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Full Length Research Paper

In vivo anti-oxidant and anti-inflammatory activities of cambial meristematic cells established from *Ginkgo biloba* L.

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Ginkgo biloba **leaves are traditionally ascribed a wide range of therapeutic attributes, including anti-oxidant, anti-cancer, and anti-inflammatory properties. We previously reported success in establishing and expanding suspension cultures of *G. biloba* cambial meristematic cells (CMCs) *in vitro*, while maintaining genetic stability and physiological homogeneity. In the present study, we tested the anti-oxidant and anti-inflammatory activities of *G. biloba* CMCs using *in vitro* and *in vivo* assay systems. Results showed that the ethanol extract of CMCs scavenged superoxide anions and hydroxyl radicals in cell-free systems. We also found that oral administration of CMC extract to mice stimulated primary anti-oxidant enzyme activity in the liver. More importantly, oral administration of CMC-containing diet significantly inhibited the inflammatory response in mouse colitis model induced by dextran sulfate sodium, as assessed by the length and status of the intestine, body weight, and IgA concentration in intestine. In addition, oral administration of CMC-containing diet significantly inhibited pro-inflammatory cytokine expression in both systemic and mucosal immune regulatory sites. In conclusion, *G. biloba* CMCs show promise as an anti-oxidant and anti-inflammatory substance.**

Key words: Anti-inflammatory activity, anti-oxidant activity, cambial meristematic cells, colitis, *Ginkgo biloba*.

INTRODUCTION

Damage by reactive oxygen species (ROS) contributes to a substantial number of adult diseases through enzyme inactivation, lipid peroxidation, and DNA mutation (Freeman and Grapo, 1982; McCord, 1987). ROS-mediated disorders cause prolonged inflammatory reactions and facilitate damage to various tissues, resulting in diverse inflammation-related diseases including cancer (Willoughby, 1975). Consequently, anti-oxidant therapies that control ROS are considered one of the most effective ways to treat inflammatory diseases. As preventive and therapeutic measures addressing inflammatory diseases gain importance, many investigators have focused their efforts on developing anti-inflammatory substances without side effects. Although, synthetic chemicals such as butylated

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Abbreviations: CMC, Cambial meristematic cell; **DPPH,** 1,1-diphenyl-1-picrylhydrazyl; **DSS,** dextran sulfate sodium; **ELISA,** enzyme-linked immunosorbent assay; **GPx,** glutathione peroxidase; **GSH,** glutathione sulfhydryl; **LDH,** lactate dehydrogenase; **MLN,** mesenteric lymph node; **SOD**, superoxide dismutase.

hydroxytoluene, butylated hydroxyanisole, and propyl gallate are considered cost-effective anti-oxidants, their general use is limited due to safety concerns (Barene, 1975; Choe and Yang, 1982). Research efforts are thus concentrated on anti-oxidant and anti-inflammatory substances derived from natural materials. Among natural materials, plants are a valuable source of a wide range of secondary metabolites, which have been used as pharmaceuticals, agrochemicals, flavors, fragrances, colors, biopesticides, and food additives (Rao and Ravishankarb, 2002). In particular, the secondary metabolites of plants are a major source of drugs, with approximately half of all pharmaceuticals in current use originating from natural plant products (Clark, 1996). *Ginkgo biloba* L. is traditionally used to treat inflammatory diseases and has gained popularity as an alternative medicine in developed countries (Koh, 2010; Rogerio et al., 2010; Lee et al., 2011). *G. biloba* nut extract was traditionally used to treat asthma and cough (Smith and Luo, 2004), while its leaf extract has shown anti-cancer (Zhang et al., 2008) and anti-oxidant (Sener et al., 2007) properties, as well the ability to improve blood circulation (Huang et al., 2004). In particular, the administration of *G. biloba* leaf extract has been found to decrease platelet aggregation, allergic reactions, and general inflammatory response (Braquet, 1987; Braquet and Hosford, 1991). However, there are few studies about the medicinal efficacy of the *G. biloba* stem compared to the well-recorded medicinal effects of leaf extract. The biological activity of plant extracts is highly variable according to the plant source, location, and harvest season, and can be adversely affected by unpredictable environmental circumstances (Roberts, 2007). Therefore, well defined plant cell cultures have been considered as an alternative method for producing useful compounds with consistent medicinal properties. We previously established the technology to isolate and culture pure cambium-derived cells from various plant tissues including *G. biloba* stem, successfully obtaining efficient and stable cell lines (Lee et al., 2010). Our preliminary studies revealed that cambium meristematic cells (CMCs) established from *G. biloba* stems exhibited the biological activities in terms of anti-oxidant and anti-inflammatory activities.

To provide a scientific basis for the biological potential of CMCs cultured from *G. biloba*, we examined the free radical-scavenging activities of CMC extract using cell-free radical generating systems. We tested the effects of CMC extract on detoxifying enzymes in the livers of mice after oral administration. Finally, we assessed the anti-inflammatory potential of dietary CMCs in mice with colitis induced by dextran sulfate sodium (DSS).

MATERIALS AND METHODS

Experimental materials

We purchased antibodies specific to mIgA, mIgG, mIL-4, mIL-6, mIFN-γ, and mTNF-α from BD Biosciences (Franklin Lakes, NJ, U.S.A.) and adult female BALB/c mice from Charles River Technology through Orient Bio (Sungnam, Korea). Mice were housed in automatically controlled conditions with a 12 h light/dark cycle at 22 ± 1°C with 45 to 55% relative humidity. All mice had free access to standard rodent food pellets and water *ad libitum*. Unless otherwise specified, chemicals and laboratory materials were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and Falcon Labware (Becton-Dickinson, Franklin Lakes, NJ, U.S.A.), respectively.

