6-gingerol modulates proinflammatory responses in dextran sodium sulfate

(DSS)-treated Caco-2 cells and experimental colitis in mice through adenosine

monophosphate-activated protein kinase (AMPK) activation

Kuei-Wen Chang[1] and Cheng-Yi Kuo[2]

[1] Department of Pediatrics, Chang Gung Memorial Hospital, Chang Gung University

College of Medicine, Taoyuan, Taiwan

[2] Department and Graduate Institute of Biology and Anatomy, National Defense

Medical Center, Taipei, Taiwan

Corresponding author: Cheng-Yi Kuo PhD

Address: 161, Sec. 6, Minquan E. Rd., Neihu Dist., Taipei City 114, Taiwan

TEL: +886-2-87923100, Email: kchengyi@gmail.com

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Running Title: 6-gingerol ameliorates DSS-induced colitis

Abstract

Background

6-gingerol has been reported for its anti-inflammatory effect in different experimental settings. The present study was aimed to evaluate the effect of 6-gingerol on dextran sodium sulfate (DSS)-induced barrier impairment and inflammation in vitro and in vivo.

Methods

Differentiated Caco-2 monolayer was exposed to DSS and treated with different concentrations of 6-gingerol (0, 1, 5, 10, 50 and 100 μM). Changes of intestinal barrier function were determined using transepithelial electrical resistance (TEER). Anti-inflammatory activity of 6-gingerol was examined as changes in the expression of pro-inflammatory cytokine using quantitative real-time PCR. Western blotting was employed to determine the activation of adenosine monophosphate-activated protein kinase (AMPK). In animal study, mice with DSS-induced colitis were orally administrated with different dosage of 6-gingerol daily for 14 days. Body weight and colon inflammation were evaluated, and level of proinflammatory cytokines in colon tissues was measured.

Results

The results showed that 6-gingerol treatment restored impaired intestinal barrier

function and suppressed the proinflammatory responses in DSS- treated Caco-2

monolayers. We found that AMPK was activated upon 6-gingerol treatment in vitro.

In animal study, 6-gingerol significantly ameliorated the DSS-induced colitis as shown

by restoration of body weight loss, reduced intestinal bleeding, and prevented colon

length shortening. In addition, 6-gingerol suppressed DSS-elevated production of

pro-inflammatory cytokine (IL-1β, TNF-α and IL-12).

Conclusion

Our findings highlight the protective effects of 6-gingerol against DSS-induced colitis.

We concluded that 6-gingerol exerts anti-inflammatory effects through AMPK

activation. It is suggested that 6-gingerol has a promising role in treatment of IBD.

Key words: 6-gingerol, AMP-activated protein kinase, dextran sodium sulfate, colitis

Introduction

Inflammatory bowel diseases (IBDs) are inflammatory disorders of the

gastrointestinal tract, which are characterized by diarrhea, bloody stools, abdominal

pain, and weight loss. Histological characteristics of IBD include crypt abscesses,

crypt distortion, ulceration, and infiltration of large numbers of neutrophils,

monocytes, and lymphocytes. Aberrant production of inflammatory mediators, such

as tumor necrosis factor-(TNF-α) and nitric oxide (NO), is strongly associated with the

pathogenesis of IBD[1-3]. In addition, dysregulated expression of matrix

metalloproteinases (MMPs) is reported to play a role in pathogenesis of human IBD

and experimental colitis [4, 5]. However, the precise pathogenesis of IBD remains

sketchy. Treatments for IBD are mainly focused on manipulating aberrant

inflammatory responses, including corticosteroids and some immunomodulators like

cyclosporine [6-8]. Despite the clinical benefits, steroid therapy has been reported to

have unwanted side effects such as infertility and developmental disability. Recently,

use of herbal remedies has been shown to be a promising approach for treating IBD [9,

10]. There is thus a great need to scientifically evaluate food ingredients that modulate

inflammatory responses in IBD as preventive or therapeutic agents.

Ginger (Zingiber officinale) has a long history of use as traditional medicine in many

countries for managing a variety of disorders. 6-gingerol, a major pungent phenolic

component in ginger, has been reported to possess various pharmacological

properties including anti-inflammation, anti-cancer and antioxidant activities [11-13]. It

has been demonstrated to have beneficial effects on gastrointestinal tract [14-17]. In

addition, recent studies have shown that 6-gingerol exerts several pharmacological

activities via activation of adenosine monophosphate activated protein kinase (AMPK)

[11, 18]. AMPK has been reported to play role in regulation of intestinal barrier function

and inflammation [19-21]. It is of interest to understand the role of ANPK activation in

6-gingerol treatment for colitis.

The present study was focused on the beneficial effect of 6-gingerol on intestinal

inflammation. We investigated the role of 6-gingerol in regulation of barrier integrity

using Caco-2 monolayers. The anti-inflammatory effect of 6-gingerol on experimental

colitis was determined with emphasis on cytokine production and gastrointestinal

pathophysiology.

