Abstract

Ulcerative colitis (UC) is a nonspecific inflammatory disorder characterized by oxidative and nitrosative stress, leucocyte infiltration, and upregulation of inflammatory mediators. Boldine is an alkaloid compound found in Boldo tree, with multiple pharmacological actions, mainly anti-inflammatory, antioxidant, antitumor, and immunomodulatory activities. Hence, the effect of boldine for its anti-inflammatory properties against dextran sulfate sodium (DSS)-induced UC in BALB/c mice was studied. Administration of boldine to DSS-induced mice protects colon damage by reduced disease activity index, spleen weight, and increased colon length. Also administration of boldine showed a reduction in the activity of myeloperoxidase (MPO) and CD 68+ expression. Boldine reduced the colon damage, with significant reductions in both the extent and the severity of the inflammation as well as in crypt damage and leukocyte infiltration in the mucosa. Analysis in vivo showed clear decrease in the production of tumor necrosis factor (TNF)-α, Interleukin (IL)-6, IL-17, and signal transducer and activator of transcription-(p-STAT3) Y705 with nuclear factor (p65-NF-κB) production being reduced significantly. Moreover, p65-NF-κB activation was reduced in mouse macrophage RAW 264.7 cells in vitro. The data demonstrated that boldine may be beneficial in colitis through selective immunomodulatory effects, which may be mediated, at least in part, by inhibition of p65-NF-κB and STAT3 signaling pathways. © 2016 BioFactors, 42(3):247–258, 2016

Keywords: ulcerative colitis; boldine; enzymic antioxidants; STAT3; NF-κB

1. Introduction

Ulcerative colitis (UC) and Crohn’s disease (CD) are the kinds of inflammatory bowel disease (IBD), and UC is an idiopathic disease characterized by the intestinal inflammation resulting from the transmural infiltration of neutrophil, macrophages or monocytes, lymphocytes accompanied by overproduction of oxygen free radicals that leading to the mucosal disruption, and ulceration [1]. It is believed that untreated chronic colitis may end up with colitis associated cancer [2]. Most prevailing therapies for UC include glucocorticosteroids, sulfasalazine, and so on [3,4]. However, the therapies have unwanted side effects and limited benefits. Consequently, looking for anti-inflammatory agents that are equally or more effective and cause no side effects are needed. Naturally derived plant products are very effective in controlling colitis with no side effects [5,6].

During the inflammatory process, a high number of immunological cells, including activated macrophages, polymorphonuclear
neutrophils, and eosinophils, infiltrate the lamina propria of the gut [7] and these cells known to produce a large amount of reactive oxygen species (ROS) [7,8]. Increased ROS leads to the oxidative damage and degrades extracellular matrix (ECM) [6,9]. Also excessive production of ROS or the impairment of antioxidant defense mechanisms may induce inflammatory and immune responses, which could directly or indirectly lead to lesions of intestinal epithelial cells and, subsequently, severe impairment in UC [10].

Nuclear factor-kappa B (NF-κB) is a key transcription factor that play vital role in the pathogenesis of IBD [6, 11]. It was a well-known phenomenon that the expression and activation of NF-κB are strongly induced in both macrophages and intestinal epithelial cells isolated from inflamed tissue specimens from patients of IBD [12] and the degree of activated NF-κB significantly correlated with the severity of inflammation in the intestine and colon [13]. On the other hand, the event of pro-inflammatory cytokine is combining with soluble IL-6 receptor (sIL-6R) which was found to be released via shedding from the surface of macrophages. After the formation of the complex of IL-6/sIL-6R is in turn activates gp130-positive cells via trans-signaling [14]. Further, IL-6 trans-signaling induces the signal transducer and activator of transcription-(STAT)-3, a downstream target of IL-6 signaling pathway [15,16]. The activation of STAT3 by IL-6 is the link between the inflammation and formation of cancer in the colon. The critical role of p65-NF-κB and IL-6/STAT3 signaling was well established in recent years and considered as a primary targets to treat IBD and colitis associated cancer (CAC) [6, 17–19].

