Appl Biol Chem (2017) 60(5):553–561
DOI 10.1007/s13765-017-0311-y

Online ISSN 2468-0842
Print ISSN 2468-0834

ARTICLE

Intestinal anti-inflammatory effects of cinnamon extracts in a co-culture model of intestinal epithelial Caco-2 cells and RAW264.7 macrophages

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Received: 28 July 2017 / Accepted: 10 August 2017 / Published online: 18 August 2017

Abstract Cinnamon is the bark of *Cinnamomum japonicum* SIEB, which possesses anti-microbial, anti-tyrosinase, and antioxidant activities. However, studies on the intestinal health benefits of cinnamon are scarce. Therefore, we were aimed at assessing the potential anti-inflammatory action of cinnamon extracts in a cellular model of intestinal inflammation. Cinnamon extracts were prepared using hot water extract and 70% ethanol extract. Prior to inducing inflammation, co-cultured Caco-2 cells with RAW264.7 cells were treated with the cinnamon extracts. Lipopolysaccharide (LPS) was treated to induce inflammation in RAW264.7 cells. Compared to LPS-treated groups, the cells treated with cinnamon extracts maintained high transepithelial electrical resistance and decreased tight junction permeability, similar to that shown by control Caco-2 cells. In addition, cinnamon extract-treated cells showed decreased levels of inflammatory cytokines, namely nitrite (NO), PGE_2, interleukin (IL)-6, IL-8, and TNF-α. These results show that cinnamon may serve as a probable agent for the development of functional supplements for maintenance of gastrointestinal health.

Keywords Caco-2 · Cinnamon · Co-culture · Gut barrier integrity · Gut inflammation

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Introduction

Intestinal cells react to inflammatory expression released by penetrated neutrophils and T cells via activation of various intracellular signal transduction pathways to limit the gene expressions associated with intestinal inflammation [1, 2]. Immune homeostasis in epithelial tissues is controlled by multiple mechanisms including integration of microbial, toxic chemicals, and allergens into immune regulatory circuits that maintain a healthy immune system, promote effective defense, and inhibit inordinate and potentially precarious inflammatory responses. [3, 4]. Several intestinal diseases such as inflammatory bowel disease (IBD) [5], nonalcoholic steatohepatitis [6], and nonalcoholic fatty acid liver disease [7] loosen tight junctions (TJs) and lead to leaky gut syndrome. IBD is characterized by increased intestinal inflammation and TJ permeability, which is closely associated with the increase in inflammatory cytokines including prostaglandin E_2 (PGE_2), interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α in the intestine and blood [8, 9]. Because the weakness of TJs contributes to various diseases or exacerbations, strengthening the function of the intestine through increase in TJs interaction can prevent or alleviate these diseases. Dietary ingredients and phytochemicals including flavonoids and phenolic chemicals have been known to prevent TJ damage induced by inflammatory cytokines in many studies [4, 10, 11].

Cinnamon is the bark of *Cinnamomum japonicum* SIEB, an evergreen tall tree belonging to the family Lauraceae, and resource plant widely used as a food owing to its spicy and sweet flavor [12]. *C. japonicum* has been known to have anti-microbial, anti-tyrosinase, and antioxidant properties [13]. Cinnamic acid and cinnamaldehyde, the key

flavor components and predominant chemical constituents of cinnamon, have been shown to regulate biological activities including anti-mutagenic [14, 15], anti-inflammatory [16], and antioxidant properties [17]. However, the intestinal health effects of cinnamon are still poorly studied at the molecular level. Therefore, a more precise in vitro assessment is required to understand the effect of cinnamon on gut barrier integrity.

A co-culture system of Caco-2 cells, an intestinal epithelial cell line, placed at the apical side of transwell and RAW264.7 cells, a macrophage cell line, placed at the basolateral side, constituted an in vitro system of gut inflammation [18].

In the co-culture model, to affect gut inflammatory processes as can be seen in human IBD, lipopolysaccharide (LPS) was applicable to the basolateral wells. The activated RAW264.7 cells induced to LPS showed a decreased transepithelial electrical resistance (TEER) that measured electrical resistance across a cellular monolayer to verify the integrity and permeability of the monolayer and increased mRNA level of IL-8 in Caco-2 cells, as well as increased TNF-α production in RAW264.7 cells [18]. In this study, the effects of cinnamon extracts on the related anti-inflammatory parameters and gut barrier integrity in Caco-2 cells following LPS-induced RAW264.7 cells were evaluated using the above-mentioned in vitro model.

Materials and methods

Materials

Fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Biowest (Nuaillé, Cholet, France). Penicillin–streptomycin, nonessential amino acid (NEAA), and HEPES (1 M) buffer solution were obtained from Gibco (Rockville, MD, USA). Fluorescein isothiocyanate (FITC)-labeled dextran (FITC-dextran), lipopolysaccharide (LPS) extracted from *Escherichia coli* 0111:B4, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), sulfanilamide, phosphoric acid, cinnamic acid, cinnamaldehyde, acetic acid, and *N*-(1-Naphthyl)ethylenediamine dihydrochloride were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of cinnamon extracts

Cinnamon extracts were provided by Korea Food Research Institute (Gyeonggi, Korea). Briefly, hot water extract (CWE) was prepared by adding a certain amount of sample to ten times its quantity of distilled water (w/v) and stirring the mixture for 3 h at 95 °C in a mantle equipped with a reflux condenser. The 70% ethanol extract (CEE) was extracted with a reflux condenser at 80 °C for 3 h similar to the preparation of CWE. Then, the extracts were filtered and lyophilized.