Materials and methods

Cell culture

The human colon adenocarcinoma cell line Caco-2 (ATCC HTB37) was obtained from

American Type Culture Collection (ATCC; Rockville, MD, USA) and propagated

Dulbecco's Modified Eagle's Medium (DMEM, Gibco-BRL, CA, USA) supplemented

with 10% fetal calf serum (FCS; Gibco-BRL, CA, USA) at 37oC in a humified 5% CO_2

atmosphere.

For treatment, cells were seeded in 6-well culture plates at an initial density of

$1x10^5$ cells/ml and grown to approximately 80% confluence. The cells were incubated

with 1% DSS and serial concentrations of 6-gingerol (0, 1, 5, 10, 50 and 100 μM)

(Sigma-Aldrich, MO, USA) in serum-free DMEM for 24 h. After the 6-gingerol

treatments, the cells were washed with phosphate-buffered saline (PBS, pH 7.2) and

collected for following analyses.

Differentiated Caco-2 cell culture

Differentiated Caco-2 monolayer was developed as previously described[22]. In order

to allow differentiation, Caco-2 cells were seeded at a density of 1×10^5 cells/ml on

6.5 mm cell culture inserts (3.0 μm pore size, Transwell, Costar, CA, USA). The inserts

were placed into 24-well tissue culture plates and cultured in DMEM supplemented

with FBS for up to 28 days. Cell culture medium was changed every other day for the

first 7 days and then daily until the cells were fully differentiated (day 28). Degree of

differentiation of Caco-2 monolayer was characterized referring to the value of TEER.

TEER of the confluent Caco-2 monolayers was determined using an EVOM ohmmeter

(World Precision Instruments, Sarasota, FL), to establish that differentiated

monolayers were formed. Cells with stable TEER readings >300 ocm2 were used. DSS

and 6-gingerol were exposed to apical sides of Caco-2 cell monolayers.

For treatment, differentiated Caco-2 monolayers were incubated with 1% DSS and

serial concentrations of 6-gingerol (0, 1, 5, 10, 50 and 100 μM) in serum-free DMEM

for 24 h. After the 6-gingerol treatments, the cells were washed with

phosphate-buffered saline (PBS, pH 7.2) and collected for following analyses.

Cell viability

Cell viability was determined using MTT assay. Briefly, cells were seeded at a density

of 4×10^{4} cells/well in a 24-well plate and cultured with serum-free DMEM for 16 h.

Then, the cells were treated with or without 1%DSS and serial concentrations of

6-gingerol (0, 1, 5, 10, 50 and 100 µM) for 24 h. Treatment at each concentration was

performed in triplicate. After treatments, the medium was aspirated and cells were

washed with PBS. Cells were subsequently incubated with MTT solution (5 mg/ml)

for 4 h. The supernatant was removed, and formazan was solubilized in isopropanol

and measured spectrophotometrically at 563 nm. The percentage of viable cells was

estimated in comparison with untreated cells.

Transepithelial Electrical Resistance assay

Caco-2 cells were seeded at a density of 1×105 cells / ml on 6.5 mm cell culture

inserts (3.0 µm pore size, Transwell, Costar, MA, USA). The inserts were placed into a

24-well tissue culture plates and cultured up to 21 days at same culturing condition

without antibiotics. Cell culture medium was changed every other day for the first 7

days and then every day until the cells were fully differentiated (day 21). TEER was

determined continuously in confluent Caco-2 monolayers using EVOM ohmmeter.

Caco-2 monolayer with TEER of 250Ω x cm2 was used in this study.

Western blotting

Cells were washed with PBS twice and then lysed with cell lysis buffer (Cell signalling,

MA, USA) containing complete protease inhibitor cocktail (Roche Applied Science,

Mannheim, Germany). The lysates were incubated on ice for 30 min and centrifuged

at 20,000 g for 15 min. The supernatants were collected and the extracted protein

concentration was measured by Qubit Protein Assay Kit (Invitrogen, CA, USA). 20μg

of protein was loaded in 15% SDS-PAGE, and transferred to polyvinylidene difluoride

(PVDF) membrane (Millipore, MA, USA) by 200mA for 1 hour. The blotted membrane

was blocked with 5% (w/v) skim milk in PBST, and then incubated for 2 h with 1/1000

dilution of antibodies against human cleaved phosphate-AMPK, AMPK, phosphate-

mammalian target of rapamycin (mTOR), mTOR and β-actin, respectively. All primary

antibodies were purchased from Cell Signaling Technology (MA, USA).

Antigen-antibody complex were detected using 1/2000 dilution of

peroxidase-conjugated secondary antibodies and blot was developed using ECL

chemiluminescence reagent (Millipore, MA, USA).

Quantitative Real-time PCR

After treatment, total RNA was extracted by using TRIzol reagent (Ambion, CA, USA).