Boldine (1,10-dimethoxy-2,9-dihydroxyaporphine), is a natural aporphine alkaloid found abundantly in Peumus boldus [20]. And it has shown to have several pharmacological activities, such as anti-inflammatory, antipyretic, anti-atherogenic, antiplatelet, anti-proliferative, anti-diabetic, and cytoprotective properties [21–23]. The compound shows a scavenging effect on hydroxyl radicals, while its effect on superoxide anion and hydrogen peroxide is unclear [24]. Boldine is known to inhibit cell proliferation and it induces apoptosis in glioma cell lines [25]. And also it induces cell cycle arrest and apoptosis in T24 human bladder cancer cell line via regulation of ERK, AKT, and GSK-3β [26]. Hence, the present study is designed to investigate whether boldine exerts protective ability against DSS-induced UC using a mouse model with reference to the regulation of IL-6/STAT3 signaling pathway.

2. Materials and Methods

2.1. Animals

Male BALB/c mice weighing 25–30 g were purchased from A Sapphire enterprises, (Serikembangan, Selangor, Malaysia), and maintained at a temperature of 25 °C at the relative humidity of 45%. The animals were allowed to access food and tap water ad libitum throughout the acclimatization and experimental periods. All animal experimental procedures and care of laboratory animals were followed in accordance with Institutional Animal Care and Use Committee (IACUC).

After 1 week of acclimation, the mice were randomly sorted into three different experimental groups and were provided with food and water ad libitum. After acclimatization, the mice in groups 2 and 3 were administered with 3.5% DSS (MP BioMedicals, OH) in drinking water for 7 days. The mice in group 3 were orally treated with boldine at the dose of 50 mg/kg body weight for 7 days. The body weights, stool consistency, and blood in the stool were monitored every day to calculate the disease activity index (DAI) [9]. The mice were sacrificed on day 8, and the colons (from the ileocecal junction to the anal verge) were removed. The colons were cut open longitudinally along the main axis and washed with phosphate-buffered saline (PBS; pH 7.4). After gross examination, the distal colons were fixed in 10% neutral-buffered formalin for histological and immunohistochemical analyses. The remaining proximal parts of colons were used for mRNA and western blot analyses.

2.2. Histological Analysis

Paraflin-embedded samples were cut into 5 μm sections and then stained with hematoxylin and eosin (H&E) for light microscopic examination. The sections of the colon were photographed with a Nikon ECLIPSE 80i (Tokyo, Japan) microscope. The samples were analyzed and scored as described previously [27,28].

2.3. Scanning Electron Microscopy (SEM)

Colon tissue samples were washed thoroughly in PBS and cut approximately in to 1 cm in length and then was fixed overnight in 2.5% phosphate buffered glutaraldehyde solution (0.1 M, pH 7.4). Furthermore, the samples were fixed in 1% osmium tetroxide solution and passed through an increasing alcohol and amyl acetate series. After drying the tissue samples with a critical-point dryer, they were coated with gold. The tissue samples were examined under Jeol 5200 JSM SEM (Tokyo, Japan).

2.4. Cell Culture

The murine macrophage-like cell line, RAW 264.7, was cultured in basal Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, low-glucose, and 80 units/mL of penicillin/streptomycin. Cells were grown on plastic cell culture flasks in a cell incubator at 37 °C and in 95% air/5% CO2. Medium was changed every day. Cells at 7–8 passages were used for the experiments until they generated an 80%-90% confluent layer.

2.5. NF-κB Activation Assay

NF-κB activation kit (Thermo Scientific, MA) was used as described by Shawish et al. [29]. RAW 264.7 cells were seeded in 96-well plate and allowed to attach for overnight. Then, boldine was added into the medium for 4 H before being stimulated with LPS for 1 H. Cells were fixed, permeabilized, and incubated with p65-NF-κB antibody for 1 H. Staining solution (containing DyLight™ 488, Goat anti-rabbit and Hoechst dye)
was then added and further incubated for 1 H. The plate with stained cells was evaluated using a Cellomics ArrayScan HCS Reader. Data were captured, extracted, and analyzed with ArrayScan II Data Acquisition and Data Viewer version 3.0 (Cellomics, PA).

2.6. Measurement of Myeloperoxidase (MPO) Activity
The activity of MPO in the colon homogenates was determined as previously described with some modifications [30]. The MPO-dependent oxidation of 3,3',5,5'-tetramethylbenzidine was measured by monitoring the absorbance at 650 nm. The protein concentrations were determined using the Pierce® BCA protein assay (Pierce Thermo Scientific, Rockford, IL).