Preparation of CMC extract

CMCs of *G. biloba* were isolated from cambium as described elsewhere (Gamborg et al., 1968; Lee et al., 2010). Establishment and expansion of CMCs were carried out according to previously described methods (Lee et al., 2010). Briefly, 20 g fresh CMCs were mixed with 1 L 80% ethanol in a flask and shaken at room temperature for 2 d. The supernatant was collected and concentrated through lyophilization (Eyela Model FDU-2100; Tokyo, Japan), eventually reaching 8 g (40% of the initial weight) of CMC extract.

Preparation of CMC-containing food pellets

We mixed freeze-dried CMC powder (33.3 g) with 1 kg ground food pellets (Orient Bio) and 500 mL distilled water. The feed mixture was pelleted as 3 x 3 x 3 cm cubes and dried at 45°C for approximately 1 d. We used the same method to produce positive control food pellets containing sulfasalazine (3.3 g per 1 kg ground pellets), an anti-inflammatory agent.

Radical scavenging assays using cell-free systems

To assess the superoxide radical ($O_2^{\cdot}$) scavenging activity of CMC extract, we used a method described by Gotoh and Niki (1992) with slight modifications. Briefly, different concentrations of CMC extract were mixed with a buffer containing EDTA, hypoxanthine, and nitro blue tetrazolium and incubated for 3 min. After addition of xanthine oxidase, we measured the absorbance of the mixture at 570 nm using a SpectraCount^{TM} enzyme-linked immunosorbent assay (ELISA) reader (Packard Instrument Co., Downers Grove, IL, U.S.A.). To determine the rate constants for the reactions between anti-oxidants and hydroxyl radicals, referred to as the non-site-specific scavenging assay, we performed a deoxyribose assay as described by Halliwell et al. (1987). We measured site-specific scavenging activity, which represents the ability of CMC extract to chelate iron ions and interfere with hydroxyl radical generation, using the same reaction buffer without EDTA. We measured the absorption of the mixtures at 532 nm using a SmartSpec^{TM} Plus spectrophotometer (Bio-Rad, Hercules, CA, U.S.A.) against a blank containing 10 mM phosphate buffer (pH 7.4).

Measurement of primary anti-oxidant enzyme and serum enzyme activities

In order to examine the effects of CMC extract on primary anti-oxidant enzymes *in vivo*, we randomly divided the mice (n = 18) into 3 groups of 6 mice each; groups were caged separately before CMC extract administration. The control group 1 received oral administration of 0.5 mL distilled water twice per week for 3 weeks. Mice in groups 2 and 3 received oral administration of distilled water with 200 mg/kg body weight of vitamin C and CMC extract, respectively, for the same period. After the last feeding, we anesthetized the mice by inhalation of diethyl ether and collected

whole blood by cardiac puncture with a syringe. We centrifuged the blood samples at 10,000 *g* for 10 min at 4°C and used the supernatants as serum samples to determine lactate dehydrogenase (LDH) activity. We also obtained liver tissue from the mice after perfusion with PBS (pH 7.4), which we processed for biochemical analysis using commercial kits. We measured the level of reduced glutathione (GSH) with a BioVision kit (Mountain View, CA, U.S.A.), catalase activity with an Amplex Red® catalase assay kit (Invitrogen, Carlsbad, CA, U.S.A.), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities with Cayman Chemical Co. kits (Ann Arbor, MI, U.S.A.), and LDH (EC 1.1.1.27) activity with an ADIVA kit (Bayer ADVIA 1650, Osaka, Japan). We performed all biochemical assays according to the manufacturers' instructions. All procedures were approved by the Institutional Animal Care and Use Committee of Chonbuk National University (Approval Number: CBU 2010-0035) and followed their suggested guidelines.

Induction of dextran sulfate sodium (DSS)-induced colitis

In order to test the *in vivo* anti-inflammatory property of CMC samples, we observed the effects of a diet containing CMCs on the disease severity of mice with DSS-induced colitis, as described previously (Okayasu et al., 1990; Dieleman et al., 1994). Briefly, mice (n=30) were divided into 5 groups with 6 mice each: control, DSS alone diet, DSS plus sulfasalazine diet, DSS plus CMC diet, and CMC pretreatment prior to a DSS plus CMC diet. Mice followed a CMC pretreatment diet for 1 week prior to 5 d of 5% DSS treatment, in which DSS was provided freely instead of drinking water. After 5 d of DSS treatment, mice received either a CMC- or sulfasalazine-containing diet for 2 weeks. The experimental procedures involving laboratory animals were approved by the Institutional Animal Care and Use Committee of Chonbuk National University (approval number: CBU 2010-0036) and followed their suggested guidelines.

Measurement of IgA in colon and serum

In order to measure colonic IgA levels, whole mouse colons were minced, put into 1.2 mL ice-cold PBS, and thoroughly mixed. We centrifuged the mixture, collected the supernatant, and measured its IgA density with an isotype-specific ELISA kit. We also performed isotype-specific ELISA on the serum of blood drawn from orbital arteries.

Measurement of cytokines produced by splenocytes and by lymphocytes of mesenteric lymph nodes

To measure the cytokine expression of the lymphocytes from CMC-treated mice, the Bank for Cytokine Research (Chonbuk National University, Jeonju, Korea) performed ELISA on several tissues. Briefly, after collecting mesenteric lymph nodes and spleens from each group of mice, lymphocytes were separated using Percoll® (Amersham Pharmacia Biotech, Piscataway, NJ, U.S.A.) density gradient centrifugation. After distributing 2 x 10^6 cells/well in a 24-well plate, cells were stimulated with 2 µg/mL concanavalin A for either 24 or 48 h, after which the cultured supernatant was subjected to each cytokine-specific OptEIA™ kit (BD Biosciences, Franklin Lakes, NJ, U.S.A.). The amount of cytokines produced was calculated using standard curves generated using known concentrations of recombinant cytokine proteins.