The amount of RNA sample was determined by Qubit® RNA Assay Kit (Invitrogen, CA,

USA). Reverse transcription was performed in a 20-μL reaction with 200 ng total RNA

using high capacity cDNA reverse transcription kits (Applied Biosystems, CA, USA).

Relative quantification of apoptosis and autophagy markers were assessed by ABI

7900HT system (Applied Biosystems, CA, USA). The housekeeping gene,

glyceraldehyde 3-phosphate dehydrogenase (GAPDH), was used as an internal

control. The primers were designed using the OligoPerfect™ Designer (Invitrogen)

and purchased from Invitrogen (CA, USA). The sequences are indicated in Table 1.

Table 1 List of primer

Gene	Forward primer (5' – 3')	Reverse primer (3' – 5')
TNF-αlpha	CACCGGCAAGGATTCCAA	CACTCAGGCATCGACATTCG
IL-6	AGCCCTGAGAAAGGAGACATGTA	AGGCAAGTCTCCTCATTGAATCC
IL-β	AATCTGTACCTGTCCTGCGTGTT	TGGGTAATTTTTGGGATCTACACTCT
GAPDH	AATGGAAATCCCATCACCATCT	CAGCATCGCCCCACTTG

DSS-induced colitis mice

The DSS-induced colitis model in mice was developed as previously described [23]. In

brief, specific pathogen free female C57BL/6 mice (aged 7 weeks, weight 18-22g,

BioLASCO, Taipei, Taiwan) used in this study were maintained in a standard cage

environment at 25°C and exposed to a 12 hour light and dark cycle. Mice were

divided into groups of 6 mice and given with 1% DSS (molecular weight:

36,000-50,000 Da；MP Biomedicals, Aurora, OH, USA) added in the drinking water [24].

Animals were administrated with either phosphate buffered saline (PBS) (Hyclone,

South Logan, MI, USA) or 6-ginerol (10, 25 or 100 mg/kg body weight) intragastrically

daily for 14 days. In the end of experiment, the mice were anesthetized by isoflurane

(Abbott Laboratories, Kent, UK) and then sacrificed by cervical dislocation. The length

of colon was measured immediately after removal. A 1-cm long segment from the

distal part of colon was cut out, followed by washing with ice-cold PBS and

immersing in 10% histological grade phosphate-buffered formalin (Mallinckrodt

Chemical, Derbyshire, UK) immediately. The protocol of animal study was reviewed

and approved by the animal ethic review board of Chung Shan Medical University.

Intestinal Bleeding Assessment

The intestinal bleeding score system was modified from Wirtz et al. [23] which

consisted of stool consistency and bleeding. Occult blood in the feces was measured

using Hemoccult Sensa kit (Beckman Coulter, Brea, CA) according to manufacturer's

instructions.

Histological Evaluation

Fixed colon tissue samples were dehydrated in ethanol and further embedded in

paraffin wax, then sectioned in 5µm thickness, followed by staining with hematoxylin

and eosin (H&E stain; Sigma-Aldrich, MO, USA).

Colon Organ Culture

Colonic cytokine production was measured by ex-vivo colon organ culture [25]. A 1-cm

colon segment was cut longitudinally and washed in ice-cold PBS with protease

inhibitor cocktails (Sigma-Aldrich, MO, USA) and 50 µg/ml penicillin, 50 µg/ml

streptomycin sulfate and 100 µg/ml neomycin sulfate (Invitrogen, Carlsbad, CA, USA).

The washed segment was cultured in 24-well culture plates (BD Biosciences, San Jose,

CA, USA) in serum free Roswell Park Memorial Institute medium-1640 medium

(Invitrogen) supplemented with protease inhibitor cocktails and 50 µg/ml penicillin,

50 µg/ml streptomycin sulfate and 100 µg/ml neomycin sulfate for 24 hours in a

humidified atmosphere of 5% CO_2 at 37°C. The supernatants were collected by

centrifugation at 12,000 rpm for 3 minutes then stored at -80°C freezer until assay.

Cytokine Production

The cytokine levels in the supernatants were measured by DuoSet ELISA

development systems (R&D Systems, Minneapolis, MN, USA) according to the

manufacturer's instructions.

Statistical Analysis

The values are given as mean ± standard deviation (SD). All results were analyzed by

Duncan's multiple range test with Statistical Analysis System (SAS Institute Inc., SAS

Campus Drive Cary, NC, USA) software. A p-value < 0.05 indicated a significant

difference.

