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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

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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\textsuperscript{1-3}. In addition, dysregulated expression of matrix metalloproteinases (MMPs) is reported to play a role in pathogenesis of human IBD and experimental colitis \textsuperscript{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 \textsuperscript{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 \textsuperscript{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 \textsuperscript{11-13}. It has been demonstrated to have beneficial effects on gastrointestinal tract \textsuperscript{14-17}. In addition, recent studies have shown that 6-gingerol exerts several pharmacological activities via activation of adenosine monophosphate activated protein kinase (AMPK) \textsuperscript{11,18}. AMPK has been reported to play role in regulation of intestinal barrier function...
and inflammation. 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 in Dulbecco’s Modified Eagle’s Medium (DMEM, Gibco-BRL, CA, USA) supplemented with 10% fetal calf serum (FCS; Gibco-BRL, CA, USA) at 37°C in a humified 5% CO₂ atmosphere.

For treatment, cells were seeded in 6-well culture plates at an initial density of 1x10⁵ 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\textsuperscript{22}. In order to allow differentiation, Caco-2 cells were seeded at a density of $1 \times 10^5$ cells/ml on 6.5 mm cell culture inserts (3.0 $\mu$m 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 $>300$ $\text{ohm}\cdot\text{cm}^2$ 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 $\mu$M) 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 × 10^5 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 cm^2 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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer (5’ – 3’)</th>
<th>Reverse primer (3’ – 5’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-αlpha</td>
<td>CACCGGCAAGGATTCCAA</td>
<td>CACTCAGGCATCGACATTCG</td>
</tr>
<tr>
<td>IL-6</td>
<td>AGCCCTGAGAAAGGAGACATGTA</td>
<td>AGGCAAGTCTCTCATTGAATCC</td>
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<tr>
<td>IL-β</td>
<td>AATCTGTACCTGTCCTGCGTGT</td>
<td>TGGGTAATTTTTGGATCTACACTCT</td>
</tr>
<tr>
<td>GAPDH</td>
<td>AATGGAAATCCCCATCCACCATCT</td>
<td>CAGCATCGCCCCACTTG</td>
</tr>
</tbody>
</table>

DSS-induced colitis mice

The DSS-induced colitis model in mice was developed as previously described. 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. 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% CO2 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 (1-100 μM) 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-gingerol 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-\(\alpha\) and IL-1\(\beta\) 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\textsuperscript{29}. Activation of AMPK leads to an increase in catabolic pathways, such as glucose uptake and decrease in metabolic pathway like glycolysis \textsuperscript{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 \textsuperscript{32, 33}. Inhibition of AMPK has been reported to enhance LPS-induced inflammatory response in vitro and in vivo \textsuperscript{34, 35}. Administration of AMPK activator has been demonstrated to attenuate DSS-induced colitis in mice \textsuperscript{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. 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. It has been found to protect the intestinal mucosa from toxic insults. 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. Intestinal mucosa plays a critical role in maintaining intestinal homeostasis through a strict regulation of permeation of subepithelial compartment. 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. In support to previous findings, 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.

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. 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.
REFERENCES

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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).
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563x1162mm (96 x 96 DPI)
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406x795mm (96 x 96 DPI)
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622x702mm (96 x 96 DPI)
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