Quantification of cinnamic acid and cinnamaldehyde using high-performance liquid chromatography (HPLC)

HPLC analyses were examined using a Shiseido SI-2 series HPLC system (Shiseido, Tokyo, Japan) equipped with a Shiseido Capcell Pak C18 MG II S-5 (250 × 4.6 mm; i.d., 5 μm) column. The mobile phase composed of water (solvent A) and acetonitrile (solvent B) both containing 1% acetic acid was applied as follows: 0–30 min, 10–60% (B); 30–40 min, 60–100% (B); 40–45 min, 100% (B), followed by re-equilibration with 10% (B) for 45–55 min at a flow rate of 1.0 mL/min and injection volume of 10 μL [19].The detection wavelength of cinnamic acid and cinnamaldehyde was set at 257 nm. Cinnamic acid and cinnamaldehyde in the cinnamon extracts were verified by contrasting their retention times with those of external standards. Stock mixed standard solution with cinnamic acid and cinnamaldehyde was prepared in methanol at the following concentrations: 0, 0.78, 1.56, 3.13, 6.25, and 12.5 μg/mL. Cinnamon extracts were immersed in 200 μL methanol, filtered, and injected into the HPLC system.

Cell culture

Human intestinal epithelial cell line Caco-2 and murine macrophage cell line RAW264.7 were obtained from the Korean Cell Line Bank. Caco-2 cells were cultured in DMEM supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 1% MEM-NEAA, and 10% FBS. RAW264.7 cells were cultured in DMEM supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 1% HEPES, and 10% FBS. Cell cultures were incubated in a 5% CO_2 humidified incubator at 37 °C.

Co-culture for determination of gut inflammation condition

Caco-2 cells were seeded at six-transwell insert plates (0.4-μm pore size; Corning Costar Corp., USA) of 1×10^5 cells/well. The cell culture medium was changed every 2–3 days for 21 days. RAW264.7 cells were seeded at the basolateral side of the transwell of 1×10^5 cells/well and incubated for 48 h to facilitate complete adherence to the well. After 48 h, all media were replaced with serum-free DMEM. To assess the anti-inflammatory activities of samples in this model, samples were treated on the apical side. The treated concentrations were confirmed to be noncytotoxic using MTT

assay [20]. After 24 h, 2 μg/mL LPS administered to the basolateral wells was incubated for 48 h.

TEER measurement

Samples were administered to the apical side and incubated for 24 h. After 24 h, LPS was added to the basolateral wells. TEER was measured before and at 0.5, 1, 3, 6, 12, 24, and 48 h after LPS addition using a Millicell® ERS instrument (Millipore, Bedford, MA, USA). TEER was recorded with three consecutive measurements and exhibited as Ω (resistance) × cm^2 (surface area of the monolayer).

Permeability measurement

The measurement of paracellular flux of FITC-dextran (4 kDa) quantified transepithelial permeability. To confirm the transportation of FITC-dextran (100 μg/mL) across the Caco-2 cell monolayer, FITC-dextran was added at the apical side and fluorescence intensity was measured at the basolateral side [21]. In brief, 48 h after the addition of FITC-dextran, the medium at the basolateral wells was collected and FITC-dextran concentrations were measured using a fluorescence spectrophotometer (PerkinElmer, Waltham, MA, USA) at excitation and emission wavelengths of 485 and 535 nm, respectively.

Measurement of cytokine secretion

Nitrite (NO), PGE_2, IL-6, IL-8, and TNF-α levels were measured in collected Caco-2 cell culture supernatants. The 100 μL of Griess reagent [1% sulfanilamide in 5% phosphoric acid/0.1% *N*-(1-naphthyl)ethylenediamine) dihydrochloride] was added to 100 μL of the supernatant and experimented at room temperature for 10 min. The absorbance was measured using a microplate reader (Bio-Tek, Winooski, Vt., U.S.A.) at 540 nm and compared to a standard calibration curve of sodium nitrite. The PGE_2, IL-6, IL-8, and TNF-α levels in the supernatant were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Rockford, IL, USA), according to the manufacturer's instructions. Each cytokine concentration was changed using the standard curve of the standard material.

RNA extraction and qRT-PCR

Total RNA was isolated from the cultured cells using TRIzol (Life Technologies, Rockville, MD, USA). The cells were added to TRIzol, followed by the addition of chloroform after 5 min, and mixed slightly with an equivalent volume of isopropanol. cDNA was synthesized by reverse transcription using a High Capacity RNA-to-cDNA Kit and measured to qRT-PCR using the Universal Probe Library (UPL) probe method with a Step-One-Plus RT-PCR system (Hoffmann La Roche, Basel, Switzerland). The relative amounts of RNAs were calculated using the comparative ΔCT method by being normalized to the amount of β-actin. The sense and antisense sequences applied to qRT-PCR analysis were as follows: iNOS (sense, 5′-GACCAGTACGTTTGGCAATG-3′; antisense, 5′-TTTCAGCATGAAGAGCGATTT-3′); COX-2 (sense, 5′-CTTCACGC ATCATTTTTCAAG-3′; antisense, 5′-TCACCGTAAATATGATTTAAGTCCAC-3′); IL-6 (sense, 5′-CAGGAGCCCAGCTATGAACT-3′; antisense, 5′-GAAGGCAGCAGGC AACAC-3′); IL-8 (sense, 5′-GAGCACTCCATAAGGCACAAA-3′; antisense, 5′-ATGGTTCCTTCCGGTGGT-3′); TNF-α (sense, 5′-AGCCCATGTTGTAGCAAACC-3′; antisense, 5′-TCTCAGCTCCACGCCATT-3′); and β-actin (sense, 5′-AAGTCCCTTGCCATCCTAAA-3′; antisense, 5′-ATGCTATCACCTCCCCTGTG-3′).

Nuclear factor kappa B (NF-κB) activity

Relative nuclear p65 transcript was assessed using nuclear proteins to Abcam kit (ab133112) (Cambridge, MA, USA). In the wells, a specific double-stranded DNA sequence including the NF-κB response element was immobilized and a specific primary antibody directed against NF-κB p65 detected NF-κB p65. Then, HRP conjugate was added and measured using ELISA (Bioteck, Winooski, USA). Both nonspecific binding and positive controls were measured at 450 nm.

Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM). Statistical analyses were performed using Duncan's multiple range test one-way analysis of variance (ANOVA) (SPSS 20, SPSS Inc., Chicago, IL). P values < 0.05 were considered statistically significant (versus LPS-treated group).

Results

Quantification of cinnamic acid and cinnamaldehyde

The chromatogram of the standard compounds and the identification of cinnamic acid and cinnamaldehyde of cinnamon extracts are indicated in Fig. 1. The compounds were identified by analysis and comparison of their retention times and UV spectra with those of six standard concentrations (0, 0.78, 1.56, 3.13, 6.25, and 12.5 μg/mL). Through HPLC quantification, we found that cinnamic acid and cinnamaldehyde were present at concentrations of

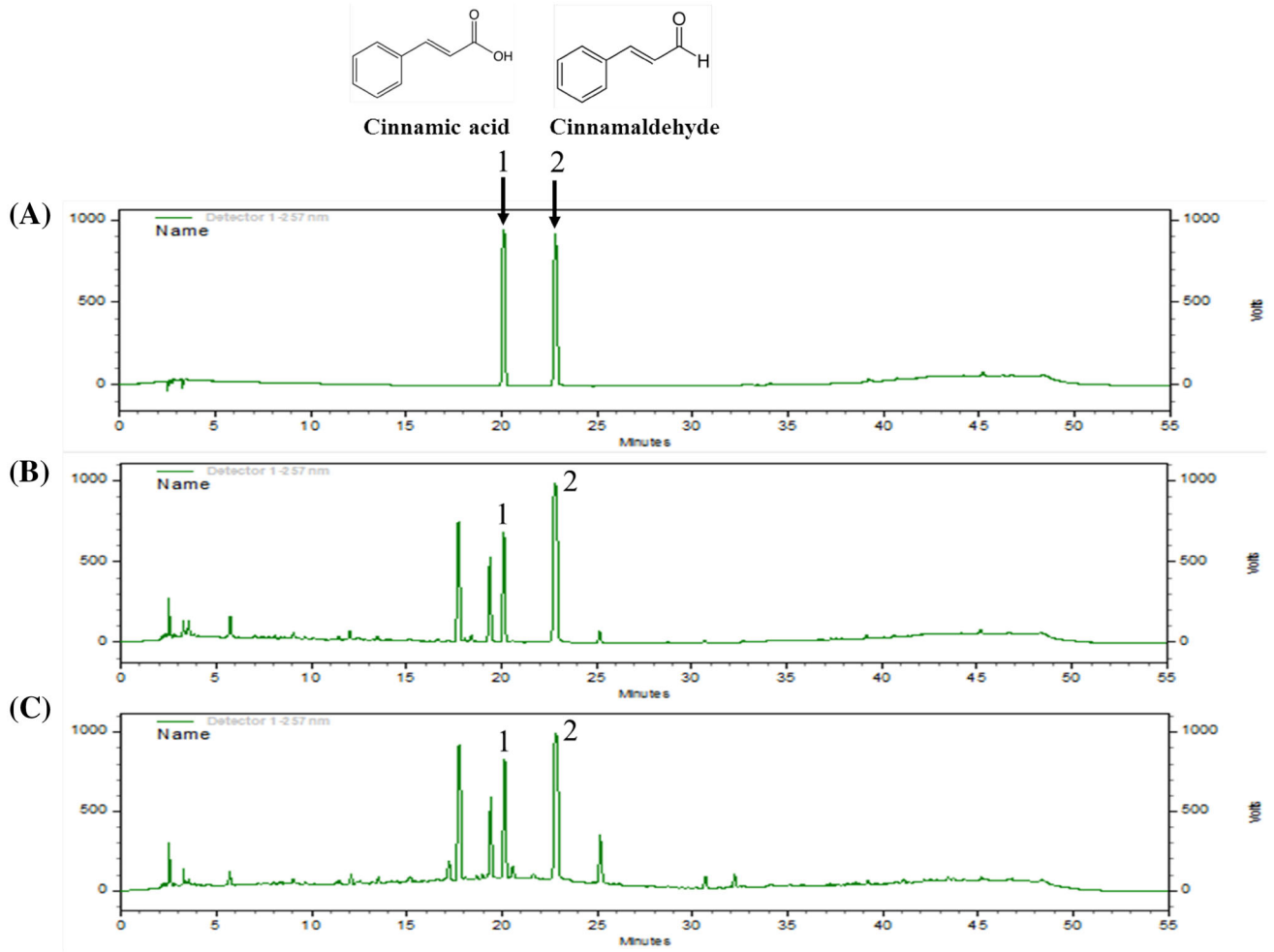

Fig. 1 Chromatograms of (**A**) cinnamic acid and cinnamaldehyde, (**B**) hot water extract (CWE), and (**C**) 70% ethanol extract (CEE)

2.4 ± 0.2 and 5.6 ± 0.3 mg/g in CWE and 2.6 ± 0.0 and 5.0 ± 0.7 mg/g in CEE, respectively. Cinnamic acid content was higher in CEE, and cinnamaldehyde content was higher in CWE, but there was no significant difference.

Intestinal TJ permeability

From the results of TEER measurement experiment, it was confirmed that the treatment of LPS significantly reduced the TEER value by approximately 25% or more than the control TEER value observed in the Caco-2 cell monolayer at 48 h ($p < 0.05$; Fig. 2A, B). Pretreatment with cinnamon extracts inhibited the LPS-induced reduction in TEER value, and the resultant TEER value was confirmed to be similar to that of the control group. TEER value increased when experimented with 50 and 100 μg/mL of both extracts. CEE had a slightly higher TEER value than CWE.

