present results in relation to these factors.

Increased levels of 8-oxo-dG have been detected in patients with IBD, and may be due to increased oxidation of DNA.[41] Sheridan et al.[33] also reported that high levels of 8-oxo-dG-positive
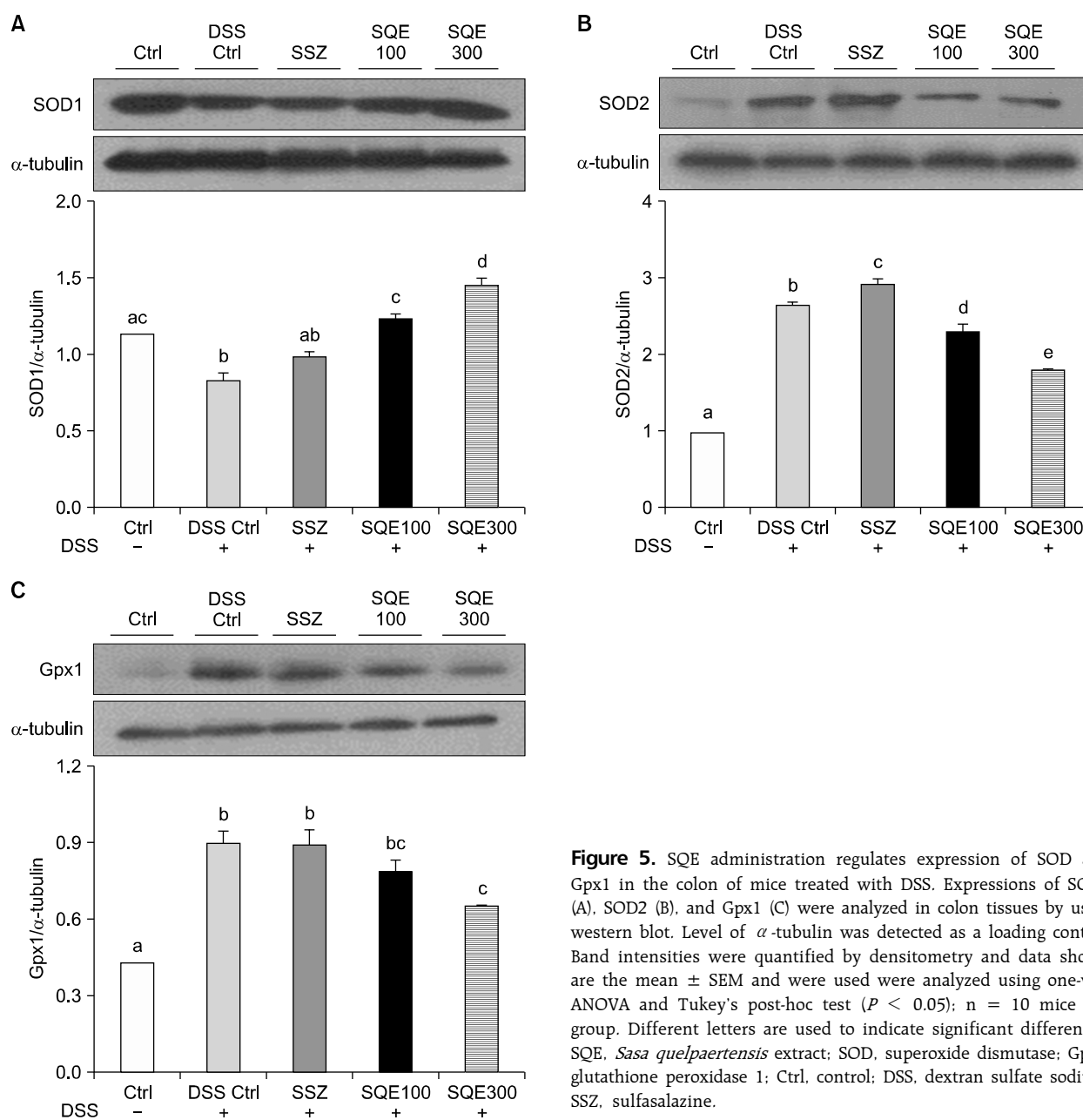
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B

C

Figure 5. SQE administration regulates expression of SOD and Gpx1 in the colon of mice treated with DSS. Expressions of SOD1 (A), SOD2 (B), and Gpx1 (C) were analyzed in colon tissues by using western blot. Level of α-tubulin was detected as a loading control. Band intensities were quantified by densitometry and data shown are the mean ± SEM and were used were analyzed using one-way ANOVA and Tukey's post-hoc test ($P < 0.05$); n = 10 mice per group. Different letters are used to indicate significant differences. SQE, *Sasa quelpaertensis* extract; SOD, superoxide dismutase; Gpx1, glutathione peroxidase 1; Ctrl, control; DSS, dextran sulfate sodium; SSZ, sulfasalazine.

cells are associated with poor survival in colorectal cancer patients. In the present study, higher levels of 8-oxo-dG were detected in the DSS Ctrl group, and these results are consistent with those of recent studies where higher levels of DNA damage were associated with the production of ROS as part of the inflammation response in a DSS-induced colitis model.[42,43] In contrast, SQE treatment resulted in the lower levels of 8-oxo-dG in the colon cells examined. Taken together, these results indicate that SQE protects cells from DNA damage resulting from oxidative stress.

