Coptisine attenuates post-infectious IBS via Nrf2-dependent inhibition of the NLPR3 inflammasome

YING XIONG[1], HONG WEI[2], CHONG CHEN[2], LU JIAO[2], JUAN ZHANG[2], YONGGANG TAN[2] and LI ZENG[2]

[1]Department of Gastroenterology, Shenzhen Longhua District Central Hospital, Shenzhen, Guangdong 518110;
[2]Department of Gastroenterology, The First Affiliated Hospital of Shenzhen University,
The Second People's Hospital of Shenzhen, Shenzhen, Guangdong 518035, P.R. China

Received February 26, 2022; Accepted August 12, 2022

DOI: 10.3892/mmr.2022.12879

Abstract. Inhibition of the activation of the NLR family pyrin domain-containing 3 (NLRP3) inflammasome has previously been reported to confer protection against post-infectious irritable bowel syndrome (PI-IBS). Coptisine, the second most abundant isoquinoline alkaloid in *Coptis chinensis*, can inhibit NLRP3 inflammasome activation; however, whether coptisine exhibits protective effects against PI-IBS remains unclear. In the present study, coptisine significantly reduced gastrointestinal motility and abdominal withdrawal reflex scores in a PI-IBS rat model that was induced using intragastric administration of *Trichinella spiralis* larvae. Coptisine treatment significantly decreased the protein levels of oxidative stress markers, 4-hydroxynonenal, protein carbonyl and 8-hydroxy-2'deoxyguanosine, and proinflammatory cytokines, TNF-α, IL-1β and IL-18 in the colon of PI-IBS rats. Moreover, coptisine treatment significantly increased nuclear factor erythroid 2-related factor 2 (Nrf2) nuclear translocation and heme oxygenase-1 protein expression levels, while significantly downregulating the protein expression levels of NLRP3, apoptosis-associated speck-like protein containing a CARD and caspase-1 in the colons of PI-IBS rats. It is important to note that the anti-inflammatory effects of coptisine were blocked by the Nrf2 inhibitor ML385. In summary, the present study indicated that coptisine potentially attenuated PI-IBS in rats via Nrf2-dependent inhibition of the NLPR3 inflammasome.

Correspondence to: Professor Li Zeng, Department of Gastroenterology, The First Affiliated Hospital of Shenzhen University, The Second People's Hospital of Shenzhen, 3002 Sungang West Road, Futian, Shenzhen, Guangdong 518035, P.R. China
E-mail: zengli0298@163.com

Key words: post-infectious irritable bowel syndrome, coptisine, nuclear factor erythroid 2-related factor 2, NLR family pyrin domain-containing 3

Introduction

Irritable bowel syndrome (IBS) is a functional bowel disorder characterized by symptoms of chronic abdominal pain and changes in dietary habits (1). Worldwide, the incidence of IBS is 11.2%, whereas the incidence in China ranges from 5-6% (2). The etiology of IBS has been well described, and it has been attributed to genetic factors, diet, exercise, drugs, stress, cognitive behavior, infectious enteritis and dysregulation of the gut-brain axis (3).

More than 10% of patients with infectious enteritis (notably protozoal enteritis, including that caused by *Trichinella spiralis* larvae) will develop IBS (4). The pathogenesis of post-infectious IBS (PI-IBS) is characterized by low-grade intestinal inflammation, increased intestinal permeability, muscle hypercontractility and visceral hypersensitivity (4,5). Treatment of IBS is often targeted towards management of the symptoms, rather than the underlying pathophysiology (3). Consequently, treatments are not sufficiently effective and the natural progression of the disorder remains unaltered by the majority of therapeutic interventions (4). The molecular mechanisms, which underpin PI-IBS, are complex and not fully understood.

Increasing evidence has indicated that oxidative stress and inflammation are involved in the pathophysiology of PI-IBS (6). The roles of nuclear factor erythroid 2-related factor 2 (Nrf2) and nuclear factor erythroid 2-related factor 2 (NLRP3) activation in PI-IBS have previously been demonstrated (7). Under normal conditions, Nrf2 is sequestered by Kelch-like ECH-associated protein 1 (Keap1) in the cytoplasm. When this Keap1/Nrf2 complex is disrupted, Nrf2 translocates to the nucleus where it binds to the antioxidant response element sequences and activates the transcription of its downstream targets, including heme oxygenase-1 (HO-1). It has been reported that activation of the Nrf2 signaling pathway confers protective effects against PI-IBS (8). Moreover, previous studies have demonstrated that NLRP3 overactivation during PI-IBS is associated with intestinal dysfunction (9,10). NLRP3 binds to apoptosis-associated speck-like protein containing a CARD (ASC) and recruits pro-caspase-1, which leads to the maturation of caspase-1. Caspase-1 cleaves pro-IL-1β into IL-1β and pro-IL-18 into IL-18 (11). Crosstalk between the Nrf2 and NLRP3 signaling pathways may exist in PI-IBS (12,13).

Coptis chinensis, a traditional Chinese medicine, has been used for thousands of years to treat type 2 diabetes mellitus, and cardiovascular, hepatic and renal disorders (14). The main constituents responsible for its bioactive properties are the isoquinoline alkaloids (15). Coptisine is the second most abundant isoquinoline alkaloid in *C. chinensis*. Coptisine exerts diverse beneficial effects, including anticancer, anti-inflammatory and antibacterial properties (16). Recently, coptisine was reported to exert suppressive effects on NLRP3 inflammasome activation in *in vivo* models (17). Coptisine has also been reported to reduce the degree of diarrhea and mucosal injury to the ileum via modulation of the IκBα/NF-κB signaling pathway (17). Therefore, it was hypothesized that coptisine may potentially exert protective effects against PI-IBS via Nrf2-dependent inhibition of the NLRP3 inflammasome. The present study aimed to assess the effect of coptisine on a rat model of IBS and investigate the underlying mechanisms.