FITC-dextran migrated from the apical well of the cell monolayer to the basolateral well through the paracellular transport path, suggesting that higher the permeability, the looser the TJ. The effect of cinnamon extracts on the migration of FITC-dextran through TJ was observed 48 h after the addition of FITC-dextran. In the LPS-treated group, the FITC-dextran flux increased by around 1.5-fold (Fig. 2C). However, the increase in LPS-induced FITC-dextran flux in the cinnamon extract pretreatment group was similar or lower than that in the untreated control cells. Higher concentrations in CEE were more effective than those in CWE.

Inhibition of cytokine production

To investigate whether cinnamon extracts affect the production of NO induced to LPS in the co-culture system, after the addition of LPS (2 μg/mL), NO levels were analyzed following treatment of cinnamon extracts on culture supernatants of the apical side. In the LPS-treated groups, approximately 30-fold increase in NO levels was observed (Fig. 3A). In contrast, cinnamon-treated groups showed a significant decrease in NO production induced to LPS.

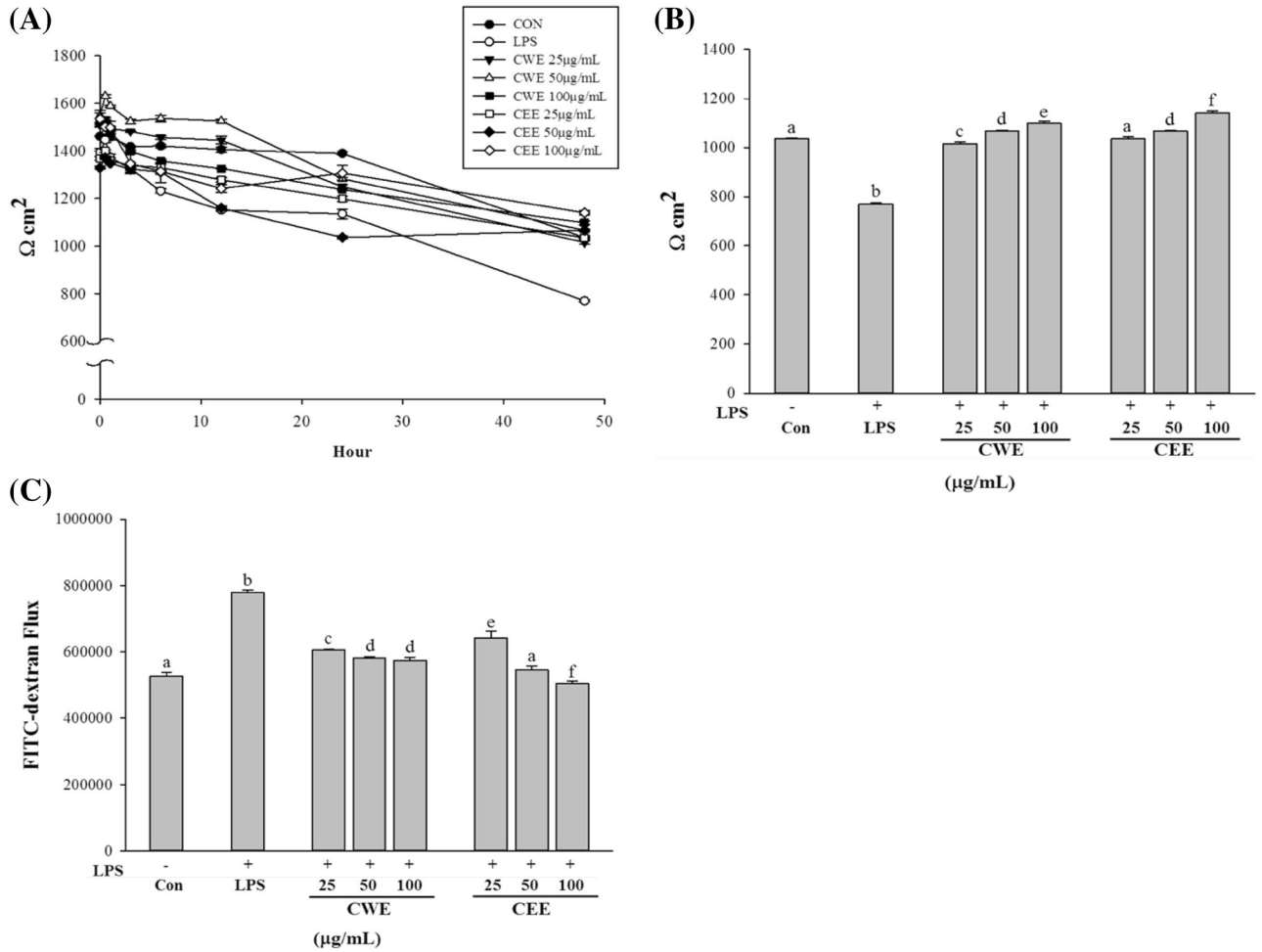

Fig. 2 (**A**) Time course of transepithelial electrical resistance (TEER) value, (**B**) protective effects of cinnamon extracts on TEER value at 48 h, and (**C**) FITC-dextran flux. Caco-2 cells were seeded in six-transwell insert plate at a density of 1.0×10^5 cells/mL and incubated with RAW264.7 cells before cinnamon extracts were added to the apical side of the co-culture system. Then, LPS (2 μg/mL) was treated to the basolateral side and incubated for 48 h. Different letters above the bars indicate significant differences (Duncan's multiple range test; $p < 0.05$). *CON* control, *LPS* lipopolysaccharide, *CWE* hot water extract, *CEE* 70% ethanol extract

Similar to NO, PGE_2 production induced by LPS decreased significantly when the cells were pretreated with cinnamon extracts ($p < 0.05$; Fig. 3B). Addition to NO and PGE_2 levels, the iNOS and COX-2 mRNA levels were confirmed as inflammatory response. In LPS-treated groups, the iNOS and COX-2 mRNA levels increased by more than 3- and 14-fold, respectively, when compared to the control groups; however, cinnamon extract decreased iNOS and COX-2 mRNA expressions similar to untreated control expressions at the highest concentration (100 μg/mL; Fig. 3C, D).