2.7. Measurement of Malondialdehyde (MDA)
The levels of MDA in the colon tissues were determined as an indicator of lipid peroxidation was estimated by the method of Ohkawa et al. [31]. Colon tissue was homogenized in 1.15% KCl solution. The sample consisted of 0.2 mL of 8.1% SDS, 1.5 mL of 20% acetic acid, 1.5 mL of 0.8% thiobarbituric acid, and 0.7 mL of distilled water. Samples were boiled for 1 H at 95 °C and centrifuged at 3,000 g for 10 Min. The absorbance of the supernatant was measured spectrophotometrically at 650 nm.

2.8. Assay of Colonic Enzymic Antioxidants
The assay of superoxide dismutase (SOD) was followed by the method of Kakkar [32] based on 50% inhibition of the formation of NADH–phenazine methosulphate–nitroblue tetrazolium (NBT) formazan at 520 nm. One unit of the enzyme activity was taken as the amount of enzyme required for 50% inhibition of NBT reduction/minute/mg protein. The activity of catalase (CAT) was determined by the method of Sinha [33]. The values of CAT activity are expressed as micromole of H₂O₂ utilized/minute/milligram protein.

2.9. Immunohistochemical Analysis
Immunohistochemical method was adopted by Pandurangan et al. [34]. Finally, the sections were counter staining with hematoxylin and the slides were photographed in light microscope (Nikon ECLIPSE 80i, Japan).

2.10. RNA Isolation and Quantitative Real-Time PCR
Total RNA of colon tissue was isolated and first-strand cDNA synthesis was performed as described previously by Pandurangan et al. [35]. Primer sequences used for cDNA amplification were as follows: mouse β-actin, 5’-GGCGGACTGTTACCTGA GCTG-3’ and 5’-CTGCAGAAGTGGTTTTGTCA-3’; TNF-α, 5’-
TGGTGACCAGGCTGTCGCTACA-3' and 5'-TACAGTCACGGCTCCCGTGGG-3'; IL-6, 5'-ATGCTGGTGACAACCACGGCC-3' and 5'-CCTCTGTGAAGTCTCCTCTCCGGAC-3'; IL-17, 5'-CGTGGCCTCGATTGTCCGCC-3' and 5'-GGTTTCTTAGGGGTCAGCCGCG-3'. Quantitative real-time PCR with SYBR green PCR Master Mix (Applied Biosystems, Foster City, CA) was performed in a MicroAmp Fast Optical 96-Well reaction plate (Applied Biosystems, Foster City, CA) using the 7300 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) for thermal cycling and real-time fluorescence measurements. The PCR cycle protocol consisted of 10 Min at 95 °C, and 40 two-step cycles of 15 Sec each at 95 °C and of 1 Min at 60 °C. Positive and negative controls were included in all the experiments and each sample was run in triplicates for each PCR. Threshold cycle (C_T) values were recorded as a measure of initial template concentration. Relative fold changes in RNA levels were calculated by the ΔΔC_T method. The range for the target, relative to a calibrator sample was calculated by 2^{-ΔΔC_T}.

2.11. Preparation of Cytosol/Nuclear Extract

The preparation of cytosol/nuclear extract was followed by Ananda Sadagopan et al. [36]. Immediately after the isolation it was transferred the supernatant (nuclear extract) fraction to a clean pre-chilled tube. And all the extracts were stored at −80°C until use. Cytoplasmic and nuclear protein extracts were used for Western blot analysis.

FIG 2

Boldine suppresses the colonic inflammation. (A) Histopathology of the colon of control and experimental mice. The slides from the control and experimental mice were stained with H&E (20×). Control mice showed normal architecture of villi’s in the colon. DSS-induced mice showed an increased signs of ulceration, infiltration of neutrophils, and edema. Administration of boldine to the DSS-induced mice reduced the all above mentioned signs. (B) Histological scoring of colon in control and experimental mice. The histological scoring revealed that DSS-induced animals showed increased inflammation, ulceration, edema, and neutrophil infiltration. Boldine administration reduced these symptoms significantly. The values are expressed as the means ± S.D. Comparisons: aControl versus DSS, bDSS versus DSS + Bol. *# denotes a statistically significant difference at P < 0.05, and ns indicates a non-significant difference. (C) SEM photomicrograph of mouse colon tissues. Normal mice showed intact epithelial surface (350×). Mice with DSS-induced colitis showed abnormal crypt structure in the epithelium, depletion of goblet cells, widened grooves, and fibrous connective tissue (500×). DSS-induced colitic mice treated with boldine showed the reconstruction of micro villi and reduced pathological symptoms (600×).
2.12. Immunoblot Analysis

Immunoblot analysis was performed according to the method of Saadadoust et al. [37]. Protein–antibody complexes were detected by the Amersham™ ECL™ Prime western blotting detection reagent (GE Healthcare, Buckinghamshire). Immunoblots were quantified using ImageJ software (NIH, Bethesda, MD).