Materials and methods

Reagents. Coptisine (cat. no. HY-N0430) was purchased from MedChemExpress. The Nrf2 inhibitor ML385 (cat. no. SML1833) was purchased from Sigma-Aldrich (Merck KGaA). The following enzyme-linked immunosorbent assay (ELISA) kits were purchased from Abcam: Protein Carbonyl ELISA Kit (cat. no. ab238536), 8-hydroxy-2-deoxyguanosine (8-OHdG) ELISA Kit (cat. no. ab201734), Rat TNF-α ELISA Kit (cat. no. ab236712), Rat IL-1β ELISA Kit (cat. no. ab255730), Rat IL-18 ELISA Kit (cat. no. ab213909), and the Lipid Peroxidation 4-hydroxynonenal (4-HNE) Assay Kit (cat. no. ab238538). Primary antibodies, including anti-Nrf2 antibody (cat. no. ab92946), recombinant anti-HO-1 antibody (cat. no. ab68477), recombinant anti-NLRP3 antibody (cat. no. ab263899), anti-ASC antibody (cat. no. ab180799), recombinant anti-pro-caspase-1 and cleaved caspase-1 antibody (cat. no. ab179515), anti-histone H3 antibody (cat. no. ab1791) and anti-β-actin antibody (cat. no. ab8226) were all purchased from Abcam. The secondary antibodies goat anti-mouse IgG heavy chain and light chain (H&L) (HRP; cat. no. ab6789) and goat anti-rabbit IgG H&L (HRP; cat. no. ab6721) were also purchased from Abcam. Protein extraction kits were purchased from Santa Cruz Biotechnology, Inc. The bicinchoninic acid (BCA) assay and Pierce™ ECL Plus Western Blotting Substrate were purchased from Pierce (Thermo Fisher Scientific, Inc.).

Animals and ethics. The protocol used in the present study was approved by the Ethics Committee for Animal Use of The First Affiliated Hospital of Shenzhen University, The Second People's Hospital of Shenzhen (approval no. 20190902002). Male, Sprague-Dawley rats (n=80; 250±10 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. and housed in a specific pathogen-free laboratory environment (temperature, 22±2˚C; relative humidity, 55±15%; 12 h light/dark cycle) with *ad libitum* access to food and water.

Animal treatment. After 7 days of environmental adaptation, 80 rats were randomly divided into the following four groups (Fig. 1A): i) Control; ii) PI-IBS model; iii) coptisine treatment; and iv) coptisine + ML385 treatment. A total of 20 animals were used in each group. The PI-IBS rat model was established as previously described (9). *Trichinella spiralis* larvae were obtained from the Institute of Pathogen Biology, Chinese Academy of Medical Sciences and Peking Union Medical College. In brief, the rats were intragastrically administered 350-400 *Trichinella spiralis* larvae (diluted in 0.2 ml saline). On day 14 post-infection, abdominal withdrawal reflex (AWR) scores were assessed to determine whether the model was successfully established. The rats in the coptisine group were administered coptisine (50 mg/kg) intragastrically for 14 consecutive days. The rats in the coptisine + ML385 group were injected with ML385 (30 mg/kg) intraperitoneally and treated with coptisine (50 mg/kg) intragastrically 30 min later for 14 consecutive days. The rats in the control and PI-IBS model groups were administered intraperitoneally and intragastrically an equivalent volume of saline. On day 14 post-infection, the body weight and fecal water content of all rats were assessed. On day 14 post-infection, 10 rats from each group were randomly selected for the gastrointestinal motility assay. The remaining 10 rats from each group were used for the assessment of AWR. After gastrointestinal mobility or AWR tests, 14 rats were randomly selected and were anesthetized using isoflurane/oxygen (induction, 5% isoflurane; maintenance, 2% isoflurane) and a Matrix VIP 3000 calibrated vaporizer (Midmark Corporation). Rats were subsequently decapitated individually with a rodent guillotine and then transcardially perfused with saline. The proximal colon tissues were collected for ELISA (n=8/group), western blotting (n=3/group) or fixed in glutaraldehyde for transmission electron microscopy (n=3/group). The remaining rats (n=6/group) were anesthetized with isoflurane (induction, 5%; maintenance, 2%) and decapitated individually with a rodent guillotine prior to perfusion with 100 ml of cold (4˚C) 4% paraformaldehyde following saline perfusion. The proximal colon tissues were collected for hematoxylin and eosin (H&E) staining.

Gastrointestinal motility. On day 14 post-infection, gastrointestinal motility was assessed by feeding the rats charcoal meals and determining the distance traveled by the charcoal in a fixed amount of time. Prior to the measurement of gastrointestinal motility, the rats (n=10/group) were fasted overnight and were subsequently administered 0.2 ml 10% charcoal in 5% acacia gum via oral gavage. The rats were euthanized 30 min later and the intestinal tracts were carefully removed. The total length from the pylorus to the cecum was measured. Gastrointestinal motility was expressed as the percentage of the total intestinal length traversed by the charcoal. At least three separate experiments were performed.

AWR score. Visceral sensory functions in rats were evaluated via AWR scores on day 14 post-infection. Briefly, the rats (n=10/group) were fasted for 24 h and anesthetized with isoflurane/oxygen (induction, 5%; maintenance, 2%). A Vaseline-coated latex, double-lumen catheter attached to a balloon dilator (6 Fr; external diameter, 2 mm; RWD Life Science Co., Ltd.) was slowly inserted into the descending colon. Colorectal distention was performed following adaptation. The rats were fixed on a table and provided 20, 40, 60 and 80 mmHg pressure stimulation every 5 min. These conditions were maintained for 20 sec. The balloon was deflated

and withdrawn following the assessment of the AWR score. AWR scoring was a double-blind procedure completed by the operator and the observer. The AWR scoring criteria were as follows: 0, no behavioral response; 1, the body remained immobile with a simple head movement; 2, abdominal contraction of muscles that did not leave the ground; 3, abdominal muscle contraction was accompanied by the lifting of the abdomen; and 4, arching of the body along with the lifting of the pelvis and scrotum. The same pressure stimulations were performed three times and the median score was calculated.