ROS are produced by activated neutrophils and macrophage and can be assessed by measuring the levels of MDA in colon

tissues.[44] MDA levels increase in the presence of ROS thereby lead to lipid peroxidation.[38] Mice treated with DSS have been found to have elevated MDA level.[45] Also, Rise et al.[46] reported that levels of MDA were increased in the colon of an UC mouse model that was induced by acetic acid. In the present study, MDA levels were higher in the DSS Ctrl group, whereas the SQE group exhibited a significant decrease in MDA levels. These results indicate that administration of SQE may reduce MDA activity in plasma by protecting the lipid peroxidation process.

Activation of phagocytic leukocytes is induced by inflammation in the mucosa, and the production of ROS increases, including

that of superoxide anions.[3] Uncontrolled overproduction of ROS can disturb protective mechanisms and can lead to cellular oxidative damage.[47] SOD is an antioxidant enzyme that converts the highly reactive superoxide anion, O_2^-, to less reactive species such as H_2O_2 and O_2.[10] During inflammation, high levels of ROS can disturb SOD antioxidant enzymes and decrease SOD enzyme activity, as observed in IBD patients with active disease.[16] In the present study, SOD activity was significantly reduced in the plasma samples obtained from the DSS Ctrl group, whereas SOD activity was maintained following administration of SQE. It is possible that the latter observation may contribute to reduced tissue damage.

In mammalian cells, three isoforms of SOD have been identified: SOD1 (Cu-Zn SOD), SOD2 (Mn SOD), and SOD3 (Extracellular-SOD).[48] In the present study, the effect of SQE on the expression of SOD1 and SOD2 was studied in a DSS-induced inflammation animal model. SOD1 (Cu-Zn SOD) is considered to have an important role in the first line of antioxidant defense. SOD1 is also located throughout the cytoplasm, yet is absent in mitochondria.[47] Previous studies have shown that SOD1 protein expression and activity are decreased in inflamed mucosa from IBD patients.[7,49] Moreover, high levels of ROS have been reported to disturb SOD1 antioxidant enzymes during inflammation. In the present study, lower levels of SOD1 expression were consistently detected in the DSS Ctrl group compared with the Ctrl group, whereas higher levels of SOD1 expression were detected in the administration of SQE group.

In contrast with SOD1, SOD2 (Mn-SOD) exclusively localized to mitochondria, and oxygen radicals in the respiratory chain are removed by SOD2.[47] It has been suggested that SOD2 is a new type of tumor suppressor gene.[50] Unlike SOD1, SOD2 is easily induced and is up-regulated under inflammatory conditions following exposure to various oxidants. Thus, levels of SOD2 are increased under inflammation conditions involving ROS.[51,52] In the present study, expression levels of SOD2 were consistently up-regulated in the DSS group, whereas these levels were suppressed with administration of SQE. These results suggest that administration of SQE regulates expression of SOD antioxidant enzymes under inflammatory conditions.

Gpx and catalase normally remove H_2O_2 and share H_2O_2 as substrate, however both enzymes have different features. Gpx localizes to intestinal epithelial cells and acts as a primary defense against low concentrations of H_2O_2.[53] Iantomasi et al.[54] reported that the mucosa of IBD patients' exhibited higher levels of Gpx activity. In the present study, higher levels of Gpx expression were detected in colon tissues from the DSS Ctrl group compared with colon tissues from the Ctrl group, and the levels of Gpx were suppressed in tissues from the SQE group. Catalase efficiently converts H_2O_2 to water and O_2 in peroxisomes and also protects cells from oxidative stress due to H_2O_2.[55] Experimental studies have shown that exposure to ROS can lead to a rapid inhibition of catalase, but not Gpx activity.[56,57] In the present study, lower levels of catalase expression were detected in the DSS Ctrl group tissues compared to the Ctrl group, whereas the levels of catalase expression were higher in the tissues from the SQE group. Taken together, these results provide further evidence that SQE has the ability to restore balance among the antioxidant enzymes that are affected by an oxidative stress environment and negatively affect IBD progression.

Many studies have reported that natural polyphenols provide beneficial effects in colitis models.[58,59] Accordingly, recent studies have reported that various Sasa leaves and their bioactive compounds mediate antioxidant effects against oxidative stress.[60-63] For example, one of major components of SQE, *p*-coumaric acid, has been shown to mediate an antioxidant effect on low-density lipoprotein cholesterol oxidation.[63] However, the antioxidant effects of individual bioactive compounds of SQE were not analyzed in the present study. Therefore, further studies are warranted to investigate the antioxidant properties of the individual bioactive compounds of SQE in IBD models.

In conclusion, the results of the present study indicate that SQE can mediate an inhibition of IBD progression and a reduction in DNA damage in a mouse model of DSS-induced colitis. Furthermore, SQE can regulate antioxidant enzymes that play key roles in the antioxidant defense system of a cell. Insight into the mechanistic details of these processes will improve our understanding of the antioxidant effect of SQE and will facilitate development of the natural therapeutic strategies for IBD patients.

ACKNOWLEDGMENTS

CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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