Statistical analysis

All data are expressed as mean ± standard error (SE). We applied one-way analysis of variance (ANOVA; SPSS version 16.0, San Rafael, CA, U.S.A.) followed by Scheffe's *post hoc* test to determine differences between groups. A value of $p<0.05$ was considered significant.

RESULTS

Anti-oxidant activity of CMC extract

We defined efficient superoxide radical scavenging activity of CMC extract as the inhibition of 73.8% of superoxide anion production by addition of 100 µg/mL of sample (Figure 1A). Of note, the radical scavenging activity of CMC extract was higher than that of vitamin C, a well-known anti-oxidant. CMC extract also displayed efficient dose-dependent inhibition of hydroxyl radical-induced deoxyribose degradation in both site-specific and non site-specific manners. Specifically, addition of 50 µg/mL CMC extract resulted in approximately 93.1 and 44.1% inhibition of site-specific and non site-specific hydroxyl radical-induced deoxyribose degradation, respectively (Figure 1B). Additionally, CMC extract exhibited dose-dependent scavenging of DPPH radicals (data not shown). These results suggest that CMCs contain substances with anti-oxidant potential.

Stimulation of hepatic anti-oxidant enzyme activities by oral CMC treatment

In order to evaluate the *in vivo* effects of CMCs on primary anti-oxidant enzyme activity, we performed several biochemical analyses on liver tissue homogenates prepared from mice fed with CMC extract (Figure 2). As shown in Figure 2A, mice fed CMC extract (200 mg/kg body weight) twice a wk for 3 weeks had significantly more GSH in their liver tissue ($p < 0.05$, 1.2 ± 0.24 µg/mL) compared to control mice (0.5 ± 0.04 µg/mL). The level of GSH in mice fed vitamin C, a well-known anti-oxidant, was significantly higher than that of control mice (Figure 2A). Mice fed CMC extract also exhibited significant enhancement in the activity of enzymes involved in the maintenance of intracellular redox balance (Figures 2B-D) compared to control mice. However, CMC-fed mice, as vitamin C-fed mice, had similar serum LDH to control mice (Figure 2E). In light of these results, CMCs appear to contain efficient anti-oxidant substances.

Anti-inflammatory effect of CMCs in a mouse model of DSS-induced colitis

Research has shown that a substantial number of anti-oxidant chemicals have anti-inflammatory activity. In light of this, we tested the anti-inflammatory activity of CMCs *in vivo* using a mouse model of DSS-induced colitis (Figure 3). Initial observation of the mice showed

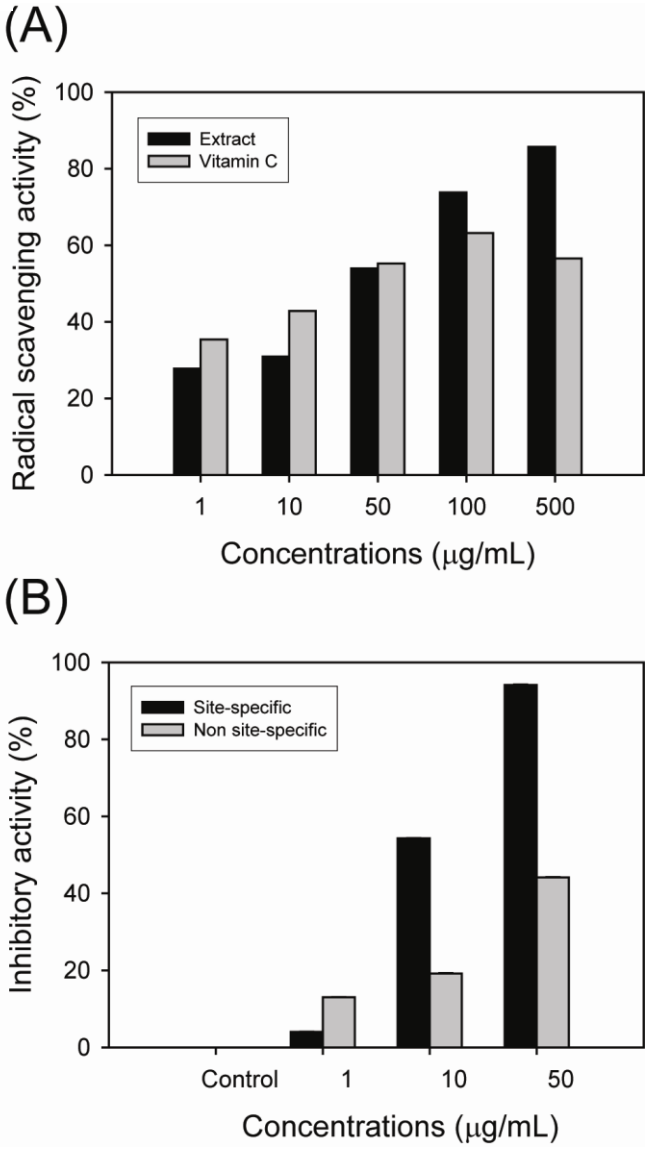

Figure 1. Radical scavenging activity of CMC extract. The scavenging activity of various concentrations of CMC extract on superoxide anions was measured by monitoring the inhibition of NBT reduction using the hypoxanthine-xanthine oxidase system as described in MATERIAL AND METHODS (A). The site-specific and non-site-specific scavenging activities of CMC extract on hydroxyl radicals were determined using a deoxyribose assay system as described in MATERIAL AND METHODS (B). Results are expressed as % activity of a maximal value.

erythema of the intestine, suspicious for bleeding (Figure 3A). In addition, their feces were soft and watery, confirming the induction of colitis by DSS treatment. In addition, the mean intestinal length of DSS-treated mice was reduced to 51 mm secondary to severe inflammation, in comparison to a normal length of 56 mm. Conversely, the intestines of mice fed sulfasalazine or CMCs after DSS treatment had near-normal shape and length, although their feces remained softer than normal. Interestingly, mice fed CMCs before and after DSS treatment had completely normal intestines 64 mm in length.