ELISA. Excised colons were homogenized in RIPA buffer (Takara Bio, Inc.) containing 1% phosphatase inhibitors and complete protease inhibitor cocktail (Roche Diagnostics). The homogenates were subsequently centrifuged at 3,000 x g for 20 min at 4˚C and the protein concentrations in the supernatant of the homogenates were determined using a BCA assay kit. The levels of the specific oxidative stress markers, 4-HNE, protein carbonyl and 8-OHdG, and the proinflammatory cytokines TNF-α, IL-1β and IL-18 in the colon homogenates were assessed using ELISA and specific assay kits according to manufacturer's protocol. At least three separate experiments were performed.

H&E staining. The colon tissues were fixed with 4% paraformaldehyde at 4˚C for 48 h, and then embedded in paraffin and dissected into 5-μm coronal sections. The sections were dewaxed in xylene and then rehydrated using a graded alcohol series (100, 90, 80 and 70%) at room temperature for 5 min. Rehydrated sections were stained using H&E. The slides were scanned using a PANNORAMIC MIDI scanner (3DHISTECH, Ltd.) and blind evaluated by two histopathologists.

Transmission electron microscopy analysis. Colon tissues were fixed using 1% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) overnight at room temperature. Following fixation, the colons were treated with reduced 1% osmium tetroxide, followed by 1% tannic acid in 0.1 M sodium cacodylate buffer at room temperature for 1 h. The colons were subsequently stained using a 2% aqueous solution of uranyl acetate at room temperature for 30 min, dehydrated in a graded ethanol series and processed for embedding in PolyBed (Polysciences, Inc.). The blocks were sectioned at a 90-nm thickness, post-stained with Venable's lead citrate at room temperature for 5 min and imaged using a transmission electron microscope (JEOL, Ltd.) by observers who were blinded to the experimental groups.

Western blotting. Fresh colon tissues were homogenized and lysed in RIPA buffer (Takara Bio, Inc.) containing 1% phosphatase inhibitors and complete protease inhibitor cocktail (Roche Diagnostics). The total cytosolic and nuclear protein extract were prepared using cell nuclear protein extraction kits (Thermo Fisher Scientific, Inc.). The samples were subsequently centrifuged at 3,000 x g for 20 min at 4˚C and the protein concentrations in the supernatant of the homogenates were determined using a BCA assay kit. An equal amount of protein (20 μg) was separated by SDS-PAGE on 8-12% gels and transferred to polyvinylidene difluoride membranes. The membranes were blocked at 4˚C using 5% non-fat milk solution in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 1 h and incubated overnight at 4˚C with anti-Nrf2 (1:1,000), anti-HO-1 (1:1,000), anti-NLRP3 (1:1,000), anti-ASC (1:1,000), anti-pro-caspase-1 and cleaved caspase-1 (1:1,000), Histone H3 (1:2,000) and anti-β-actin (1:2,000) antibodies. After washing with TBST, the membranes were then incubated with goat anti-rabbit IgG H&L (HRP; 1:2,000) or goat anti-mouse IgG H&L (HRP; 1:2,000) antibodies at room temperature for 1 h. The protein bands were developed using enhanced chemiluminescence kits and were visualized using the ChemiDoc Touch Imaging System (Bio-Rad Laboratories, Inc.). The bands were semi-quantified via Image Lab Software (version 6.1; Bio-Rad Laboratories, Inc.). The protein band density of cytosolic protein was normalized compared with that of β-actin. The protein band density of nuclear protein was normalized compared with that of Histone H3. At least three separate experiments were performed.

Statistical analysis. Data were analyzed using SPSS (version 20; IBM Corp.). AWR scores are presented as the median and range, and were analyzed using Kruskal-Wallis and Dunn's post hoc test. Continuous data are presented as the mean ± SD and were analyzed using one-way ANOVA followed by Tukey's post-hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Coptisine prevents intestinal dysmotility in PI-IBS rats. A diagram of the establishment and grouping of animal models is shown in Figure 1A. The body weight (Fig. 1B) was significantly decreased, whereas fecal water content (Fig. 1C) was significantly increased in PI-IBS rats compared with those in the control group ($P<0.01$). Treatment with coptisine (50 mg/kg) and co-treatment with ML385 for 14 days did not significantly affect the body weight and fecal water content of PI-IBS rats ($P>0.05$). Gastrointestinal motility was assessed by feeding rats charcoal meals and determining the distance traveled by the charcoal in a fixed amount of time. Gastrointestinal motility was significantly increased in PI-IBS rats compared with the that in the control group ($P<0.01$; Fig. 1D). Treatment of the rats with coptisine (50 mg/kg) for 14 days significantly decreased gastrointestinal motility compared with that in the PI-IBS model group ($P<0.01$). The visceral sensitivity of colorectal distention in rats was assessed using the AWR scores. The PI-IBS group exhibited significantly higher AWR scores compared with those in the control group following pressure stimulation at 40 and 60 mmHg ($P<0.01$; Fig. 1E). The AWR scores of the PI-IBS group at 20 and 80 mmHg were comparable with the control group, which suggested that too much or too little pressure could affect the AWR scores. However, the coptisine-treated rats demonstrated significantly lower AWR scores compared with those in the PI-IBS group following pressure stimulation at 40 and 60 mmHg ($P<0.01$). The decreased gastrointestinal motility and the lower AWR scores following pressure stimulation at 40 and 60 mmHg mediated by coptisine were abolished by ML385 treatment. These results indicated that coptisine significantly alleviated gut hypersensitivity of rats in the PI-IBS rats.

