ประสิทธิภาพในการปกป้องของสารสกัดและส่วนที่ผ่านกระบวนการย่อยภายในร่างกายของบัวบกต่อเซลล์ลำไส้มนุษย์ที่ถูกเหนี่ยวนำให้เกิดภาวะการอักเสบ และภาวะเครียดออกซิเดชันด้วย IL-1β

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บทคัดย่อ

สารก่อการอักเสบและอนุมูลอิสระเป็นสาเหตุและมีบทบาทสำคัญในการก่อให้เกิดโรคไม่ติดต่อเรื้อรังหลายชนิด การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบประสิทธิภาพในการต้านการอักเสบและต้านอนุมูลอิสระของสารสกัด (CAE) และส่วนที่ผ่านกระบวนการย่อยภายในร่างกาย (BFC) ของบัวบกต่อเซลล์ลำไส้มนุษย์ (Caco-2) ที่ถูกเหนี่ยวนำให้เกิดภาวะการอักเสบและภาวะเครียดออกซิเดชันด้วย IL-1β ไทรเทอร์พีนอยด์ที่พบใน CAE และ BFC คือ เอเชียติค แอซิด (421 และ 185 ไมโครกรัมต่อกรัมน้ำหนักแห้งของตัวอย่างแห้ง) และ มาเดแคสสิค แอซิด (583 และ 285 ไมโครกรัมต่อกรัมน้ำหนักแห้งของตัวอย่างแห้ง) ฟลาโวนอยด์ที่พบใน CAE และ BFC คือ เควอซิทิน (1,632 และ 987 ไมโครกรัมต่อกรัมน้ำหนักแห้งของตัวอย่างแห้ง) และ แคมพ์เฟอรอล (749 และ 427 ไมโครกรัมต่อกรัมน้ำหนักแห้งของตัวอย่างแห้ง) เซลล์ลำไส้มนุษย์ถูกเลี้ยงด้วยCAE ที่ความเข้มข้น 250-1000 ไมโคกรัมต่อมิลลิลิตร หรือ BFC เป็นเวลา 4 ชั่วโมง ก่อนจะถูกเหนี่ยวนำด้วย IL-1β ที่ความเข้มข้น 10 นาโนกรัมต่อมิลลิลิตร เป็นเวลา 24 ชั่วโมง ผลการศึกษาพบว่า Caco-2 cells ที่ได้รับ CAE ก่อนถูกเหนี่ยวนำด้วย IL-1β สามารถลดการหลั่ง IL-6, IL-8 and TNF-α และ ROS ได้อย่างมีนัยสำคัญทางสถิติ ($p<0.05$) และพบว่าเป็นไปในแบบแปรผันตรงกับความเข้มข้น นอกจากนั้นยังพบว่า BFC ก็ยังสามารถลด IL-6, IL-8 and TNF-α และ ROS ได้อย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ใน Caco-2 cells ที่ถูกเหนี่ยวนำด้วย IL-1β ได้เช่นเดียวกัน ผลทั้งหมดนี้แสดงให้เห็นว่าสารสกัดจากบัวบกมีประสิทธิภาพในการต้านการอักเสบและภาวะเครียดออกซิเดชันและถึงแม้ว่าบัวบกจะถูกนำไปผ่านกระบวนการย่อยภายในร่างกาย ส่วนที่ผ่านกระบวนการย่อยของร่างกายก็ยังมีประสิทธิภาพเหล่านั้นอยู่ แต่อย่างไรก็ตามการศึกษานี้เป็นเพียงการศึกษาในหลอดทดลอง ดังนั้นเพื่อให้ได้ประโยชน์ทางด้านสุขภาพของบัวบกนั้นควรจะต้องมีการศึกษาในรูปแบบของสัตว์ทดลองและในมนุษย์ต่อไป

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Protective Effects of Ethanol Extract and Bioaccessible Fraction of *Centella asiatica* against IL-1β-Induced Inflammation and Oxidative Stress in Human Intestinal Caco-2 Cells