Results

6-gingerol ameliorates DSS-Induced decrease in TEER across Caco-2 monolayer

We examined the cytotoxic effect of 6-gingerol at various concentrations on Caco-2

cell viability using MTT assay. As shown in Figure 1A, treatments of Caco-2 cells with

a serial of concentration of 6-gingerol (1-100μM) for 24 hours were found

well-tolerated in the experimental setting. The cytotoxic effects of 6-gingerol on

DSS-treated Caco-2 cells were determined. Treatment with 6-gingerol had

insignificant influence on the viability of Caco-2 cells treated with 1% of DSS (Figure

1B). We then explored the effects of 6-gingerol on the DSS-induced intestinal barrier

impairment. As shown in Figure 1C, exposure of Caco-2 monolayer to 1% DSS

resulted in a significant decrease in TEER. Treatment with 6-gingerol restored the

integrity of Caco-2 monolayer with a significant increase in TEER compared with that

of DSS group in a dose-dependent manner.

6-gingerol attenuated DSS-induced pro-inflammatory responses in Caco-2 monolayer

through AMPK activation

It is evident that DSS induces proinflammatory responses that contribute to the

barrier impairment and pathogenesis of colitis. To evaluate the anti-inflammatory

effects of 6-gingerol, DSS-treated Caco-2 monolayers were treated with or without

6-gingerol and measured for the changes in levels of proinflammatory cytokines

using qPCR. Exposure of Caco-2 monolayer to 1% DSS resulted in remarkably

increased expression of proinflammatory cytokines including IL-1β, IL-6 and TNF-α

(Figure 2). The elevated expressions were decreased in response to 6-gingerol

treatment in a dose-dependent manner. AMPK has been demonstrated to play a role

in homeostasis of intestinal barrier. As 6-gingerol activates AMPK, it was

hypothesized that 6-gingerol exerts the anti-inflammatory activity through AMPK

activation. As shown in Figure 2D, 6-gingerol induced a significant phosphorylation of

AMPK and dephosphorylation of mTOR. The AMPK activation induced by 6-gingerol

in Caco-2 monolayer was inhibited in response to the treatment with AMPK inhibitor

compound C.

6-gingerol ameliorated DSS-induced colitis in mice

To investigate the possible protective efficacy of 6-gingerol on the development of

colitis, the effect of 6-gingero on DSS-induced colitis was tested in BALB/c mice. A

significant decrease in body weight was observed in the mice treated with 1% DSS

(Figure 3A). Administration of 6-gingerol at a concentration of 100 mg/kg body

weight resulted in a significant restoration of body weight loss caused by

DSS-induced colitis. Moreover, the macroscopic examination revealed that the

administration of 1% DSS caused a significant reduction of colon length compared to

that of control. The colon shortening induced by DSS exposure was improved upon

6-gingerol treatment. In addition, DSS-treated mice exhibited severe intestinal

bleeding. 6-gingerol treatment promoted a significant reduction of severe intestinal

bleeding in DSS colitis mice. According to the histological examination, DSS-induced

colitis in mice was characterized with high degree of inflammatory cell infiltration

and severe damage of epithelium along with crypt destruction (Figure 4B). Intestinal

samples from DSS-treated mice receiving 6-gingerol were found to have attenuated

inflammation, evidenced by reduced infiltration of inflammatory cells and intact

epithelium (Figure 4).

6-gingerol reduced cytokines secretion in DSS-induced colitis

The profile of inflammatory cytokine production in colonic organ culture ex vivo

supported the anti-inflammatory effects of 6-gingerol. Secretion of three

pro-inflammatory cytokines, IL-1β, TNFα and IL-12 were significantly increased in

mice which had DSS-induced colitis (Figure 5). Increased production of

proinflammatory cytokine in response to DSS exposure was abrogated upon

6-gingerol treatment in a dose-dependent manner.

Discussion

In the present study, we demonstrated that 6-gingerol improved the barrier

impairment and reduced proinflammatory responses in DSS-treated Caco-2

monolayer. Our results indicate that 6-gingerol induces AMPK activation involved in

the pharmacological activity. 6-gingerol treatment effectively ameliorated severity of

DSS-induced colitis.

Intestinal epithelium constitutes a physical and functional barrier separating lamina

propria from external environment. It serves as a first line of defense for the mucosal

immune system and regulates paracellular passage of solutes and antigens [26, 27].

Compromised intestinal barrier function is associated with the development of

inflammatory diseases such as IBD. The barrier dysfunction has been suggested to

contribute to the intestinal inflammation through influx of luminal antigens into the

lamina propria. Proinflammatory cytokines including TNF-α and IL-1β have been

characterized as key players in the pathogenesis of IBDs in a consideration of

increased paracellular permeability. The DSS-induced colitis model is generally

accepted and utilized for study of intestinal inflammatory disorders. It is suggested

that DSS initiates colonic inflammatory through inducing lesions in distal colon. In the

present study, we demonstrated that the exposure of differentiated Caco-2

monolayer to DSS resulted in a compromised barrier function with increased

permeability. Our results are in agreement with previous study that DSS induced a

marked expression and production of proinflammatory cytokines in epithelial cells [28].