To elucidate whether cinnamon extracts influence other cytokines related to intestinal inflammation, the profiles of proinflammatory cytokines, namely IL-6, IL-8, and TNF-α, were also analyzed (Fig. 4). In agreement with iNOS and COX-2 levels, elevated proinflammatory cytokine and protein expressions were shown. In contrast, pretreatment with cinnamon extracts caused a significant, dose-dependent decrease in the both of mRNA and protein levels of IL-6, IL-8, and TNF-α ($p < 0.05$; Fig. 4). When compared to the both extracts, higher concentrations of CEE were more effective than those of CWE, but no statistically significant difference was founded.

NF-κB inhibition of cinnamon extracts

To evaluate the effects of cinnamon extracts on NF-κB transcription repression, the nuclear NF-κB activity was measured. In LPS-treated cells, the nuclear NF-κB activity increased approximately nine times compared to normal control cells (Fig. 5). In contrast, pretreatment of cells with cinnamon extracts significantly inhibited NF-κB activity ($p < 0.05$). CEE exhibited weaker inhibitory activity than CWE.

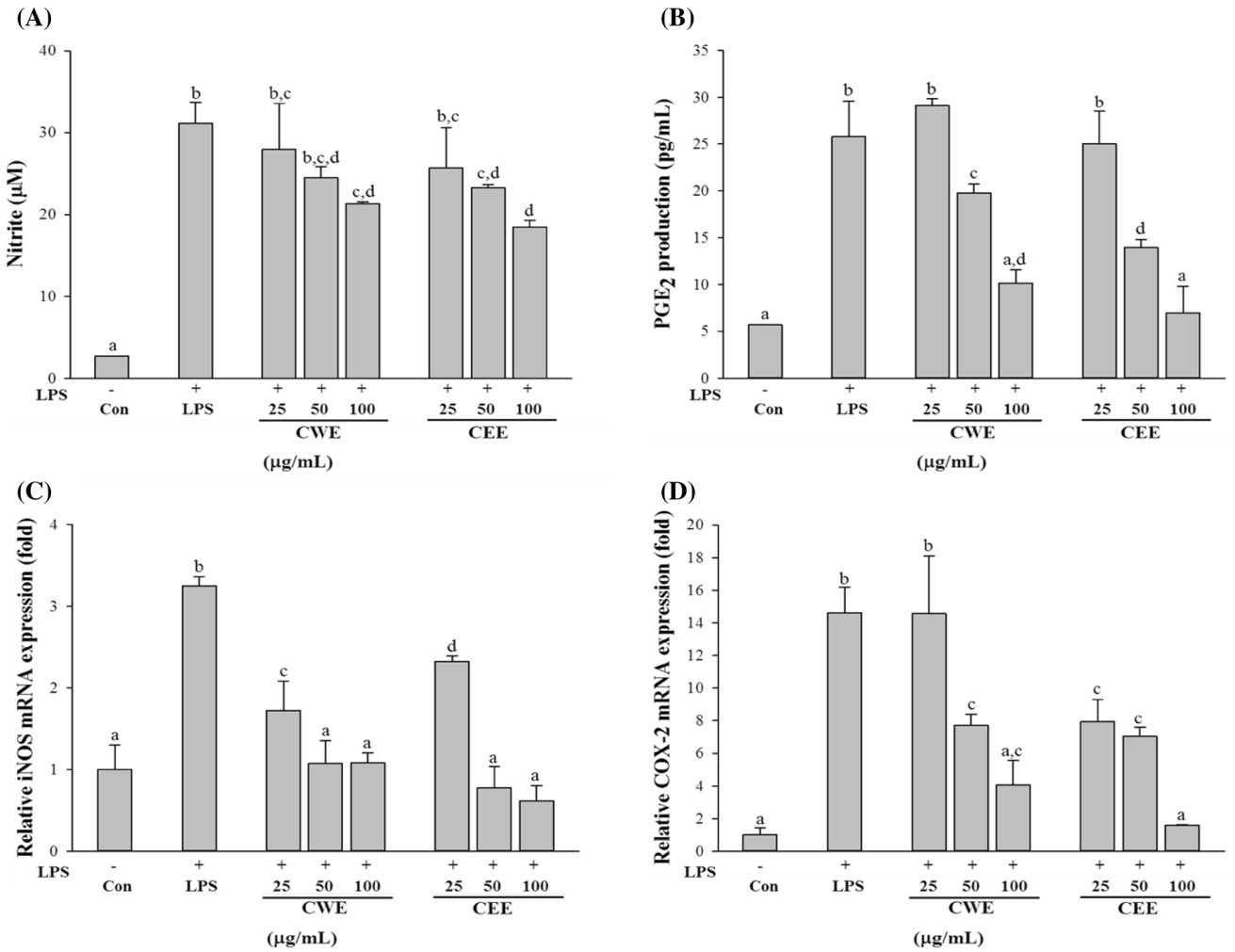

Fig. 3 Concentration profile of cytokines and relative mRNA expression levels of the genes. Caco-2 cells were seeded in six-transwell insert plate at a density of 1.0×10^5 cells/mL and incubated with RAW264.7 cells before cinnamon extracts were added to the apical wells of co-culture system. Then, LPS (2 μg/mL) was treated to the basolateral side and incubated for 48 h. The levels of (**A**) nitrite and (**B**) PGE_2 were analyzed from the collected culture supernatants using commercially available ELISA kits. Relative mRNA expression profiles of (**C**) iNOS and (**D**) COX-2 were measured by qRT-PCR as described in the experimental procedures. Different letters above the bars indicate significant differences (Duncan's multiple range test; $p < 0.05$). *CON* control, *LPS* lipopolysaccharide, *CWE* hot water extract, *CEE* 70% ethanol extract