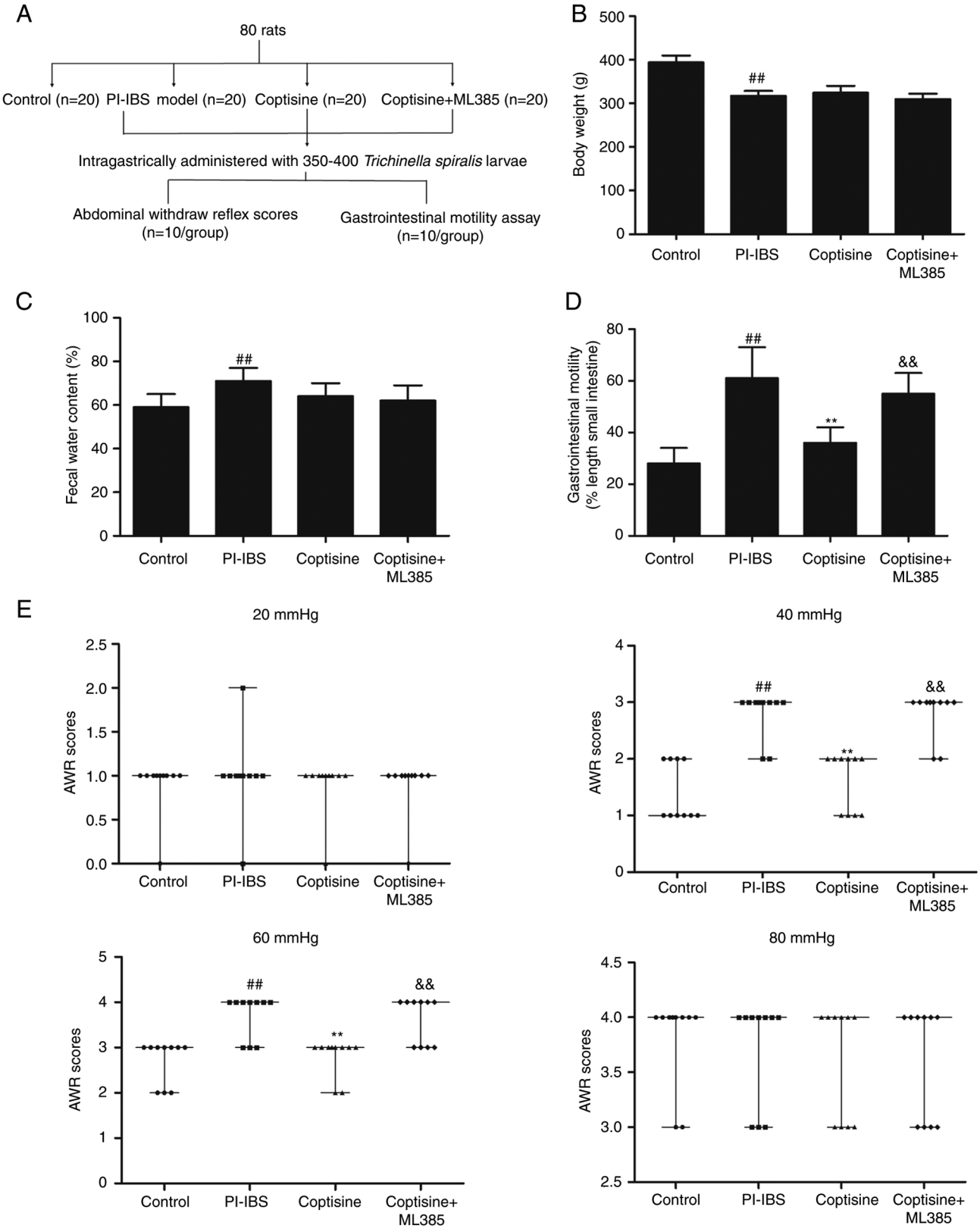

Figure 1. Coptisine attenuates intestinal dysmotility in PI-IBS rats. (A) Diagram of the establishment and grouping of animal models. Effects of coptisine on (B) body weight, (C) fecal water content, (D) gastrointestinal motility and (E) AWR scores in PI-IBS rats. $^{\#\#}$P<0.01 vs. control; **P<0.01 vs. PI-IBS; $^{\&\&}$P<0.01 vs. coptisine. PI-IBS, post-infectious-irritable bowel syndrome; AWR, abdominal withdrawal reflex.

Coptisine improves colonic ultrastructure in PI-IBS rats. No significant pathomorphological changes were noted in the colon tissues of rats in the PI-IBS and coptisine-treatment groups compared with in the control (Fig. 2A). The colonic ultra-structures of the rats were assessed via transmission electron microscopy. The mitochondria in the control rats were oval-shaped with clear and regular cristae (Fig. 2B). However, the mitochondria became swollen with larger sizes