CMC treatment had another apparently beneficial effect on the inflammatory response in the colitis mouse model in terms of body weight. In comparison to DSS-treated mice, which had a mean body weight of 17.8 g at 2 weeks after finishing the 5-d DSS treatment, the mean body weight of CMC-treated mice was approximately 20 g, which began to improve 5-6 d after DSS treatment. The DSS-treated mice failed to regain weight during the entire experimental period (Figure 3B).

Regarding intestinal IgA levels (Figure 4), we found that those of DSS-treated mice were approximately 7-fold higher than those of normal mice. However, mice which were fed CMCs after DSS treatment had intestinal IgA levels similar to those of control and sulfasalazine-treated mice. As shown in Figure 3, mice treated with CMCs before and after DSS treatment had the lowest levels of intestinal IgA. Thus, we observed that oral feeding of CMCs appeared to both inhibit and cure DSS-induced colitis in mice.

Reduction of pro-inflammatory cytokines in mesenteric lymph node lymphocytes with CMC treatment

Given that the inflammation in DSS-induced colitis was localized to the gut, we examined the effects of a CMC-containing diet on inflammatory indicators in gut-associated lymphoid tissue. To this end, we prepared lymphocytes from mesenteric lymph nodes and characterized their cytokine expression profiles (Figure 5).

The balance of helper T-cell immune response profiles in DSS-treated mice was opposite from the normal Th2-skewed immune response: the expression of mIL-4, an indicator of Th2 response, was dramatically reduced (Figure 5A). Conversely, expression of mIFN-γ, an indicator of Th1 response, was dramatically increased in DSS-treated mice (Figure 5B). However, mice fed a CMC-containing diet after DSS treatment had completely normal levels of mIL-4 and mIFN-γ. These results suggest that CMC treatment can aid in the immune recovery of gut-associated lymphoid tissue after DSS treatment.

We also monitored expression levels of the pro-inflammatory cytokines mIL-6 and mTNF-α (Figures 5C and D). As expected, the levels of pro-inflammatory cytokines were increased between 3- and 7-fold by DSS treatment. Importantly, the levels in CMC-treated mice were significantly reduced to nearly those of normal controls. These results strongly suggest that oral administration of CMCs, both before and after DSS treatment, effectively inhibits DSS-induced colitis in mice.

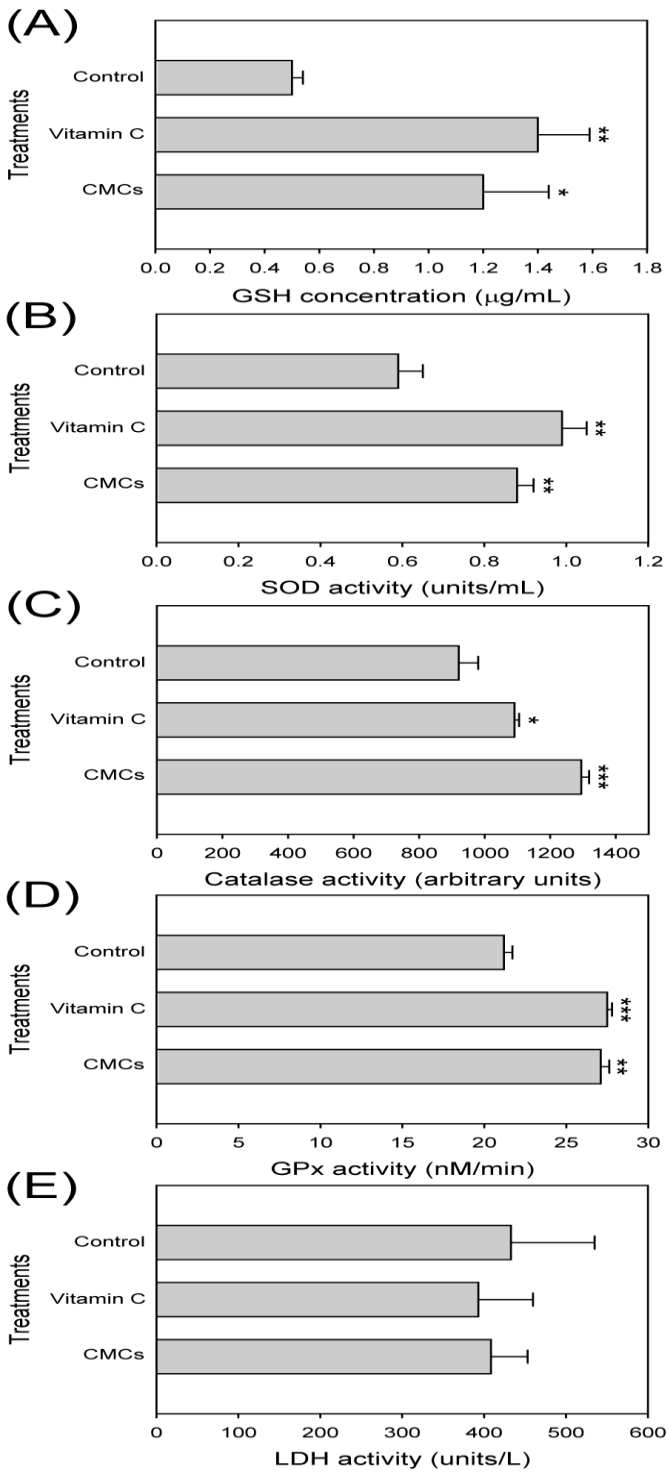

Figure 2. Effect of oral CMC treatment on the activity of anti-oxidant enzymes in mice. The effect of oral CMC extract on hepatic anti-oxidant enzymes was analyzed as described in MATERIAL AND METHODS and compared with those of a representative anti-oxidant, vitamin C (A-D). The effect of oral CMC extract on serum LDH was compared with that of a representative anti-oxidant, vitamin C (E). The control represents the result from mice fed plain water. Values represent mean ± S.E. (n = 6) and $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ indicate significant differences.