2.13. Statistical Analysis

Statistical analyses were processed according to conventional procedures using the Statistical Program of Social Sciences (SPSS) (SPSS Inc., Chicago, IL) software for Windows, Version 12.0 (Post-hoc, Tukey’s test). A P value less than 0.05 was considered statistically significant.

3. Results

3.1. Boldine Attenuates DSS-Induced Colitis

All experimental animals survived throughout the study period. Figure 1A depicts the DAI in the control and experimental mice. DSS-induced mice had an increased DAI score (body weight loss, stool consistency, and blood in the stool) compared with the control mice. DSS-induced mice that received oral boldine treatment had obviously less weight loss and lower DAI scores than the untreated DSS-induced mice.
The spleen weights of the mice administered with DSS were significantly ($P < 0.05$) higher compared with those of the control group, and boldine treatment significantly decreased the spleen weights ($P < 0.05$; Fig. 1B). The shrunken colon length is the major indicator of the severity of inflammation [38,39]. DSS-induced mice showed significantly decreased colon length (Fig. 1C) when compared with control mice and this decrease was suppressed by the boldine administration.

3.2. Boldine Improves the Histological Alterations in DSS-Induced Mice

The histological and morphological characteristics of the colons were assessed after H&E staining, and representative results as well as the microscopic scores are shown in Figs. 2A and 2B. The colons presented a normal morphology of crypts, abundant goblet cells, a small number of lamina propria mononuclear cells, no signs of mucosal thickening, and complete absence of ulcerations was observed in control mice. However, the DSS-induced mice presented severe epithelial damage with extensive cellular infiltration into the lamina propria, depletion of the goblet cells, and partial destruction of the architecture, resulting in a high microscopic damage score (Fig. 2B). In contrary, treatment with boldine tend to protect the colon from damage by partially blocking the inflammatory cells infiltration with minimal loss of epithelial cells, which resulted in a very low microscopic damage score, compared with the colons from mice treated with DSS.

SEM observations of the colonic mucosa in control mice showed a normal epithelium with crypts and some granulated mast cells (Fig. 2C). The mucosal surface appeared to be subdivided by well-defined concave grooves and regular-shaped crypt openings containing mucin-like material. The mice treated with DSS showed degenerated epithelium, severe inflammatory cell infiltration, widened grooves, and depletion of goblet cells, leaving an irregular crater like area. Relevantly, treatment with boldine following 7 days of DSS treatment significantly restored the architecture of the colon epithelium with a marked decrease in inflammatory cell infiltration compared with the DSS-induced group.

3.3. Boldine Reduces the Expression of CD $68^+$ in DSS-Induced Mice

The immunohistochemical expression of CD $68^+$ was depicted in Figs. 3A and 3B. We noted that there was an increase in the expression of CD $68^+$ in DSS-induced mice when compared with the control mice. In contrast, boldine treated mice decreased the expression of CD $68^+$. To confirm further the quantification of CD $68^+$ was performed, that showed the similar results. This indicates that boldine effectively controls the infiltration of macrophages/monocytes during the administration of DSS.
3.4. Boldine Decreased the Activity of MPO in DSS-Induced Mice

MPO activity, a marker for leukocyte infiltration into the inflamed tissue, was low in the colonic tissues of control mice (group 1) and markedly increased in mice with DSS-induced colitis (Fig. 3C). These results confirmed the histologic assessment, which showed increased leukocyte infiltration in mice with DSS-induced colitis. The increased MPO activity in mice with DSS-induced colitis was significantly reduced after administration of boldine.