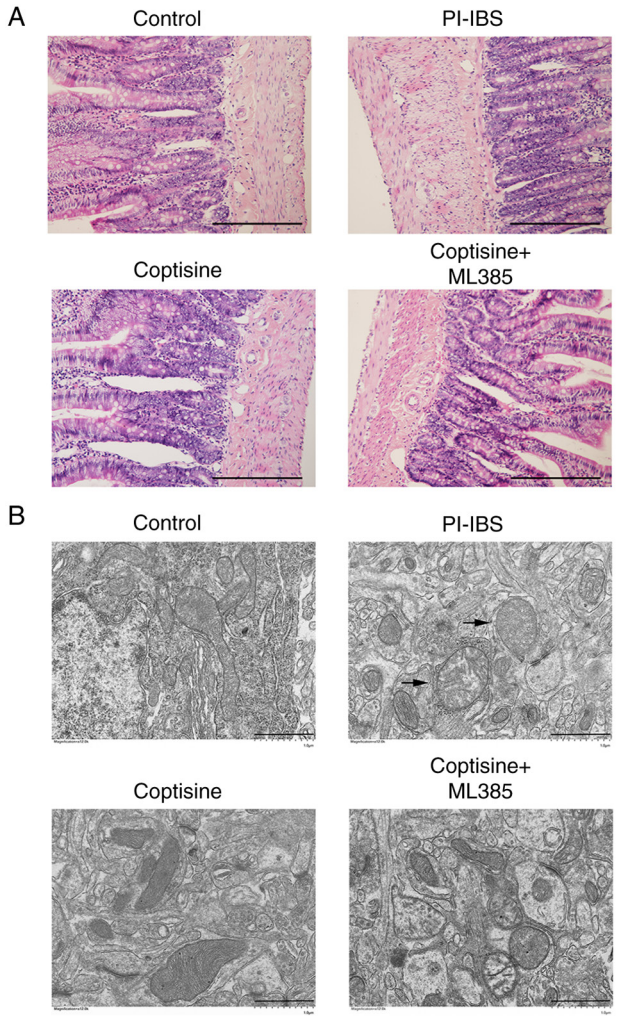

Figure 2. Coptisine improves colonic ultrastructure in PI-IBS rats. (A) Pathological and morphological condition of the colon tissues of the rats was assessed using hematoxylin and eosin staining (scale bar, 50 μm). (B) Colonic ultrastructure of the rats was assessed using transmission electron microscopy (scale bar, 1 μm). Arrows indicate the defective mitochondria. PI-IBS, post-infectious irritable bowel syndrome.

and blurred inner crests in the colons of the PI-IBS rats. Coptisine treatment markedly reversed the aforementioned changes in the colonic ultrastructure of PI-IBS rats. The improvements in colonic ultrastructure mediated by coptisine were abolished by ML385 treatment. These results suggested that coptisine significantly improved colonic ultrastructure in PI-IBS rats.

Coptisine suppresses oxidative stress and inflammation in PI-IBS rats. Oxidative stress and inflammation serve pivotal roles in the occurrence and persistence of PI-IBS symptoms (7,9). In the present study, the levels of oxidative stress and inflammation markers were detected using ELISA in colon tissue lysates from the different treatment groups. The PI-IBS group exhibited significantly increased levels of 4-HNE (Fig. 3A), 8-OHdG (Fig. 3B) and protein carbonyl (Fig. 3C) compared with those in the control group (P<0.01). However, the coptisine treatment group exhibited significantly lower levels of 4-HNE, protein carbonyl and 8-OHdG levels compared with those in the PI-IBS group (P<0.01). Furthermore, *T. spiralis* infection caused a significant increase in the levels of proinflammatory cytokines (TNF-α, IL-1β and IL-18) in the colon compared with those in the control group (P<0.01; Fig. 3D-F). Treatment with coptisine significantly reduced the levels of TNF-α (Fig. 3D), IL-1β (Fig. 3E) and IL-18 levels (Fig. 3F) compared with those in the PI-IBS group (P<0.01). The decreased levels of 4-HNE, 8-OHdG, protein carbonyl, TNF-α, IL-1β and IL-18 mediated by coptisine were abolished by ML385. These results indicated that coptisine significantly suppressed oxidative stress and inflammation in PI-IBS rats.

Coptisine attenuates NLRP3 inflammasome activation via Nrf2 signaling. The protein expression levels of nuclear Nrf2 and HO-1 were significantly decreased, whereas the protein expression level of cytoplasm Nrf2 was significantly increased in the colon tissues of the PI-IBS group compared with those in the control group (Fig. 4A; P<0.01). Treatment of the rats with coptisine significantly increased the protein expression levels of nuclear Nrf2 and HO-1 and significantly decreased cytoplasm Nrf2 compared with those in the PI-IBS group (P<0.01). In addition, he protein expression levels of NLRP3 and ASC were significantly increased in the PI-IBS group compared with those in the control group (Fig. 4B; P<0.01). Coptisine treatment significantly decreased the protein expression levels of NLRP3 and ASC compared with those in the PI-IBS group. *T. spiralis* infection significantly increased the level of cleaved caspase-1, which was markedly decreased by coptisine treatment (P<0.01). The increased levels of nuclear Nrf2 and HO-1, and the decreased levels of cytoplasm Nrf2, NLRP3, ASC and cleaved caspase-1, that were mediated by coptisine, were abolished following the treatment of rats with ML385. These results suggested that coptisine significantly attenuated NLRP3 inflammasome activation via Nrf2 signaling.

Discussion

PI-IBS is a common, chronic and multifactorial gastrointestinal disorder associated with recurrent abdominal pain (18), the pathogenesis of which has not been fully characterized. Intestinal dysmotility symptoms, such as abdominal pain, diarrhea and tenesmus, are frequently reported in patients with PI-IBS (19). In the present study, *T. spiralis*-infected rats were used as a PI-IBS animal model and developed symptoms similar to those of patients with PI-IBS, including gut hypersensitivity. Gastrointestinal motility and AWR scores were significantly increased in PI-IBS rats. However, treatment of the rats with coptisine significantly decreased both gastrointestinal motility and AWR scores.

It has previously been demonstrated that oxidative stress and inflammation are important in the development of PI-IBS (8,20). Oxidative stress results from a disturbance of redox homeostasis, in which the production of reactive oxygen species is beyond the capability of the endogenous antioxidant enzyme system to manage, which results in lipid peroxidation, protein carbonylation and DNA oxidation (21). HO-1, a potent anti-oxidative enzyme, is primarily regulated by Nrf2 (9). Under normal conditions, Nrf2 is sequestered in the cytoplasm by Kelch-like ECH associating