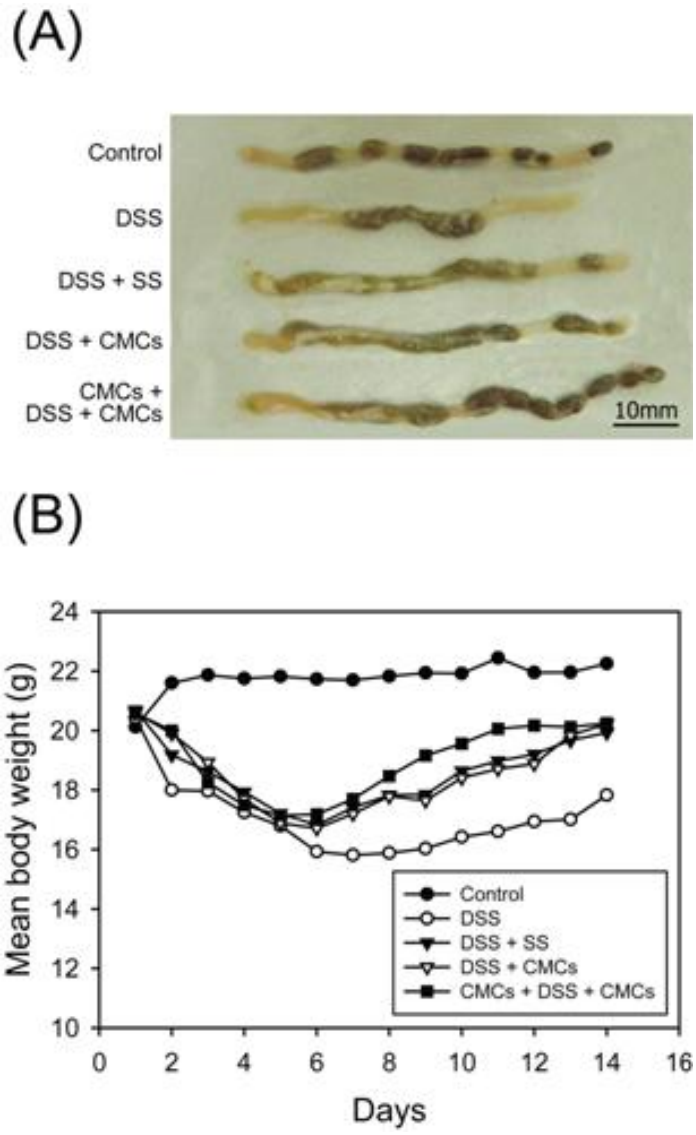

Figure 3. Effect of oral CMC treatment on the intestinal health and body weight of mice with DSS-induced colitis. We recorded changes in the length and condition of the large intestine, including stool softness and the presence of fecal blood, in the mice treated as indicated (A). Body weight was recorded daily during treatment (B). Each group consisted of 6 BALB/c mice. The control group received a normal rodent diet and water without any additives. The experimental treatments were abbreviated as follows: DSS = mice fed DSS alone, DSS + SS = mice fed DSS and then SS, DSS + CMCs = mice fed DSS and then CMCs, and CMCs + DSS + CMCs = mice fed CMCs both before and after DSS treatment. Each treatment was performed as described in MATERIAL AND METHODS.

Effect of oral CMC treatment on cytokine expression in systemic lymphoid organs

The mesenteric lymph nodes collect afferent lymph for transfer to systemic and mucosal immune systems. Hence, we studied the influence of oral CMC treatment on the cytokine expression profile of systemic lymphoid organs using splenocytes (Figures 6 and 7). Regarding serum IgG, mice with DSS-induced colitis had significantly lower levels than those of controls (Figure 6A). Additionally, the serum IgG of mice treated with CMCs before and after DSS treatment was significantly higher than that of mice treated with DSS alone. Conversely, the serum IgA of mice treated with CMCs

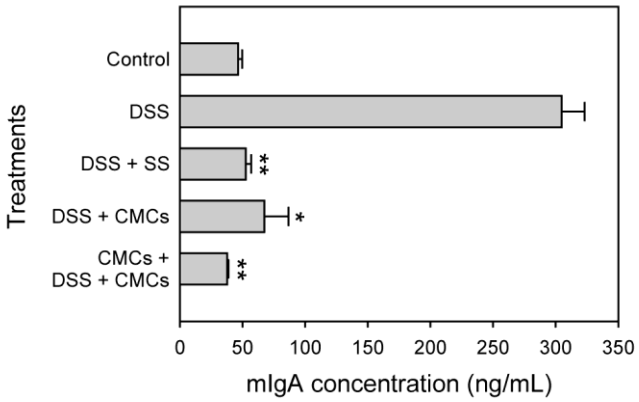

Figure 4. Effect of oral CMC treatment on colonic IgA level in mice. As described in MATERIAL AND METHODS, we used isotype-specific ELISA to measure the IgA levels in the supernatant of a preparation of colonic tissue collected from the experimental mice. The experimental groups and their treatments are described in Figure 3. Mice fed pure water were used as controls. Values represent mean ± S.E. (n = 4-6); $^{*}p < 0.05$ and $^{**}p < 0.01$ indicate significant differences.

before and both before and after DSS treatment was significantly lower than that of mice treated with DSS alone (Figure 6B). Sulfasalazine treatment did not affect the recovery of either serum IgG or IgA levels after DDS treatment.