Discussion

Cinnamon exhibits multiple biological activities, including anti-microbial, anti-tyrosinase, and antioxidant activities [13]. However, there is poorly understood to demonstrate that cinnamon weaken gut inflammatory response. To our knowledge, this is the first study to show that cinnamon mediates intestinal barrier function and exerts anti-inflammatory activity in the intestinal system. A gut inflammation was constituted using an in vitro model of Caco-2 co-cultured with RAW264.7 cells [18]. In the present study, cinnamon extract reduced LPS-induced inflammatory response, specifically by inhibiting NO, PGE_2, IL-6, IL-8, and TNF-α levels and iNOS and COX-2 expressions in Caco-2 co-cultured with inflammatory macrophage, RAW264.7 cells. Increased activation of immune cells and elevated production of proinflammatory cytokines are generally observed in IBD [22]. An increase in proinflammatory cytokines, namely IL-6, PGE_2, iNOS, and COX-2, accompanies high levels of IL-1β and TNF-α [1]. Upregulation of COX-2 induced by LPS increases PGE_2 synthesis because COX-2 mediates various inflammatory processes [23]. During inflammatory process, NO overproduction is catalyzed by iNOS [24]. In addition, the COX-2 promoter region consists of various transcription factor binding sites, such as NF-κB [25]. Bacterial-derived LPS has been reported to bring about impaired barrier function, prompting leak flux diarrhea by increased antigens uptake across the intestinal epithelial TJs [26]. A defect in the gut epithelial barrier function is connected

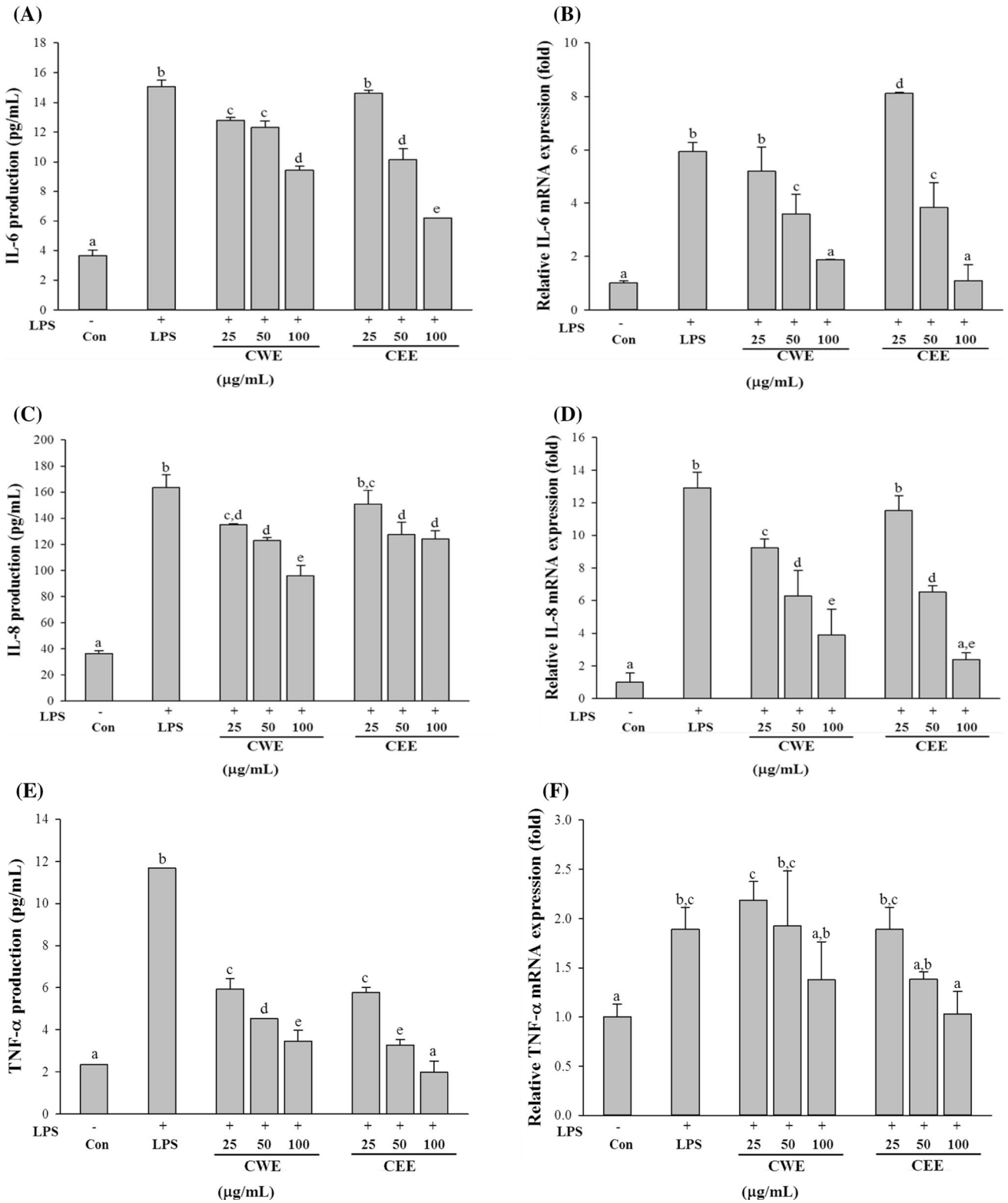

Fig. 4 Concentration profile of cytokines and relative mRNA expression levels of the genes. Caco-2 cells were seeded in six-transwell insert plate at a density of 1.0×10^5 cells/mL and incubated with RAW264.7 cells before cinnamon extracts were added to the apical side of co-culture system. Then, LPS (2 μg/mL) was treated to the basolateral side and incubated for 48 h. The levels of (**A**) IL-6, (**C**) IL-8, and (**E**) TNF-α were analyzed from the collected culture supernatants using commercially available ELISA kits. Relative mRNA expression levels of (**B**) IL-6, (**D**) IL-8, and (**F**) TNF-α were measured by qRT-PCR as described in the experimental procedures. Different letters above the bars indicate significant differences (Duncan's multiple range test; $p < 0.05$). *CON* control, *LPS* lipopolysaccharide, *CWE* hot water extract, *CEE* 70% ethanol extract