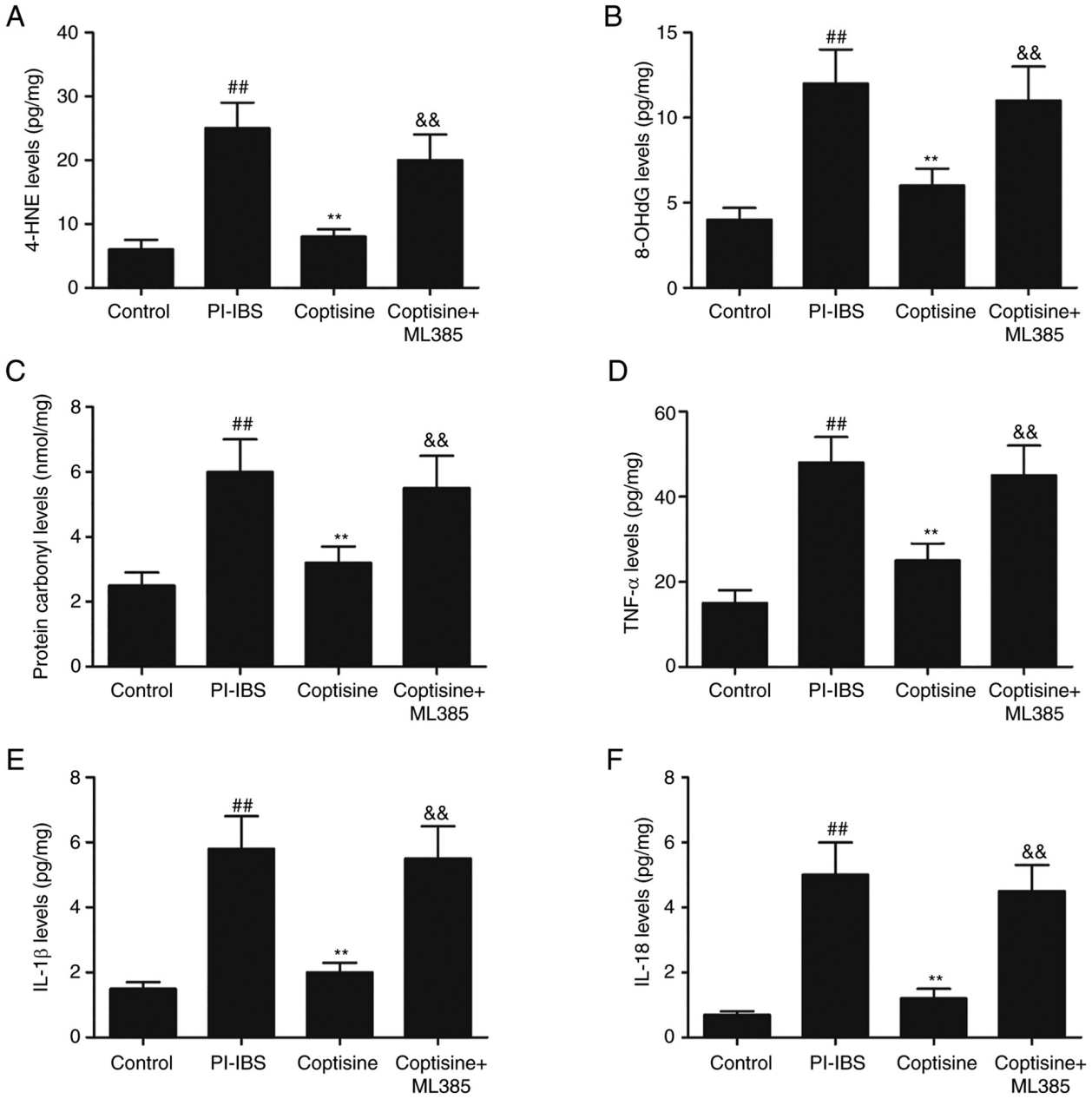

Figure 3. Coptisine suppresses inflammation in PI-IBS rats. Effects of coptisine on the levels of (A) 4-HNE, (B) 8-OHdG, (C) protein carbonyl, (D) TNF-α, (E) IL-1β and (F) IL-18, in the colon as detected via ELISA. $^{\#\#}$P<0.01 vs. control; **P<0.01 vs. PI-IBS; $^{\&\&}$P<0.01 vs. coptisine. 4-HNE, 4-hydroxynonenal; 8-OHdG, 8-hydroxy-2'deoxyguanosine; PI-IBS, post-infectious irritable bowel syndrome.

protein 1 (Keap1). Nrf2, exposed to various insults and stresses, including drugs, free radicals and ROS, dissociates from Keap1 and translocates to the nucleus where it induces HO-1 expression (22). Overexpression of HO-1 can also suppress inflammation. Induction of the inflammatory cascade can cause damage to intestinal epithelial cells and the formation of crypt abscesses, followed by an increase in intestinal permeability (7). Excessive inflammation in the intestine is due to disruption of the intestinal epithelial barrier causing deterioration of the colonic mucus layer, which is recurrent in patients with intestinal disorders (9). It has been reported that coptisine exhibits multiple pharmacological effects, including antioxidative and anti-inflammatory activities (23,24). The LD_{50} value of coptisine has previously been reported as 880.10 mg/kg (25), which indicates that the maximum dose used in the present study included a wide margin of safety. To the best of our knowledge, the present study is the first to demonstrate that coptisine attenuated PI-IBS. The precise mechanisms by which coptisine regulates this process requires further investigation.

The NLR inflammasome is involved in the intestinal inflammation characteristic of PI-IBS (2). NLRP3 is one of the members of the NLR family, which forms the inflammasome following binding to ASC and caspase-1 (26). The activation of caspase-1 promotes the release of proinflammatory cytokines, such as IL-1β, TNF-α and IL-18. In the present study, it was demonstrated that coptisine treatment significantly reduced the protein expression levels of NLRP3, ASC and cleaved caspase-1. Furthermore, coptisine significantly increased the