We also measured cytokine expression in splenocytes (Figure 7). Mice that received CMC treatment before and both before and after DSS treatment had normal levels of mIL-4 and mIFN-γ expression, cytokines significantly affected by DSS treatment (Figures 7A and B). Furthermore, the expression levels of the pro-inflammatory cytokines mIL-6 and mTNF-α were significantly lower in mice fed the CMC-containing diet than those that received DDS treatment alone. This recovery of pro-inflammatory cytokine levels did not occur in mice fed the sulfasalazine-containing diet (Figures 7C and D).

DISCUSSION

As chemopreventive measures gain importance in the treatment of inflammatory diseases, numerous studies have focused on developing anti-inflammatory substances from natural sources. Although plants are excellent sources of bioactive materials, their biologically active compounds differ according to many factors, such as growing location, time of harvest, and environmental conditions (Roberts, 2007; Li et al., 2010). In response to this imprecision, research has identified plant cell cultures as a useful way to produce plant-derived bioactive compounds in a reliable manner (Roberts, 2007). In a previous report, we established the technology to isolate and expand CMCs from several plant stems to obtain efficient and stable cell lines. In this study, we analyze the biological activity of these cell lines. Specifically, we examined the biological activities of CMCs obtained from *G. biloba*, and discovered significant anti-oxidant and anti-inflammatory potential using *in vitro* and *in vivo* experimental systems.

We measured the ability of CMCs to scavenge superoxide anions, the most common free radical *in vivo*. Produced by auto-oxidation or enzymatic processes, the concentration of superoxide anions increases under conditions of oxidative stress (Mates and Sanchez-Jimenez, 2000). Superoxide anions themselves produce other cell-damaging free radicals and oxidizing agents (Liu and Ng, 2000). In our study, we found that CMC extract inhibited superoxide anions in a dose-dependent manner (Figure 1A). We also found that CMC extract inhibited hydroxyl radical-induced deoxyribose degradation in both site-specific and non-site-specific assays, although the inhibition was greater in the site-specific assays (Figure 1B). This result suggests that CMC extract inhibits deoxyribose degradation by chelating ferric ions rather than by directly scavenging hydroxyl radicals (Lee et al., 2002).

Our findings also revealed significant increases in GSH levels, as well as in SOD, catalase, and GPx activity, with oral administration of CMC extract (Figure 2). The antioxidant defense mechanisms in living organisms consist of a network of enzymatic and non-enzymatic components that control the levels of exogenous and endogenous free radicals. For example, SOD converts superoxide radicals into H_2O_2, while catalase hydrolyzes H_2O_2 into water and oxygen (Izawa et al., 1996). SOD also helps maintain cellular GSH levels (Hayes and McLellan, 1999). GSH is a cysteine-containing tripeptide that plays a critical role in protecting tissues from genotoxins by directly interacting with free radicals, in addition to acting as a cofactor for enzymatic detoxification (Larsson et al., 1994; Janssen et al., 1993). We also found that oral feeding of CMC extract did not increase LDH activity, an important measure of hepatic damage (Figure 2E). Taken together, these findings suggest that CMCs increase the activities of primary hepatic anti-oxidant enzymes without toxic effects.

An increase in pro-inflammatory cytokines is a central phenomenon in the process of inflammation (Munhoz et al., 2008). Although the precise mechanisms leading to ulcerative colitis remain unclear, accumulated evidence has documented increased expression of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α in its pathogenesis (Papadakis and Targan, 2000). Colitis is characterized by colonic tissue edema, increased colonic epithelial permeability, and extensive infiltration of leukocytes. In the present study, we demonstrated the anti-inflammatory potential of CMCs using a mouse model of DSS-induced colitis, as shown by increased colonic length and body weight in DSS-treated mice fed a CMC-containing diet (Figure 3). ELISA results revealed