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Abstract

Pro-inflammatory mediators and free radicals play a critical role in the pathogenesis of several chronic diseases. This study aims to investigate the anti-inflammatory and antioxidant effects of ethanol extracts (CAE) and bioaccessible fraction from simulated gastrointestinal digestion of *Centella asiatica* (BFC) leaves on IL-1β induced inflammation and oxidative stress Caco-2 cells. Triterpenoids and flavonoids were found in CAE and BFC from HPLC analysis. Triterpenoids in CAE and BFC were asiatic acid (421 and 185 µg/g dry weight of freeze-dried, respectively) and madecassic acid (583 and 285 µg/g dry weight of freeze-dried, respectively). Flavonoids in CAE and BFC were quercetin (1,632 and 987 µg/g dry weight of freeze-dried, respectively) and kaempferol (749 and 427 µg/g dry weight of freeze-dried, respectively). Caco-2 cells were treated with CAE at concentration 250-1000 µg/ml or BFC for 4 h prior to induce with 10 ng/ml IL-1β for another 24 h. The results showed that pre-treatment Caco-2 cells with CAE significantly ($p<0.05$) decreased pro-inflammatory mediators concentration (IL-6, IL-8 and TNF-α) and ROS formation in a dose-dependent manner. Moreover, the BFC significantly ($p<0.05$) decreased IL-1β induced pro-inflammatory mediators (IL-6, IL-8 and TNF-α) levels and ROS formation. These results showed that both CAE and BFC have anti-inflammatory and antioxidant activities in intestinal cells. This implies that *C. asiatica* leaves even after digestion still retains anti-inflammatory and antioxidant activities. However, these biological functions of *C. asiatica* leaves should be further investigated in animals and humans.

Keywords: *Centella asiatica*, Caco-2 cells, Pro-inflammatory mediators, Free radicals, Simulated digestion

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Introduction

Inflammation is a physiological response against invading pathogens or damaged cells. During an inflammatory response, blood vessels increase permeability and leukocytes, including neutrophils and macrophages are recruited to eliminate invaded pathogens or remove damaged tissues[1]. Chemical mediators, such as reactive oxygen species, chemokines and cytokines, are produced by such leukocytes to stimulate biological functions of leukocytes, endothelial and epithelial cells. In some condition, when inflammation could not be resolved, it becomes chronic. Excess inflammatory mediators produced during chronic inflammatory process have been shown to play crucial roles in the pathological development of some chronic diseases, such as inflammatory bowel diseases, atherosclerosis, cancer, type 2 diabetes and Alzheimer[2].

Intestinal epithelial cells are the first line of defense as they are directly exposed to nutrients, xenobiotics and harmful pathogens, such as bacteria and parasites[3]. The protective function of the intestinal mucosa is achieved by forming a physical barrier and secretion of pro-inflammatory cytokines and chemokines to eliminate invading pathogens. However, over production of these mediators may damage intestinal cells and nearby tissues, causing chronic inflammatory response including Crohn disease and ulcerative colitis leading to increased risk of colorectal cancer[4]. The etiologies of these diseases include genetic susceptibility, dysfunction of the immunity, oxidative stress condition, gastrointestinal infection and intestinal epithelium barrier dysfunction[5]. Reducing the frequency and extent of inflammation may be an alternative effective strategy to prevent or alleviate the severity of inflammation- associated chronic diseases.

Epidemiological data have been demonstrated that high consumption of fruits and vegetables decreases prevalence of some chronic diseases[6]. Phytochemicals in vegetables and fruits have been shown to play various biological functions [2, 7-10]. *Centella asiatica (C. asiatica)*, a local herbal vegetable, is commonly consumed either as freshly squeezed juice or as fresh vegetable side dish in habitual Thai diet. *C. asiatica* has long been used as a traditional medicine to cure various diseases including hypertension and Alzheimer[11]. Its pharmacological activities include wound healing[12, 13], anti-inflammatory activity[14], anti-cancer activity[15], antioxidant and anti-lipid peroxidation activity[16]. *C. asiatica* has

been claimed to be able to treat vascular diseases by lowering blood pressure and is often referred to as a rejuvenating medicament in Ayurvedic Pharmacopeias[17]. The active constituents present in *C. asiatca* include triterpenes and phenolic compounds, which have been demonstrated strong antioxidant and anti-inflammatory activities[18]. So far, there is no data demonstrating the antioxidant and anti-inflammatory activities of *C. asiatica* leaves after subjected to gastrointestinal digestion. Therefore, the present study aimed to assess antioxidant and anti-inflammatory activities of the ethanol extract and bioaccessible fraction following simulated digestion treatment from *C. asiatica* leaves