3.5. Boldine Attenuated Pro-Inflammatory Cytokines

Cytokines are the key players in the intestinal immune system, and immune cells, such as macrophages, T cells, dendritic cells, and intestinal epithelial cells, are known to secrete various types of cytokines and they regulate inflammatory response in UC [40,41]. The qRT-PCR analysis of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-17 in the control and experimental groups were depicted in Fig. 4. The expression of TNF-α, IL-6, and IL-17 inflammatory cytokines in the mice treated with DSS was increased compared with the untreated mice. Boldine treatment significantly attenuated the expression of all of tested cytokines ($P < 0.05$).

3.6. Boldine Attenuates the Level of MDA

The level of MDA was depicted in Fig. 5A. We observed a significant ($P < 0.05$) increase in the level of MDA in DSS-induced mice compared with control mice. Additionally, treatment of DSS-induced mice with boldine resulted in a significant reduction in the level of MDA.

3.7. Boldine Increases the Activities of Enzymic Antioxidants

Figures 5B and 5C present the levels of enzymic antioxidants such as SOD and CAT in the colonic mucosa of mice in the control and experimental groups. The activities of enzymic antioxidants were significantly ($P < 0.05$) reduced in DSS-induced mice compared with the control mice (group 1). DSS-induced mice administered boldine (group 3) showed significantly increased enzymic antioxidant activities compared with the untreated DSS-induced mice (group 2).

3.8. Boldine Directly Inhibits of p65-NF-κB Activity

In Vivo and In Vitro

A significant increase in the protein expression of p65-NF-κB was observed in colonic tissues of DSS-induced model group (Figs. 6A and 6B). Furthermore, the degradation and phosphorylation of IκBα was induced in colonic tissues of DSS-exposed mice, compared with mice control. By contrast, administration...
of boldine reduced p65-NF-κB expression in DSS-induced colitis, and the phosphorylation/degradation of IκBα was effectively suppressed. We also checked the effect of boldine on LPS-induced p65-NF-κB activation in RAW 264.7 mouse macrophage cells. Our western blot results revealed that boldine (25 and 50 μg/mL) inhibited NF-κB p65 nuclear translocation, as indicated in LPS-stimulated mouse macrophage RAW 264.7 cells (Fig. 7A). We further confirmed the inhibition of NF-κB nuclear translocation by immunofluorescence analysis that showed similar results (Figs. 7B and 7C). Hence it was clear that boldine suppresses the expression of p65-NF-κB in RAW 264.7 mouse macrophage cell lines. These results indicated that boldine significantly inhibited p65-NF-κB expression in DSS-induced colitis and LPS-induced RAW 264.7 macrophage cells by suppressing IκBα phosphorylation/degradation and blocking p65-NF-κB nuclear translocation.

3.9. Boldine Inhibits the Activation of STAT3

The western blot expression of p-STAT3 Y705 and T-STAT3 was shown in Fig. 8. The DSS-induced mice showed increased expression of p-STAT3 Y705 when compared with the control mice. However, treatment with boldine inhibited the phosphorylation of STAT3 at Tyr705 (Figs. 8A and 8B) and the translocation into the nucleus (Fig. 8C). We have not noted any changes in the expression of T-STAT3 in all the experimental groups.

4. Discussion

To study the molecular and cellular mechanisms of inflammation, animal models of intestinal inflammation is vital to understand the pathophysiological mechanisms and different animal models of chemically induced experimental colitis have
Boldine inhibits p65-NF-κB expression in RAW 264.7 mouse macrophage cells. (A) Western blot analysis of p65-NF-κB in the cytosolic and nuclear fractions in RAW 264.7 macrophage cells. The isolation of nuclear and cytosolic fractions was mentioned in detail in the “Materials and Methods” section. The RAW 264.7 cells treated with LPS (10 ng/mL) showed increased expression of p65-NF-κB in the nucleus. In contrast, pretreated with 25 and 50 μg/mL of boldine along with LPS activated RAW 264.7 cells showed reduced expression of p65-NF-κB in the nucleus. (B) Average relative intensity of Nucleus/p65-NF-κB ratio. Comparisons: aControl versus LPS, bLPS versus LPS+Bold (25 μg/mL), cLPS versus LPS+Bold (50 μg/mL), #P < 0.05.
been developed [42]. There are several animal models available among these the most widely used preclinical model is the DSS-induced colitis model, which mimics human UC with some important immunological and histopathological aspects [43] and is frequently used to evaluate the effect of novel anti-inflammatory drugs. In this study, we showed that boldine suppresses the inflammation in the colon by modulating pro-inflammatory cytokines, NF-κB, and STAT3 signaling.