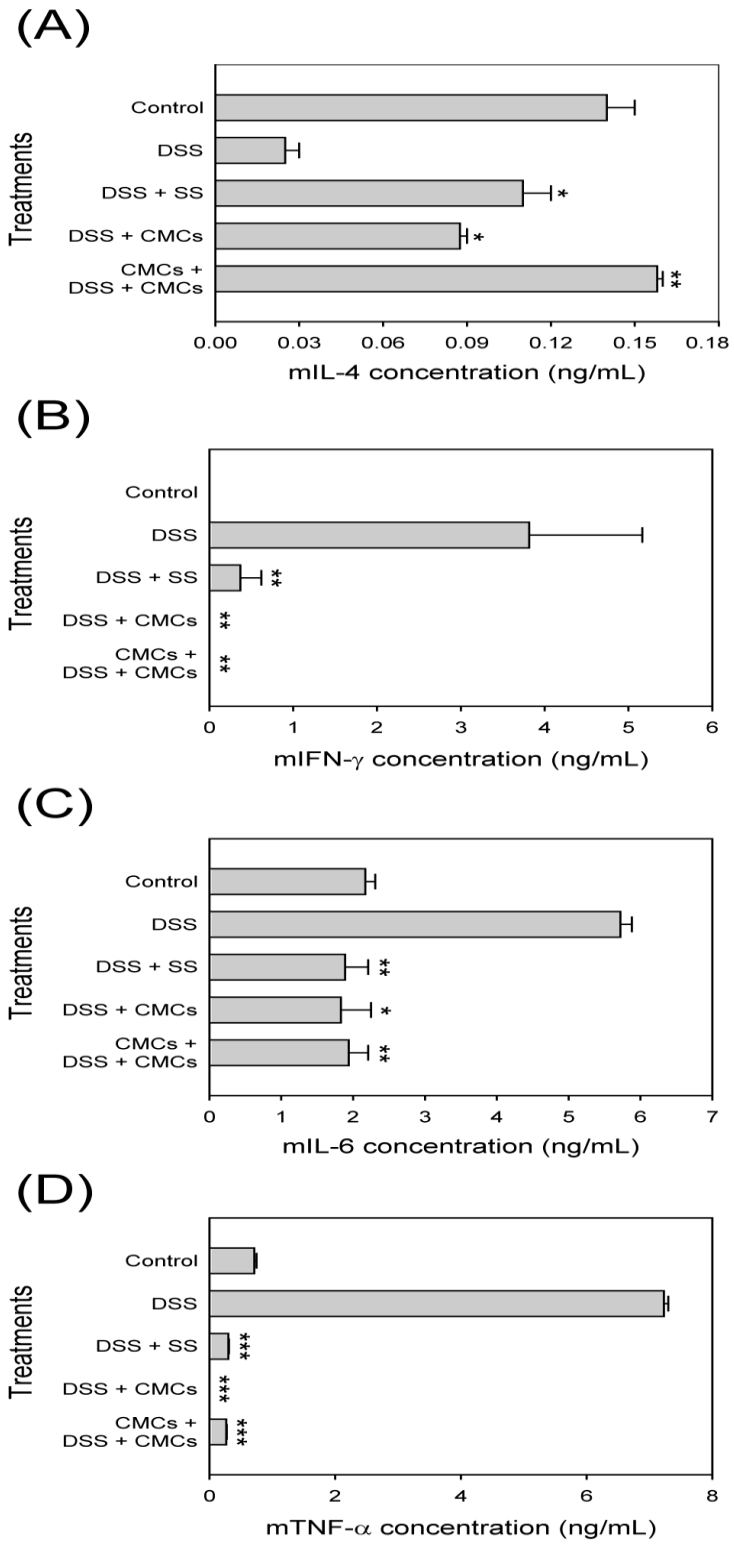

Figure 5. Effect of oral CMC treatment on cytokines expressed by lymphocytes from mesenteric lymph nodes in mice. As described in MATERIAL AND METHODS, we measured the levels of mIL-4 (A), mIFN-γ (B), mIL-6 (C), and mTNF-α (D) in stimulated lymphocytes from the mesenteric lymph nodes prepared from the experimental mice. The experimental groups and their treatments are described in Figure 3. Values represent mean ± S.E. (n = 4-6); $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ indicate significant differences.

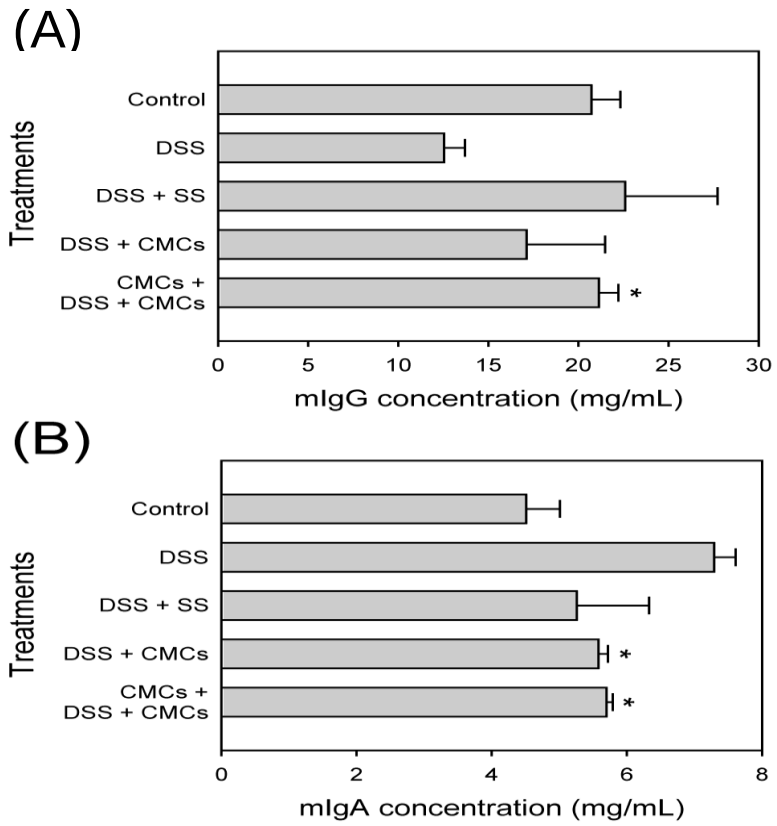

Figure 6. Effect of oral CMC treatment on serum IgG and IgA in mice. As described in MATERIAL AND METHODS, we measured the levels of serum mIgG (A) and mIgA (B) in the experimental mice. The experimental groups and their treatments are described in Figure 3. Values represent mean ± S.E. (n = 4-6); $^{*}p < 0.05$ indicates significant differences.

that the protective effects of the CMC diet were associated with a reduction in mIgA in the colon andserum, along with reductions in mIL-6 and mTNF-α produced by lymphocytes in the lymph nodes and spleen (Figures 4 and 5). Moreover, DSS-treated mice fed a CMC-containing diet did not experience a reduction in mIL-4, nor an increase in mIFN-γ levels in the culture supernatants of lymphocytes in the lymph nodes and spleen (Figures 5 and 7).