Materials and Methods

Chemicals

All chemical and reagents were purchased from Sigma Chemical (St. Louis, MO) unless indicated otherwise. Fetal bovine serum was obtained from Hyclone (Cramlington, USA), and penicillin, streptomycin, amphotericin B, L-glutamine, and non-essential amino acids were bought from Gibco (Invitrogen, CA).

Sample preparation

Fresh *C. asiatica* leaves were purchased from Salaya market, Nakhon Pathom Province, Thailand. They were extensively washed with tap water and distilled water and drained. They were homogenized by a kitchen electric blender prior to freeze-dried and stored at -20 °C in aluminum foil until used.

Sample extraction from ethanol

The extraction protocols were followed those of Inamdar et al.[17], and Jiang et al.[18] In brief, freeze-dried *C. asiatica* were extracted with 90% ethanol (0.1 g dry wt/10ml) and centrifuged at 4,500 x *g* for 10 min at room temperature and evaporated by rotary evaporator under vacuum at 35-40° C until dryness. The dried film of extract was stored at -20°C until used. Dried film was dissolved with DMSO:Ethanol or EtOH (1:1) mixture (0.5 mg/ml) as a stock solution before serially diluted with DMEM basal medium for addition to cell cultures monolayer (final concentration of 0.2% DMSO:EtOH mixture in cultures).

In vitro simulated digestion

Simulated gastrointestinal digestion was carried out as previously described by Ferruzzi et al.[19] and Chitchumroonchokchai

et al[34]. In brief, the gastric phase, 0.6-0.8 g of freeze-dried *C. asiatica* were homogenized with 20 ml of 120 mM NaCl in 50 ml polypropylene tube by homogenizer (model T25D, Germany), then adjusted pH to 2.0 ± 0.1 with 1 M HCl. Pepsin (40 mg/ml in 100 mM HCl) 2 ml was added. Then the volume was adjusted to 40 ml with 120 mM NaCl, blanked remaining tube with nitrogen gas, tightly cap and sealed with parafilm and incubated in a shaking water bath for 1 h at 37ºC, 95 rpm. After incubation for 1 h, the small intestinal phase was followed by bringing up pH to 6.0 ± 0.1 with 1 M sodium bicarbonate (Na_2HCO_3). Crude bile extract (40 mg/ml in 100 mM Na_2HCO_3) 3 ml, pancreatin (10 mg/ml in 100 mM Na_2HCO_3) 2 ml, lipase (5 mg/ml in 100 mM Na_2HCO_3) 2 ml were added to the reaction tube. The pH of sample was increased to 6.5 ± 0.1 with 1 M Na_2HCO_3. Then the tube was filled with nitrogen gas tightly cap and sealed with parafilmed before putting in a shaking water bath for 2 h at 37ºC, 95 rpm.

After completion of the simulated small intestinal phase of digestion, sample solution was centrifuged (Becton Dickinson Dynac Centrifuge, Sparks, MD, U.S.A.) at 4,000g for 1 h at room temperature to isolate the aqueous fraction (supernatant). Control digestion without *C. asiatica* was also conducted to assess the possible cytotoxic effects of digested compounds in the aqueous fraction. The supernatant after centrifugation was filtered (0.22 μm pores; polytetrafluoroethylene (PTFE) membrane; Millipore Corp., Cork, Ireland) to obtain the fraction with mixed micelles or bioaccessible fraction of *C. asiatica* (BFC). The BFC was used to assess anti-inflammatory activity in differentiated Caco-2 cells monolayers.