Number of reports stating that oxidative stress is one of the vital causative of UC and colitis associated cancer [6,35]. Damaging macromolecules such as proteins, DNA, and lipids was the consequence of oxidative stress. MDA is the end product of lipid peroxidation, acts as a marker of oxidative stress [44]. It was well known that the level of MDA was elevated when the rodents exposed to DSS [6,9]. In consistent with the previous reports the level of MDA was elevated in DSS-induced mice (Fig. 5A). By contrast, administration of boldine reduced the level of MDA in DSS-induced mice. Kringstein and Cederman [45] reported that boldine was shown to prevent lipid peroxidation without inhibiting enzymes, such as CYP450 or its reductase or by diverting electrons away from the peroxidative process. Also boldine prevents the non-enzymatic peroxidation of microsomal lipids initiated by Fe²⁺ or the enzyme-catalyzed peroxidation. So, it was confirmed that boldine inhibits lipid peroxidation in DSS-induced mice.

Enzymic antioxidants such as SOD and CAT were the first line defense against ROS in many diseases including UC and the levels of enzymic antioxidants were decreased in UC. It

**FIG 8**

**Boldine reduced the expression/activation of STAT3.** (A) The DSS-induced animals showed increased expressions of p-STAT3Y705 (lane 2) compared with the control mice (lane 1). Boldine treatment (lane 3) decreased the expression of p-STAT3Y705 compared with the DSS-induced mice. The total STAT3 was unaltered in all of the experimental groups of mice. (B) Quantification of the respective blots using the ImageJ software. (C) Immunohistochemical analysis of p-STAT3Y705. The values are expressed as the means ± S.D. in three mice. In comparison of Control versus DSS, #DSS versus DSS + Bol. *#* denotes a statistically significant difference at P < 0.05 and ns indicates a non-significant difference.
was well known that enzymic antioxidants were depleted in DSS-induced UC [46,47]. We noted that, the levels of SOD and CAT were decreased in DSS-induced mice (Figs. 5B and 5C). SODs convert $O_2^-$ into $H_2O_2$ and Oxygen. Although SOD scavenges $O_2^-$, its product, $H_2O_2$, can be converted to more active OH• and leads to lipid peroxidation if there are not enough catalase or peroxidase to decompose $H_2O_2$ [48]. CAT catalyzes the conversion of $H_2O_2$ into $H_2O$ and $O_2$. And also CAT was found to be inactivated by high OH• production [49]. Administration of boldine increased the levels of SOD and CAT in DSS-induced mice. We believed that since boldine was a potent antioxidant [22], which eliminates the MDA and restores the enzymic antioxidants.

In general, NF-κB is the crucial regulator of the immune and inflammatory responses. Since NF-κB, is a transcription factor, it controls an array of pro-inflammatory genes involved in inflammatory signaling cascade. In the pathogenesis of UC, NF-κB plays a vital role and the levels of NF-κB was increased in the rodent and human models [6,[50,51]]. We observed that the expression of p65-NF-κB was increased in DSS-induced mice (Fig. 6). Boldine also showed the inhibition of p65-NF-κB in the LPS-activated RAW 264.7 macrophage cells (Fig. 7). We previously reported that boldine inhibits the p65-NF-κB activation and nuclear translocation in MDA-MB1 breast cancer cells [23]. Hence, we hypothesized that the anti-inflammatory effect of boldine in response to DSS-induced colitis correlated with the blockade of p65-NF-κB activation in DSS-induced colitis and LPS-activated RAW 264.7 mouse macrophage cells.

STAT3 is known to be involved in colonic inflammation as well as colitis associated cancer and also activated by variety of cytokines and growth factors [2,6,[19]]. STAT3 is activated through phosphorylation of Tyr705 at the COOH terminus. Upon activation, STAT3 translocates to the nucleus, where it through phosphorylation of Tyr705 at the COOH terminus.

In conclusion, boldine ameliorates the DSS-induced UC and inhibits activation of p65-NF-κB signaling in both DSS-induce mouse model and RAW 264.7 macrophage cells, and inhibits the activation and translocation of p-STAT3Y705 signaling in the colon. Molecular mechanisms underlying suppressive effects of boldine on p65-NF-κB and STAT3 remain elusive. These findings suggest that boldine might be a potential therapeutic agent for IBD.

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