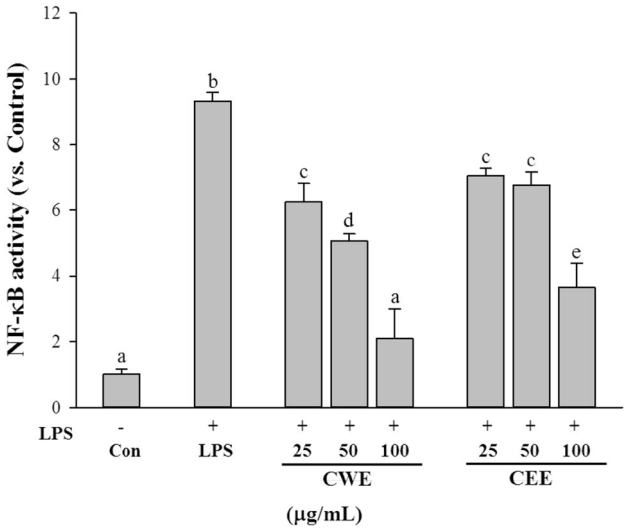

Fig. 5 Effect of cinnamon extracts on NF-κB activity. Caco-2 cells were seeded in six-transwell insert plate at a density of 1.0×10^5 - cells/mL and incubated with RAW264.7 cells before cinnamon extracts were added to the apical side of co-culture system. Then, LPS (2 μg/mL) was treated to the basolateral side and incubated for 48 h. NF-κB activity was quantified using an ELISA kit. Different letters above the bars indicate significant differences (Duncan's multiple range test; $p < 0.05$). *CON* control, *LPS* lipopolysaccharide, *CWE* hot water extract, *CEE* 70% ethanol extract

with most types of gut inflammation. In this study, we observed that pretreatment of the cells with cinnamon extracts inhibited the LPS-induced reduction in TEER value and FITC-dextran flux. Thus, besides its anti-inflammatory effect against co-cultured Caco-2 cells with RAW264.7 cells, cinnamon extracts enhanced intestinal barrier integrity.

The anti-inflammatory and antioxidant properties of cinnamaldehyde, cinnamic alcohol, and cinnamic acid have been demonstrated using macrophages and a mouse model of topical inflammation induced by LPS, respectively, in in vitro and in vivo [27, 28]. However, there are few reports on the anti-inflammatory activity of cinnamon extract in a gastrointestinal system. Recently, cinnamon ethanol extract has been reported to downregulate fibrotic symptoms, matrix metalloproteinases in murine colitis (IL-$10^{-/-}$ mice), and fibrotic marker expression via modulation of mast cell function [29, 30]. In the present study, direct modulation of inflammatory responses by cinnamon extracts in Caco-2 cells was confirmed. Cinnamaldehyde and cinnamic acid, known as the key compounds of cinnamon extracts, were identified and quantified using HPLC analysis. These findings might provide supportive evidence for the anti-fibrotic effect of cinnamon extract in murine colitis model. In addition, we observed that pretreatment of cells with cinnamon extracts significantly inhibited NF-κB activity ($p < 0.05$). Both CWE and CEE contained similar levels of cinnamaldehyde and cinnamic acid, and the anti-inflammatory activities of both extracts were similar. Thus, the present study suggested that CWE and CEE could be developed as gut health-promoting functional food ingredients.

However, this study has certain limitations. First, the activity of cinnamic acid and cinnamaldehyde was not compared to that of the extracts. Thus, we could not identify which was the main active compound. Second, the effects of cinnamon extracts on localization and expression of TJ-related proteins, such as claudin, occludin, junctional adhesion molecule (JAM), and zonula occludens (ZO) families, and their exact mechanism of action were not assessed.

Based on the results of this study, we suggest that cinnamon extracts can increase TJ barrier integrity and attenuate intestinal inflammation, pointing to the inhibition of cytokines, namely NO, PGE_2, IL-6, IL-8, and TNF-α, by decreasing the levels of iNOS, COX-2, IL-6, IL-8, and TNF-α. It is necessary to examine the activity of the bioactive constituents and cinnamon extracts for intestinal disease of gut health in in vitro and in vivo system.

Acknowledgments This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through High Value-added Food Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (Project No. 116012-3). We express our sincere appreciation for the support.

References

1. Romier-Crouzet B, Van De Walle J, During A, Joly A, Rousseau C, Henry O, Larondelle Y, Schneider YJ (2009) Inhibition of inflammatory mediators by polyphenolic plant extracts in human intestinal Caco-2 cells. Food Chem Toxicol 47:1221–1230
2. Olejnik A, Kowalska K, Kidon M, Czapski J, Rychlik J, Olkowicz M, Dembczynski R (2016) Purple carrot anthocyanins suppress lipopolysaccharide-induced inflammation in the co-culture of intestinal Caco-2 and macrophage RAW264.7 cells. Food Funct 7:557–564
3. Wullaert A, Bonnet MC, Pasparakis M (2011) NF-κB in the regulation of epithelial homeostasis and inflammation. Cell Res 21:146–158
4. Kim Y, Kim DM, Kim JY (2017) Ginger extract suppresses inflammatory response and maintains barrier function in human colonic epithelial Caco-2 cells exposed to inflammatory mediators. J Food Sci 82:1264–1270
5. Heller F, Florian P, Bojarski C, Richter J, Christ M, Hillenbrand B, Mankertz J, Gitter AH, Burgel N, Fromm M, Zeitz M, Fuss I, Strober W, Schulzke JD (2005) Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. Gastroenterology 129:550–564
6. Brun P, Castagliuolo I, Di Leo V, Buda A, Pinzani M, Palu G, Martines D (2007) Increased intestinal permeability in obese mice: new evidence in the pathogenesis of nonalcoholic steatohepatitis. Am J Physiol Gastrointest Liver Physiol 292:G518–G525