HPLC analysis of triterpenoids

Dried film from ethanol extract was dissolved in 1 ml of absolute methanol. A 10 ml of BFC was extracted with 15 ml of 90% EtOH by sonicating for 10 min, followed by vortexing for 2 min and evaporated until dryness by rotary evaporator (Buchi Rotavapor R-124, Switzerland) at 35-40 °C. The dried film was suspended in a 2 ml of absolute methanol. Triterpenic acids were analyzed by HPLC, using C_{18} Symmetry reverse phase column (4.6 x 150 mm, 5 μm, Waters, Ireland) with Sentry guard column and equipped with a photodiode array (PDA) detector (Agilent 1100, USA) as previously described by Inamdar et al[17]. Separations were conducted by an isocratic elution of 45% acetonitrile in HPLC-grade

water containing 0.05% trifluoroacetic acid (TFA) at a flow rate of 0.6 ml/min at 25 ºC by monitoring wavelength at 210 nm. Triterpenoids were identified based on retention times and spectrum comparing with pure standards. Concentration of triterpenic acids were calculated from areas under the curve using standard curves (10 - 100 µg/ml) of pure triterpenoid standards (asiatic and madecassic acids).

HPLC analysis of flavonoids

Dried film from ethanol extract was dissolved in 2 ml of 62.5% methanol: 6M HCl (4:1) solution and shaken at 70 °C for 2 h. BFC was extracted with 6M HCl containing 62.5% MeOH and 0.5 g/L of tert-butylhydroquinone (tBHQ) and shaken at 70 °C for 2 h. Flavonoids were analyzed by HPLC using Zorbax Eclipase XDB-C18 column (4.6 mm x 150 mm, 5 µm, Agilent Technologies, USA) with guard column and equipped with PDA detector (Agilent 1100, USA). Column temperature was controlled at 30 °C. Flavonoids were eluted by a gradient program as previously described by Merken and Beecher [24], with 100% water containing 0.5% (w/w) TFA (solvent A), 100% methanol containing 0.5% (w/w) TFA (solvent B) and 100% acetonitrile containing 0.5% (w/w) TFA (solvent C). Concentration of flavonoids were calculated from areas under the curve using standard curves (10 - 200 µg/ml) of pure flavonoid standards (quercetin and kamepferol).

Caco-2 cell and treatment

Human intestinal-like Caco-2 cells were obtained from the American Type Culture Collection (Rockville, MD). Caco-2 cells (passage 25-35) were cultured in a complete medium (DMEM supplemented with 15% heat inactivated FBS (v/v), 1% L-glutamine (v/v), 1% nonessential amino acids (v/v), 1% penicillin and streptomycin (v/v) and 0.2% amphotericin B). Cells were seeded in 6-well plate at a density of $4x10^4$ cells/well. Cells were incubated at 37ºC in a humidified atmosphere of 95% air/5% CO_2. The serum content of the complete medium was decreased to 7.5% once the cultures reached confluency. The complete medium was changed every other day. At 11–14 days after monolayers became confluent. Differentiated Caco-2 cell monolayers were treated either with *C .asiatica* extract (CAE) (250-1000 µg/ml of serum free media) or with BFC diluted 1:3 (v/v) with serum free media for 4 h. Cells were washed with serum free media and treated with 10 ng/ml IL-1β for 24 h. Culture media were collected for the determination of IL-8, IL-6 and TNF-

α concentrations by ELISA. Cell viability was determined by MTT assay.

Cell viability (MTT) assay

Cell viability was measured using the 3-(4, 5-dimethlthiazol-2-yl)-2, 5-diphenyltetrazolium bromide tetrazolium (MTT) assay. The MTT substrate is converted into a purple-colored formazan product from incubation with cells, which is proportional to the number of live cells. After cells reached their respective experimental time points, media was removed, and cells were washed one time with phosphate buffer saline (PBS) and incubated with PBS containing 0.5 mg/ml of MTT at 37oC for 4 hours. After incubation, the MTT solution was removed from the cells and DMSO was added to solubilize formazan crystal produced from MTT by viable cells. Cells were incubated at 37ºC for 10 mins, after which absorbance was measured at 540 nm using a microplate reader (SPECTROstarNano, BMG LABTECH, Germany).