Given these findings, damage of intestinal epithelium is postulated as an initiating

step in the vicious cycle of inflammatory bowel diseases and barrier protection has a

role in improvement of IBD. The argument is supported by our findings obtained with

6-gingerol demonstrating its ability to restore the integrity of intestinal epithelium.

We demonstrate that 6-gingerol prevents barrier dysfunction induced by DSS

functionally.

AMPK, a critical cellular energy sensor, plays a role in regulating systemic and cellular

energy status. It is activated by phosphorylation of a threonine residue (Thr172) in

response to elevated AMP/ATP ratio[29]. Activation of AMPK leads to an increase in

catabolic pathways, such as glucose uptake and decrease in metabolic pathway like

glycolysis [30, 31]. Accumulating evidence has indicated the role of AMPK in

inflammation and immunity with focus on the effect of AMPK activation in

inflammatory disorders [32, 33]. Inhibition of AMPK has been reported to enhance

LPS-induced inflammatory response in vitro and in vivo [34, 35]. Administration of AMPK

activator has been demonstrated to attenuate DSS-induced colitis in mice [36, 37]. In this

study, we discovered that AMPK in DSS-treated differentiated Caco-2 monolayer was

activated in response to 6-gingerol treatment. Our data revealed that the activation

was associated with a reduction of inflammatory response and a corresponding

improvement of barrier integrity. Conversely, AMPK inhibition using compound C

abolished the inhibitory effect of 6-gingerol in DSS-treated differentiated Caco-2

monolayer. It is suggested that the beneficial effect of 6-gingerol on intestinal

inflammatory disorder is attributed to AMPK activation. Activation of AMPK has been

shown to contribute to intestinal epithelial barrier function [38]. For example, that

AMPK activation by butyrate and AICAR could enhance barrier function by increasing

transepithelial electric resistance (TEER) value of Caco-2 intestinal barrier cell

monolayer in vitro through redistribution of tight junction protein ZO-1. These results

indicate that activation of AMPK in intestinal epithelial was important to maintaining

normal barrier function and to reduce cytokine induced damage.

6-gingerol has been reported to exert various pharmacological activities including

anti-inflammatory[17, 39]. It has been found to protect the intestinal mucosa from toxic

insult insults[40, 41]. In the present study, administration of 6-gingerol was shown to

ameliorate signs and symptoms of inflammation, including colon shortening, weight

loss, and increased proinflammatory cytokine production. The finding is consistent

with previous study in which beneficial effect of 6-gingerol on DSS-induced colitis

was demonstrated with physiological and pathological observations[17]. Intestinal

mucosa plays a critical role in maintaining intestinal homeostasis through a strict

regulation of permeation of subepithelial compartment [41,42]. It is hypothesized

that compromised integrity of intestinal epithelium represents the initiating site for

both infection and establishment of inflammation in the gut. We showed that

6-gingerol protected intestinal membrane from DSS-induced damage and reduced

the DSS-induced inflammatory response. Once the mucosal barrier is breached,

lamina propria is exposed to luminal stimuli, including food and bacteria, resulting in

increased production in proinflammatory cytokines such as TNF-α and IL-1β. We

showed that the administration of 6-gingerol significantly ameliorated DSS-induced

colitis in mice. The beneficial effects were supported by histological results including

crypt regeneration, epithelial restitution and reduced neutrophil infiltration in the

colon. The neutrophil infiltration has been considered as an important feature of

histological evaluation in mucosal region of colitis [42]. In support to previous findings[17],

this evidence suggests that 6-gingerol has a potent direct anti-inflammatory effect

and may lead to inhibition of neutrophil accumulation in the colonic mucosal region

in DSS-colitis. The acute DSS-induced colitis is known to be toward a Th1 response [42].

A recent study has reported that AMPK activator AICAR also elicited

anti-inflammatory effects in DSS-treated mice, through interfering RNA or protein

expressions of Th1 and Th17 related inflammatory cytokines in colonic tissue [43]. Our

findings that 6-gingerol treatment induced AMPK activation and reduced colonic

inflammatory cytokines (e.g. IL-1β, IL-12 and TNF-α) are in agreement with the

previous study. Activation of AMPK has been indicated to inhibit transcription factor

NF-κB activation and further TNF-α production as well as important gene expression

related to inflammation such as inducible nitric oxide synthase (iNOS), monocyte

chemoattractant protein-1 (MCP-1), cyclooxygenase-2 (COX-2) and

metalloprotease-9 in macrophage cells activated by different stimuli [44-48].

Furthermore, the inhibition of TNF-α activity reduce colonic inflammation in

DSS-induced colitis [49], implying that inhibition of TNF-α activity or production by

AMPK activation might be a potential therapeutic way to inflammatory bowel

disease.