7. Giorgio V, Miele L, Principessa L, Ferretti F, Villa MP, Negro V, Grieco A, Alisi A, Nobili V (2014) Intestinal permeability is increased in children with non-alcoholic fatty liver disease, and correlates with liver disease severity. Dig Liver Dis 46:556–560
8. Neurath MF (2014) Cytokines in inflammatory bowel disease. Nat Rev Immunol 14:329–342
9. Kim CY (2016) Inhibition of interleukin-1α-induced intestinal epithelial tight junction permeability by curcumin treatment in Caco-2 cells. J Life Sci 26:1082–1087
10. Suzuki T, Hara H (2011) Role of flavonoids in intestinal tight junction regulation. J Nutr Biochem 22:401–408
11. Ulluwishewa D, Anderson RC, McNabb WC, Moughan PJ, Wells JM, Roy NC (2011) Regulation of tight junction permeability by intestinal bacteria and dietary components. J Nutr 141:769–776
12. Lee JM, Lee DG, Park JY, Chae SW, Lee SH (2015) Analysis of the trans-cinnamic acid content in *Cinnamomum spp*. and commercial cinnamon powder using HPLC. J Agric Chem Environ 4:102–108
13. Lin CC, Yang CH, Wu PS, Kwan CC, Chen YS (2011) Antimicrobial, anti-tyrosinase and antioxidant activities of aqueous aromatic extracts from forty-eight selected herbs. J Med Plants Res 5:6203–6209
14. Rutten B, Gocke E (1988) The 'antimutagenic' effect of cinnamaldehyde is due to a transient growth inhibition. Mutat Res 201:97–105
15. Dvorackova E, Snoblova M, Chromcova L, Hrdlicka P (2015) Effects of extraction methods on the phenolic compounds contents and antioxidant capacities of cinnamon extracts. Food Sci Biotechnol 24:1201–1207
16. Chao LK, Hua KF, Hsu HY, Cheng SS, Lin IF, Chen CJ, Chen ST, Chang ST (2008) Cinnamaldehyde inhibits pro-inflammatory cytokines secretion from monocytes/macrophages through suppression of intracellular signaling. Food Chem Toxicol 46:220–231
17. Point D, Coudert P, Leal F, Rubat C, Sautou-Miranda V, Chopineau J, Couquelet J (1998) Antioxidant activity of some ascorbic and cinnamic acids derivatives. Farmaco 53:85–88
18. Tanoue T, Nishitani Y, Kanazawa K, Hashimoto T, Mizuno M (2008) In vitro model to estimate gut inflammation using co-cultured Caco-2 and RAW264.7 cells. Biochem Biophys Res Commun 374:565–569
19. Kim JH, Seo CS, Shin HK (2015) Development of validated determination of the eleven marker compounds in Gyejibokryeong-hwan for the quality assessment using HPLC analysis. Arch Pharm Res 38:52–62
20. Lee EH, Cho JH, Kim DH, Hong SH, Kim NH, Park MJ, Hong EJ, Cho YJ (2017) Anti-inflammatory activity of manassantin A from ultra-fine ground *Saururus chinensis* in lipopolysaccharide-stimulated RAW 264.7 cells. Appl Biol Chem 60:63–71
21. Shao Y, Wolf PG, Guo S, Guo Y, Gaskins HR, Zhang B (2017) Zinc enhances intestinal epithelial barrier function through the PI3K/AKT/mTOR signaling pathway in Caco-2 cells. J Nutr Biochem 43:18–26
22. Kim KM, Kim YS, Lim JY, Min SJ, Ko HC, Kim SJ, Kim Y (2015) Intestinal anti-inflammatory activity of Sasa quelpaertensis leaf extract by suppressing lipopolysaccharide-stimulated inflammatory mediators in intestinal epithelial Caco-2 cells co-cultured with RAW 264.7 macrophage cells. Nutr Res Pract 9:3–10
23. Pettus BJ, Bielawski J, Porcelli AM, Reames DL, Johnson KR, Morrow J, Chalfant CE, Obeid LM, Hannun YA (2003) The sphingosine kinase 1/sphingosine-1-phosphate pathway mediates COX-2 induction and PGE2 production in response to TNF-alpha. FASEB J 17:1411–1421
24. Aktan F (2004) iNOS-mediated nitric oxide production and its regulation. Life Sci 75:639–653
25. Appleby SB, Ristimaki A, Neilson K, Narko K, Hla T (1994) Structure of the human cyclo-oxygenase-2 gene. Biochem J 302:723–727
26. Wu SJ, Don TM, Lin CW, Mi FL (2014) Delivery of berberine using chitosan/fucoidan-taurine conjugate nanoparticles for treatment of defective intestinal epithelial tight junction barrier. Mar Drugs 12:5677–5697
27. Liao JC, Deng JS, Chiu CS, Hou WC, Huang SS, Shie PH, Huang GJ (2012) Anti-inflammatory activities of cinnamomum cassia constituents in vitro and in vivo. Evid Based Complement Altern Med 2012:429320
28. Lee HS (2009) Chemical composition of cinnamomum cassia leaf oils and suppression effect of cinnamyl alcohol on nitric oxide synthase. Appl Biol Chem 52:480–485
29. Hagenlocher Y, Satzinger S, Civelek M, Feilhauer K, Koninger J, Bischoff SC, Lorentz A (2017) Cinnamon reduces inflammatory response in intestinal fibroblasts in vitro and in colitis in vivo leading to decreased fibrosis. Mol Nutr Food Res. doi:10.1002/mnfr.201601085
30. Hagenlocher Y, Hosel A, Bischoff SC, Lorentz A (2016) Cinnamon extract reduces symptoms, inflammatory mediators and mast cell markers in murine IL-$10^{(-/-)}$ colitis. J Nutr Biochem 30:85–92