Evaluation of ROS production

DCFH-DA was used to detect and quantify intracellular ROS activity. Caco-2 cells were seeded into black 96-well, clear bottom plates at a cell density of 1.0 x 10^4 cells/well and cultured until confluence. Media was then removed, and cells were washed with serum free media. Cells were treated with CAE (250-1000 µg/ml of serum free media) or BFC diluted 1:3 (v/v) with serum free media for 4 h. Cells were washed with serum free media and treated with 10 ng/ml IL-1β for 24 h. The media was removed and cells were washed with serum-free media. Next, cells were incubated with 10µM DCFH-DA in serum-free media at 37ºC for 20 mins. Finally, cells were washed twice with PBS, and ROS production was measured in the plates using the Fluostar Optima plate reader (BMG Labtech, Aylesbury, UK) with the excitation/emission settings of 485nm/530nm, respectively.

Evaluation of IL-8, IL-6 and TNF-α levels by ELISA

IL-8, IL-6 and TNF-α produced by IL-1β-induced Caco-2 cells were determined by quantitative “sandwich” ELISA using paired antibodies purchased from eBioscience Inc. (San Diego, CA, USA) as previously described by Muangnoi et al[35]. In brief, capture antibodies for IL-8, IL-6 and TNF-α were coated in 96-well plates. After incubation for overnight at 4°C, plates were incubated with 1% bovine serum albumin (BSA) in phosphate buffered saline. Culture

medium or various concentrations of recombinant human IL-8, IL-6 and TNF-α protein were incubated at 25°C for 2 h prior to adding biotinylated detecting antibodies to the wells. After 2 h at 25°C, immune complex was detected by reacting with streptavidin horseradish–HRP–tetramethyl benzidine detection system. Reactions were terminated by addition of 2 M H_2SO_4, and absorbance at 450 nm was determined using a microplate reader. Concentrations of IL-8, IL-6 and TNF-α in samples were calculated by comparing absorbance with the standard curve.

Statistical analysis

All experiments were conducted at least in four replications. Statistical analyses were performed using SPSS (version 13.0) (SPSS Inc., USA). Data are presented as mean ± standard deviation (SD). Means were compared by One-way Analysis of Variance (ANOVA) with Duncan's post hoc test. Difference is considered significant at $P < 0.05$.

Results

Triterpenoids content in CAE and BFC

The majority of bioactive triterpenoids in *C. asiatica* are asiaticoside, madecassoside, asiatic acid and madecassic acid[21]. Walsh et al.[22] showed that triterpenoid glycosides are converted to their corresponding aglycones during simulated digestion. This study focused only on the aglycones (asiatic and madecassic acids) in CAE and BFC. The concentration of asiatic acid in CAE and BFC was 421 ± 35.8 and 185 ± 12.7 μg/g dry weight of freeze-dried respectively, and that of madecassic acid 583 ± 51.2 and 285 ± 25.8 μg/g dry weight of freeze-dried respectively.

Flavonoids content in CAE and BFC

Detectable flavonoids in CAE and BFC were quercetin (1,632 ± 124.5 and 987 ± 78.5 μg/g dry weight of freeze-dried, respectively) and kaempferol (749 ± 52.3 and 427 ± 38.7 μg/g dry weight of freeze-dried, respectively).

Effects of CAE and BFC on IL-6, IL-8 and TNF-α secretion in IL-1β-induced Caco-2 cells

Cytokine IL-1β has been used to induce inflammatory response of Caco-2 cells[5]. Exposure of Caco-2 cells with 10 ng/ml IL-1β significantly induced secretion of IL-6, TNF-α and IL-8 compared with

vehicle-treated or bioaccessible control, whereas there were no significant changes for cells exposed to CAE or BFC (Fig. 1). Pre-incubation with CAE (250 - 1,000 µg/ml) significantly decreased in a dose-dependent manner IL-1β-induced IL-6, IL-8 and TNF-α secretion (Fig.1A), which without exerting any cytotoxic effect (Fig.2A). Similar results were obtained with pre-incubation with BFC (diluted 1:3 (v/v) with basal media) (Fig.1B) without cytotoxicity effect (Fig.2B).