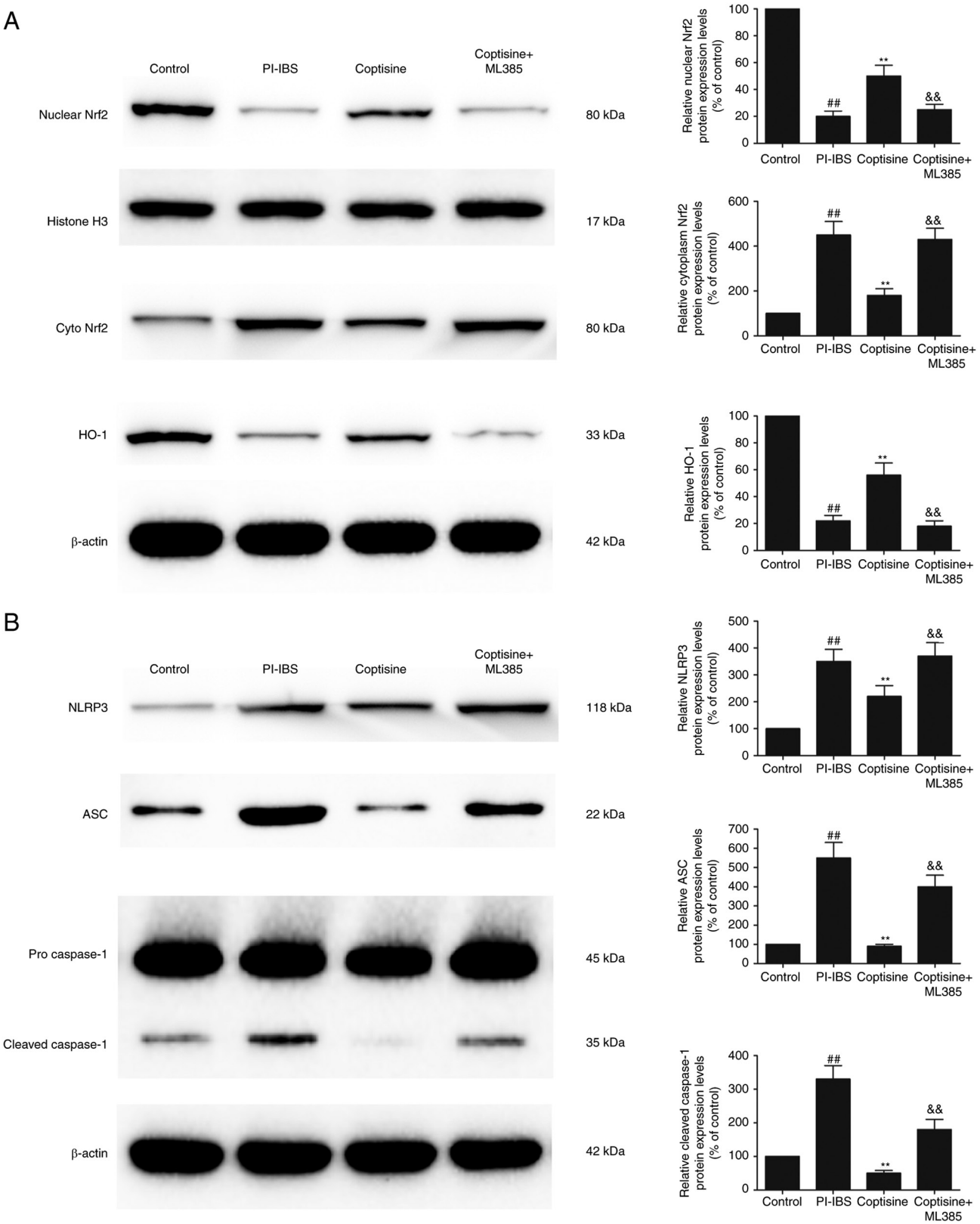

Figure 4. Coptisine attenuates NLRP3 inflammasome activation via Nrf2 signaling. Protein expression levels of (A) nuclear Nrf2, cyto Nrf2 and HO-1, and (B) NLRP3, ASC, pro-caspase-1 and cleaved caspase-1 in the colon tissues of the rats (normalized to the control). $^{\#\#}$P<0.01 vs. control; **P<0.01 vs. PI-IBS; $^{\&\&}$P<0.01 vs. coptisine. NLRP3, NLR family pyrin domain-containing 3; Nrf2, nuclear factor erythroid 2-related factor 2; cyto, cytoplasmic; HO-1, heme oxygenase-1; ASC, apoptosis-associated speck-like protein containing a CARD; PI-IBS, post-infectious irritable bowel syndrome.

nuclear translocation of Nrf2, which potentially significantly induced the upregulation of HO-1 protein expression. All of these coptisine-mediated effects were significantly abolished by the selective Nrf2 inhibitor ML385, which suggested that coptisine potentially blocked the signal transduction of the NLRP3 signaling pathway via the activation of Nrf2. The

modulation of the inflammasome pathway via coptisine markedly reduced the pathological indications of IBS. Therefore, this treatment strategy can be considered a valuable strategy to reduce the development of PI-IBS.

A limitation of the present study was the lack of investigation of the toxicity of coptisine, although no obvious abnormalities were observed in the rats administered with coptisine (50 mg/kg) for 14 days. Another limitation of the present study is that an ML385-only treatment group was not included.

In conclusion, the present study indicated that coptisine potentially exhibited a protective effect on PI-IBS via Nrf2-dependent inhibition of the NLPR3 inflammasome. The resultant protective effects indicated that coptisine may merit consideration for its use as a therapeutic agent in PI-IBS. Furthermore, the results of the present study proposed that coptisine should be explored further for its use as a prophylactic treatment of PI-IBS in humans.

Acknowledgements

Not applicable.