These results suggest that CMCs regulate inflammatory disorders either by enhancing the Th2-mediated immune response or suppressing the Th1-mediated response, given the reciprocal interaction of Th1/Th2 cytokines that maintains a balanced immune network (Parronchi et al., 1992; Spellberg and Edwards, 2001). Immune response deviation toward either the Th1 or Th2 pathway is associated with a number of pathologic conditions, such as Th2 deviation in allergic reactions, and Th1 deviation in organ-specific autoimmunity. Consequently, we propose that the CMC diet helped repair the abnormal systemic immune system induced by DSS treatment, although its role is less prominent than that of the mucosal immune system. Research has elucidated a number of beneficial effects of *G. biloba*, including improvement in neurological disorders and neural damage, increased blood flow, and anti-oxidant, anti-tumor, and anti-inflammatory effects. Our study supports anti-oxidant and anti-inflammatory roles of CMCs from *G. biloba*. Although, the mechanisms by which CMCs exert their biological effects remain unclear, we propose that their polyphenol compounds are responsible for the ability to scavenge free radicals and modify the inflammatory response. In fact, flavonoidic compounds are the main constituents of ginkgo leaves, which include kaempferol, quercetin, isorhamnetin, rutin, and myricetin, (Chan et al., 2007). In our study, the CMC extract contained 75.4 mg/g (7.54% of initial ethanol extract) of total phenolic contents (data not shown).

In summary, oxidative stress occurs when reactive oxidants are produced in excess of the capacity of primary and secondary anti-oxidant systems. This stress has been implicated in a number of degenerative diseases and inflammatory conditions. There is a close relationship between oxidative stress and inflammation,

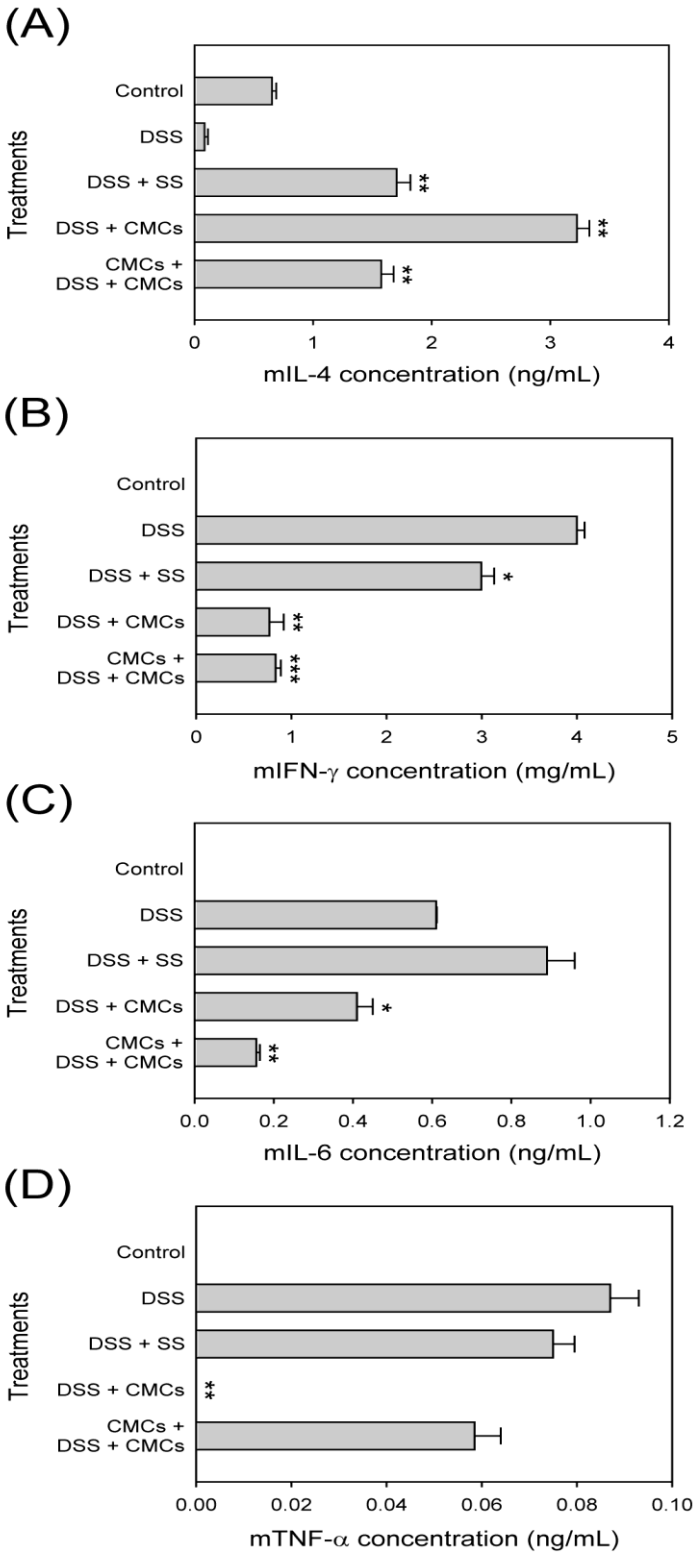

Figure 7. Effect of oral CMC treatment on the cytokine production of mouse splenocytes. As described in MATERIAL AND METHODS, we measured the levels of mIL-4 (A), mIFN-γ (B), mIL-6 (C), and mTNF-α (D) in stimulated splenocytes prepared from the experimental mice. The experimental groups and their treatments are described in Figure 3. Values represent mean ± S.E. (n = 4-6); $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ indicate significant differences.

leading many biochemical and clinical researchers to study natural bioactive materials with anti-oxidant activity in hopes that they can suppress the inflammatory response. We found that *G. biloba* CMCs hold potential as bioactive anti-oxidant and anti-inflammatory materials. Further study is warranted to identify the active components of CMCs and investigate the mechanisms involved in their anti-inflammatory activity.

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