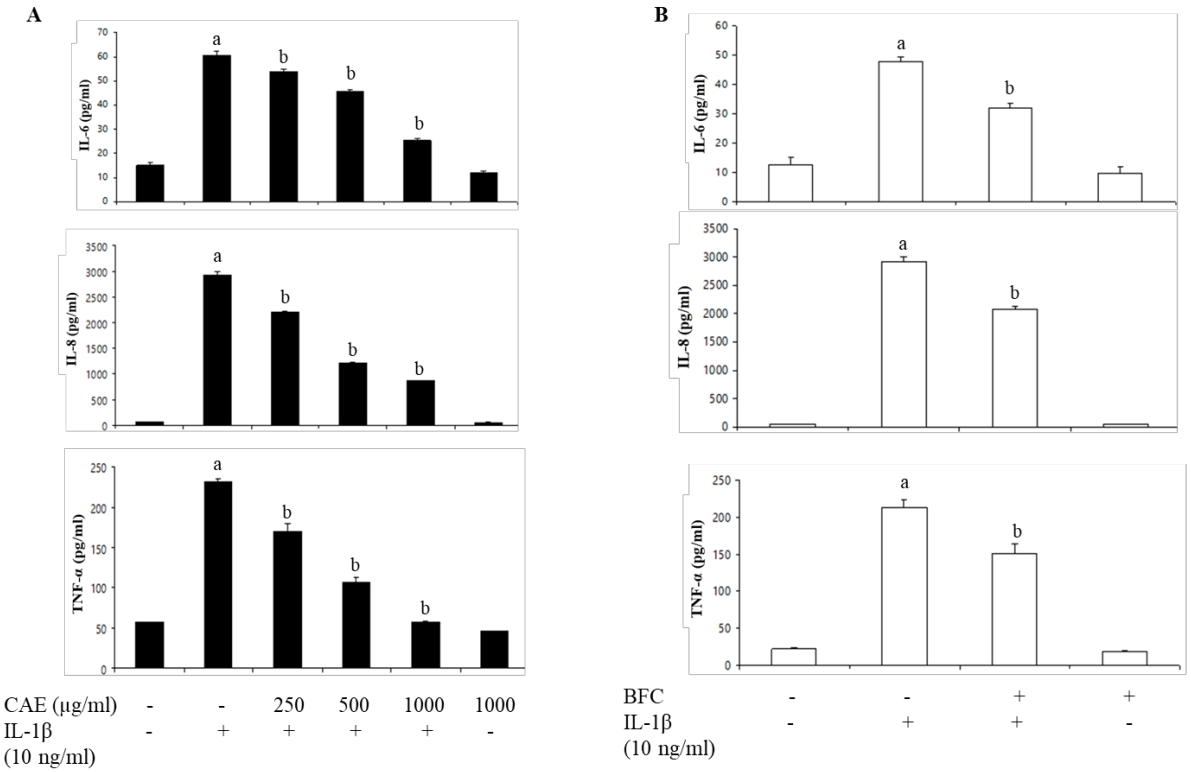


Figure 1. Effects of CAE and BFC on secretion of IL-6, IL-8 and TNF-α in Caco-2 cells induced inflammation with IL-1β. Caco-2 cells were pre-incubated with the indicated varies concentrations of CAE (A) or BFC (B) (diluted 1:3 (v/v) with basal media) for 4 h prior to stimulation with 10 ng/ml IL-1β for 24 h. Negative controls were cells treated with 0.2% DMSO and bioavail fraction without CAE for CAE and BFC, respectively. IL-6, IL-8 and TNF-α concentrations in culture media determined by sandwich ELISA. Results are reported as mean ± SD of at least four independent experiments. $^{a}p < 0.05$ compared with negative control group; $^{b}p < 0.05$ compared IL-1β treated alone group.