In conclusion, we demonstrated that 6-gingerol prevents intestinal barrier

dysfunction from toxic insults through AMPK activation. Our data suggest that

6-gingerol ameliorated DSS-induced acute colitis, evidenced by reducing clinical

symptom, decreasing macroscopic and histological severity, and suppressing

proinflammatory responses. Given ease of administration and cost effectiveness,

6-gingerol represents a promising candidate for maintaining the integrity of

intestinal epithelium and modulating inflammatory response with ulcerative colitis.

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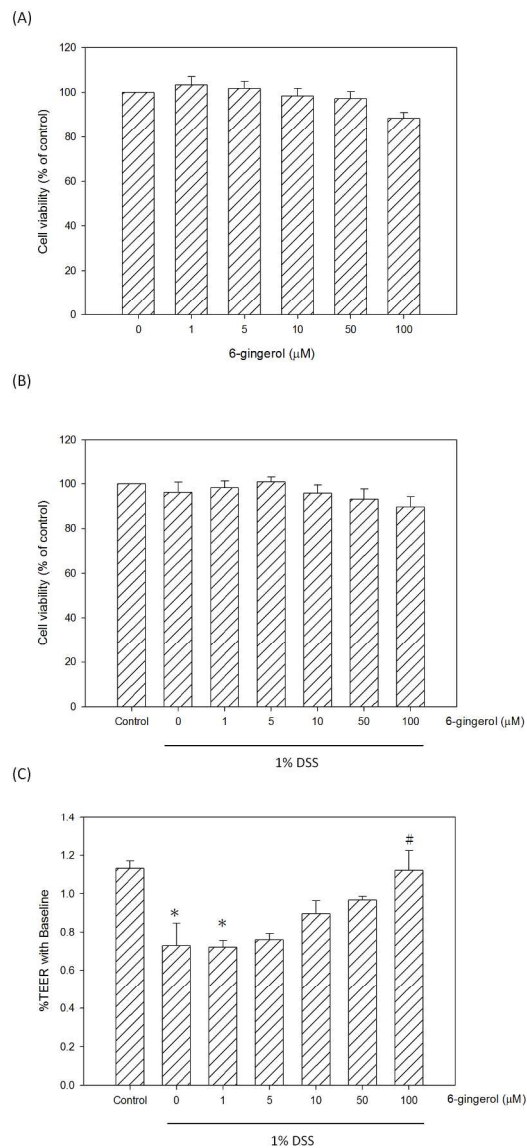
Figure legends

Figure 1. Effects of 6-gingerol on cell viability and TEER of Caco-2 cells. (A) Caco-2

cells were treated with serial concentrations of 6-gingerol (0-100 μM) for 24 h. (B)

Caco-2 cells were stimulated with 1% DSS and treated with serial concentrations of

6-gingerol (0-100 μM) for 24h. (C) Differentiated Caco-2 monolayer was exposed to 1% DSS with or without 6-gingerol treatment. Data were expressed as mean ± SEM for 3 independent experiments.#, p <0.05 as comparing to 0μM; *, p <0.05 as comparing to control (Con).

Figure 2. 6-gingerol suppressed up-regulated production of proinflammatory cytokine in DSS-treated Caco-2 cells. Cells were treated with serial concentrations of 6-gingerol (0-100 μM) for 48 h and mRNA expression of IL-1β (A), IL-6 (B) and TNF-α (C) were determined by qPCR. (D) Cells were treated with serial concentrations of 6-gingerol (0-100 μM) for 48 h and cell lysates were subject to immunoblot for detection of indicated proteins protein. Data were expressed as mean ± SEM for 3 independent experiments. #, p <0.05 as comparing to 0μM; *, p <0.05 as comparing to control (Con).

Figure 3. Effects of 6-gingerol on (A) body weight, (B) colon length and (C) intestinal bleeding score in DSS-induced colitis mice. In the end of experiment, the mice were sacrificed and colons were collected. Means for each data without a common letter differ significantly (p<0.05).

Figure 4. Representative histological photographs of distal colon section of the treated mice. (A) Control, (B) 1% DSS, (C) 1% DSS+ 10 mg/kg b.w. (D) 1% DSS+ 25 mg/kg b.w. and (E) 1% DSS+ 100 mg/kg b.w. In the end of experiment, mice were

sacrificed and distal colons were collected followed by fixation and H&E stain.

Figure 5. Ex vivo cytokine analysis of colon organ culture after 6-gingerol

administration in DSS-induced colitis in mice. (A) IL-1β , (B) TNF-α and (C) IL-12

production in the supernatant of distal colon organ culture of mice with different

treatments in DSS-induced colitis were measured. Means for each data without a

common letter differ significantly (p<0.05).