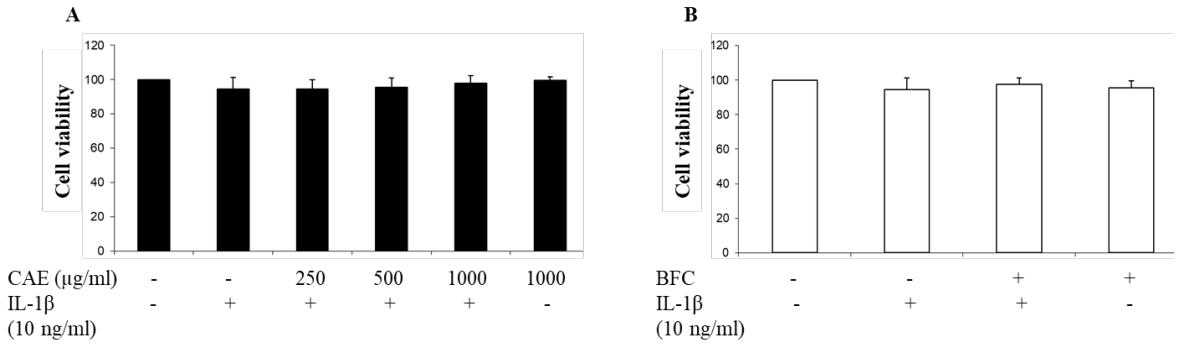


Figure 2. Effects of CAE and BFC on cell viability in Caco-2 cells induced inflammation with IL-1β. Caco-2 cells were treated with varies concentrations of CAE (A) or BFC (B) (diluted 1:3 (v/v) with basal media) for 4 h prior to stimulation with 10 ng/ml IL-1β for 24 h. Cells were washed with PBS and added MTT solution (0.5 mg/ml in PBS). After incubation for 4 h, MTT solution was removed from the cells and DMSO was added to solubilize formazan produced from MTT by viable cells. Absorbance was measured at 540 nm using a microplate reader (SPECTROstarNano, BMG LABTECH, Germany).

Effects of CAE and BFC on ROS production in IL-1β-induced Caco-2 cells

After exposure of Caco-2 cells to IL-1β, the amount of intracellular ROS significantly increases compared with vehicle-treated control cells, but there is no significant change in cells exposed to CAE or BFC (Fig. 3). Pre-incubation with CAE (250 - 1,000 µg/ml) significantly decreases in a dose-dependent manner IL-1β induced ROS production (Fig.3A). Similar result was obtained with pre-treatment of Caco-2 cells with BFC (diluted 1:3 (v/v) with basal media) (Fig. 3B).

Discussion

Phytochemicals from a variety of vegetables have been shown to suppress pro-mediators of inflammatory responses and reduce risk factors of many human chronic diseases[23]. In particular, triterpenoids and flavonoids are able to attenuate the production of various pro-inflammatory mediators, such as IL-6, IL-8 and TNFα, in many cell types, including intestinal epithelial cells[24]. Previous studies have shown that the active constituents present in *C. asiatica* including triterpenoids and flavonoids, exhibit strong anti-inflammatory and anti-oxidant activities[25]. However, to be beneficial in human, these components

must be able to exert their biological activities following digestion.

In this study we have investigated the ability of an ethanol extract of *C. asiatica*, following *in vitro* simulated gastric and intestinal digestion, to inhibit secretion of pro-inflammatory mediators (IL-6, IL-8 and TNF-α), as well as antioxidant effect, in human intestinal Caco-2 cells following stimulation with IL-1β. Digestion reduced the bioavailability of triterpenoids (asiatic and madecassic acids) and flavonoids (quercetin and kaempferol) which found in BCF by approximately one half of CAE. Nevertheless, pre-treatment of BFC was able to inhibit secretion of IL-6, IL-8, TNF-α and ROS from IL-1β-induced Caco-2 cells when comparison with pre-treatment of CAE. Moreover, we found that the both of BFC and CAE were not cytotoxic to Caco-2 cells (Fig.2) and were not effect on production of pro-inflammatory mediators (Fig.1) and ROS (Fig.3) in non-induced cells. Al-Sadi et al.[5] showed that IL-1β causes a rapid activation of NF-κB, which translocates to the nucleus where it participates in the transcription of genes involved in synthesis of pro-inflammatory mediators. Asiatic and madecassic acids act by via MAPK signaling pathway and inactivate NF-κB[26,27]. Both of quercetin and kaempferal inhibit p38 MAPK activation in IL-4 induced in Caco-2 cells[28]. However, the exact mechanisms exerted by these triterpenoids and flavonoids in IL-1β-induced Caca-2 cells need further studies.

Recent studies have indicated that the variety of phytochemicals, including flavonoids, are able to stimulate translocation into nucleus of Nrf2 , which switches on gene expression of a number of antioxidant enzymes and phase I and II detoxification enzymes[29]. Hussin et al.[30] showed that CAE and powder of *C. asiatica* are able to reduce H_2O_2-induced oxidative stress in rats by increasing the levels of antioxidant enzymes and by decreasing lipid peroxidation. Lee et al.[31] demonstrated that asiatic acid exhibits significant protective activity against carbon tetrachloride-induced hepatotoxicity in rat by preserving the level of glutathione and activities of antioxidant enzymes. Yokomizo et al.[32] showed that flavonoids, such as quercetin and kaempferal, have cytoprotective effects by scavenging ROS from H_2O_2- induced oxidative stress in Caco-2 cells. Again, the true anti-oxidant mechanisms of these phytochemicals should be explored further

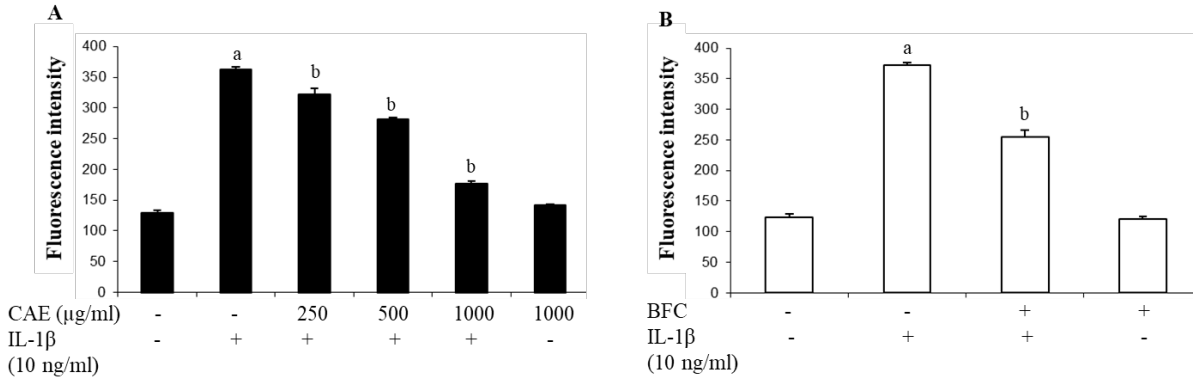


Figure 3. Effects of CAE and BFC on ROS production-2 in Caco-2 cells induced inflammation with IL-1β. Caco-2 cells were treated with varies concentrations of CAE (A) or BFC (B) (diluted 1:3 (v/v) with basal media) for 4 h prior to stimulation with 10 ng/ml IL-1β for 24 h. ROS production in cell lysate as determined using DCFH-DA assay. Results are reported mean ± SD of at least four independent experiments. $^{a}p < 0.05$ compared with negative control group; $^{b}p < 0.05$ compared IL-1β treated alone group.

Conclusion

In conclusion, the present study indicates that both extracts and bioaccessible fraction of *C. asiatica* have anti-inflammatory and antioxidant activities in the intestinal cells. This implies that *C. asiatica* even after digestion still retains anti-inflammatory and antioxidant activities. The presence of phytochemicals in *C. asiatica* is most likely responsible for the observed activities, but the mechanisms of action need to be examined in the future. However, this is an *in vitro* study. Since most phytochemicals are transformed and metabolized by detoxification phase I and II enzymes to other physiological forms by enterocytes and hepatocytes, the health benefits of *C. asiatica* should be further investigated in animals and humans.

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Conflict of Interest

The authors declare no conflict of interest.

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