Figure 1

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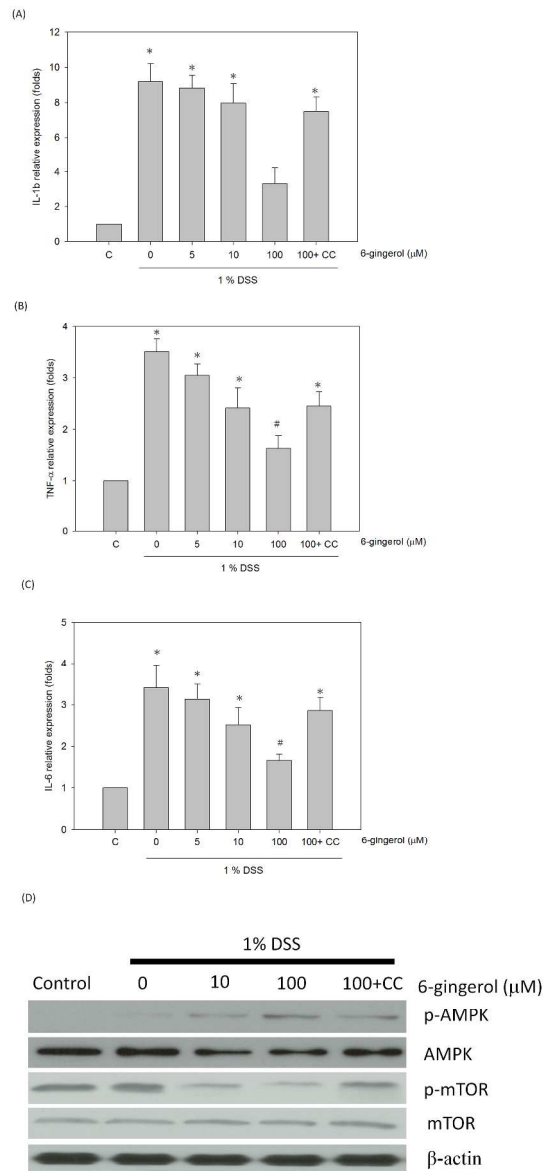
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Figure 3
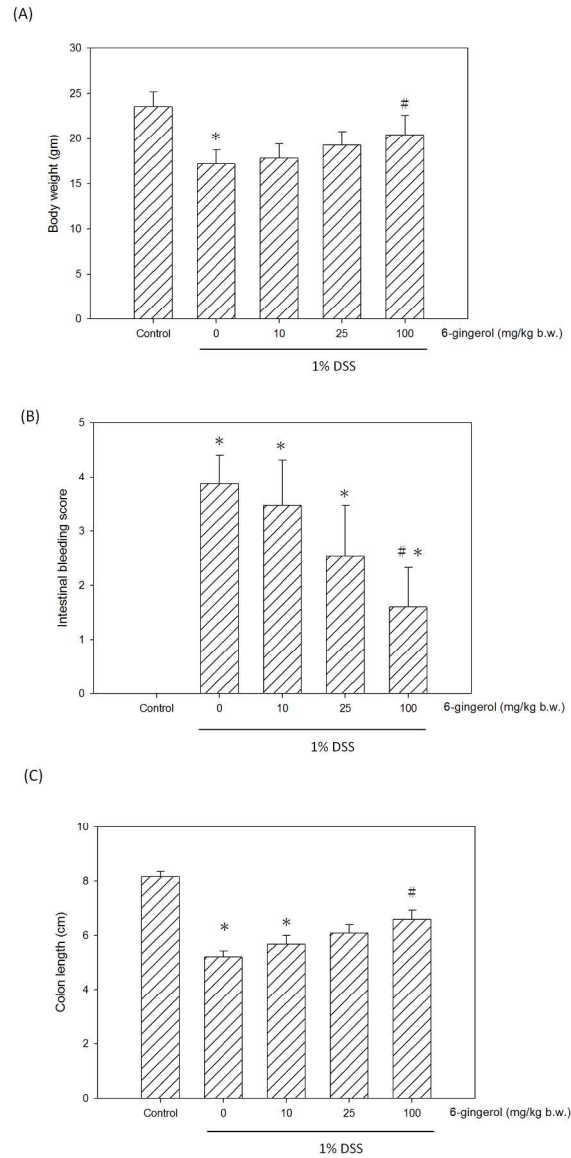
(A)

(B)

(C)

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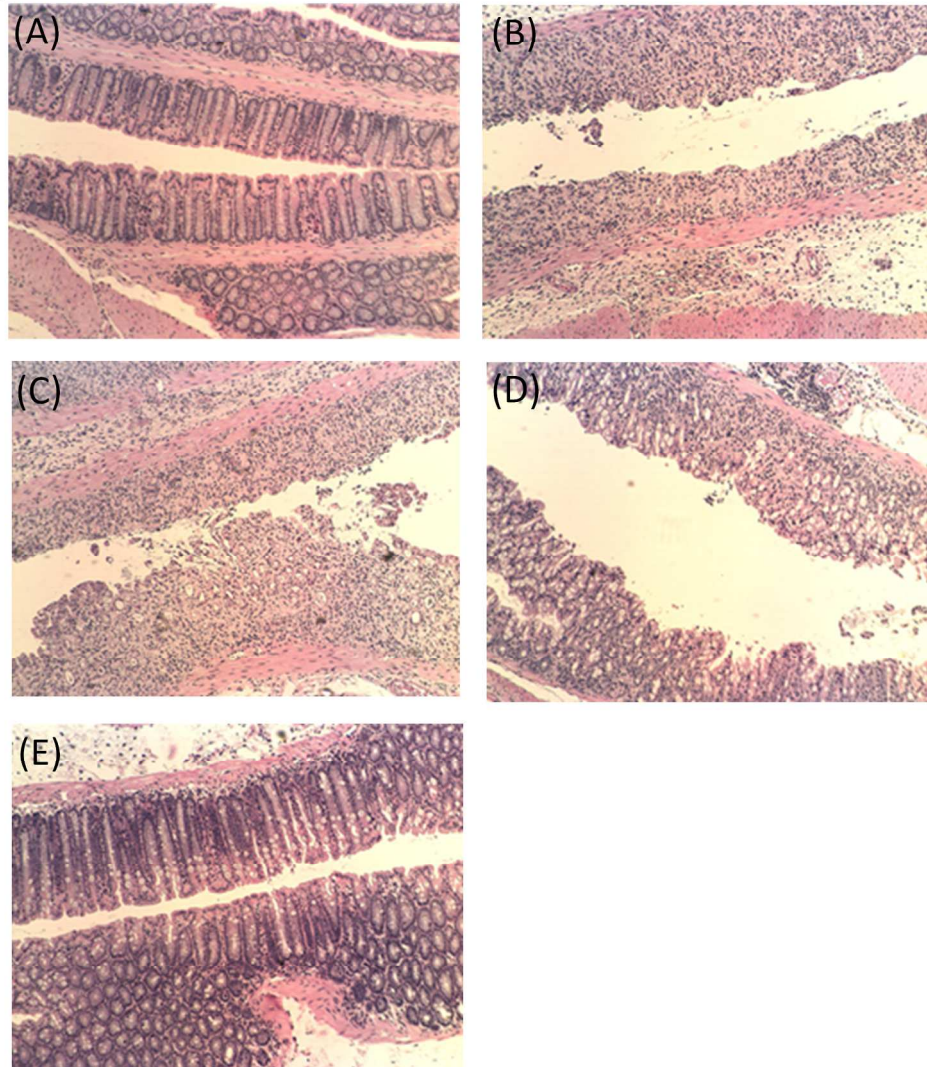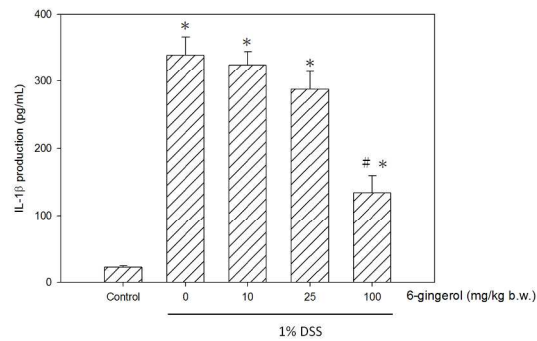406x795mm (96 x 96 DPI)

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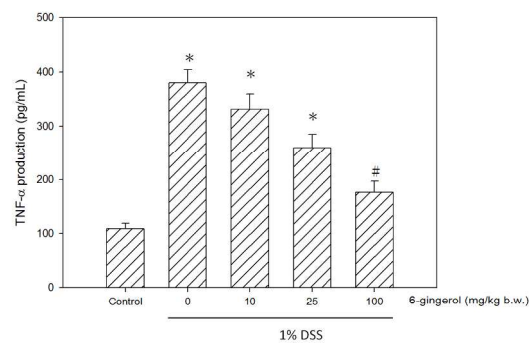622x702mm (96 x 96 DPI)
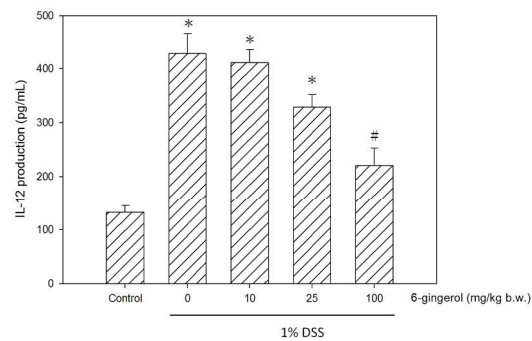
Figure 5

(A)

(B)

(C)

Figure 5. Ex vivo cytokine analysis of colon organ culture after 6-gingerol administration in DSS-induced colitis in mice. (A) IL-1β , (B) TNF-α and (C) IL-12 production in the supernatant of distal colon organ culture of mice with different treatments in DSS-induced colitis were measured. Means for each data without a common letter differ significantly (p<0.05).
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