Funding

This work was supported by the International Scientific and Technological Cooperation Projects of Shenzhen Collaborative Innovation Technology Plan (grant no. GJHZ20150316141713255), the Science and Technology Project of Shenzhen (grant nos. JCYJ20180228163012046 and JCYJ20210324103605014) and the Shenzhen Health and Family Planning Commission Scientific Research Project (grant no. 201601021).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YX and LZ contributed to the conception and the design of the study. HW, CC, LJ, JZ and YT contributed to the acquisition, analysis and interpretation of the data. YX contributed to drafting the manuscript. LZ contributed to critical revisions of the intellectual content. YX and LZ confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The protocol used in the present study was approved by the Ethics Committee for Animal Use of The First Affiliated Hospital of Shenzhen University, The Second People's Hospital of Shenzhen (approval no. 20190902002).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Aziz I and Simrén M: The overlap between irritable bowel syndrome and organic gastrointestinal diseases. Lancet Gastroenterol Hepatol 6: 139-148, 2021.
2. Bao CH, Wang CY, Li GN, Yan YL, Wang D, Jin XM, Wu LY, Liu HR, Wang XM, Shi Z and Wu HG: Effect of mild moxibustion on intestinal microbiota and NLRP6 inflammasome signaling in rats with post-inflammatory irritable bowel syndrome. World J Gastroenterol 25: 4696-4714, 2019.
3. Black CJ and Ford AC: Global burden of irritable bowel syndrome: Trends, predictions and risk factors. Nat Rev Gastroenterol Hepatol 17: 473-486, 2020.
4. Klem F, Wadhwa A, Prokop LJ, Sundt WJ, Farrugia G, Camilleri M, Singh S and Grover M: Prevalence, risk factors, and outcomes of irritable bowel syndrome after infectious enteritis: A systematic review and meta-analysis. Gastroenterology 152: 1042-1054.e1, 2017.
5. Tian Z, Zhuang X, Luo M, Yin W and Xiong L: The propionic acid and butyric acid in serum but not in feces are increased in patients with diarrhea-predominant irritable bowel syndrome. BMC Gastroenterol 20: 73, 2020.
6. Yu ZC, Cen YX, Wu BH, Wei C, Xiong F, Li DF, Liu TT, Luo MH, Guo LL, Li YX, *et al*: Berberine prevents stress-induced gut inflammation and visceral hypersensitivity and reduces intestinal motility in rats. World J Gastroenterol 25: 3956-3971, 2019.
7. Scuderi SA, Casili G, Lanza M, Filippone A, Paterniti I, Esposito E and Campolo M: Modulation of NLRP3 inflammasome attenuated inflammatory response associated to diarrhea-predominant irritable bowel syndrome. Biomedicines 8: 519, 2020.
8. Zeng L, Li K, Wei H, Hu J, Jiao L, Yu S and Xiong Y: A Novel EphA2 inhibitor exerts beneficial effects in PI-IBS in vivo and in vitro models via Nrf2 and NF-κB signaling pathways. Front Pharmacol 9: 272, 2018.
9. Gu QY, Zhang J and Feng YC: Role of NLRP3 inflammasome in Bifidobacterium longum-regulated visceral hypersensitivity of postinfectious irritable bowel syndrome. Artif Cells Nanomed Biotechnol 44: 1933-1937, 2016.
10. Liao L, Schneider KM, Galvez EJC, Frissen M, Marschall HU, Su H, Hatting M, Wahlström A, Haybaeck J, Puchas P, *et al*: Intestinal dysbiosis augments liver disease progression via NLRP3 in a murine model of primary sclerosing cholangitis. Gut 68: 1477-1492, 2019.
11. He Y, Hara H and Núñez G: Mechanism and regulation of NLRP3 inflammasome activation. Trends Biochem Sci 41: 1012-1021, 2016.
12. Hennig P, Garstkiewicz M, Grossi S, Di Filippo M, French LE and Beer HD: The Crosstalk between Nrf2 and Inflammasomes. Int J Mol Sci 19: 562, 2018.
13. Arioz BI, Tastan B, Tarakcioglu E, Tufekci KU, Olcum M, Ersoy N, Bagriyanik A, Genc K and Genc S: Melatonin attenuates LPS-Induced acute depressive-like behaviors and microglial NLRP3 Inflammasome activation through the SIRT1/Nrf2 pathway. Front Immunol 10: 1511, 2019.
14. Yang Y, Vong CT, Zeng S, Gao C, Chen Z, Fu C, Wang S, Zou L, Wang A and Wang Y: Tracking evidences of Coptis chinensis for the treatment of inflammatory bowel disease from pharmacological, pharmacokinetic to clinical studies. J Ethnopharmacol 268: 113573, 2021.
15. Song D, Hao J and Fan D: Biological properties and clinical applications of berberine. Front Med 14: 564-582, 2020.
16. Wu J, Luo Y, Deng D, Su S, Li S, Xiang L, Hu Y, Wang P and Meng X: Coptisine from Coptis chinensis exerts diverse beneficial properties: A concise review. J Cell Mol Med 23: 7946-7960, 2019.
17. Wu J, Luo Y, Jiang Q, Li S, Huang W, Xiang L, Liu D, Hu Y, Wang P, Lu X, *et al*: Coptisine from Coptis chinensis blocks NLRP3 inflammasome activation by inhibiting caspase-1. Pharmacol Res 147: 104348, 2019.
18. Adriani A, Ribaldone DG, Astegiano M, Durazzo M, Saracco GM and Pellicano R: Irritable bowel syndrome: The clinical approach. Panminerva Med 60: 213-222, 2018.

19. Ghoshal UC and Gwee KA: Post-infectious IBS, tropical sprue and small intestinal bacterial overgrowth: The missing link. Nat Rev Gastroenterol Hepatol 14: 435-441, 2017.
20. Xiong Y, Li KX, Wei H, Jiao L, Yu SY and Zeng L: Eph/ephrin signalling serves a bidirectional role in lipopolysaccharide-induced intestinal injury. Mol Med Rep 18: 2171-2181, 2018.
21. Forman HJ and Zhang H: Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. Nat Rev Drug Discov 20: 689-709, 2021.
22. Puentes-Pardo JD, Moreno-SanJuan S, Carazo Á and León J: Heme Oxygenase-1 in gastrointestinal tract health and disease. Antioxidants (Basel) 9: 1214, 2020.
23. Feng M, Kong SZ, Wang ZX, He K, Zou ZY, Hu YR, Ma H, Li XG and Ye XL: The protective effect of coptisine on experimental atherosclerosis ApoE$^{-/-}$ mice is mediated by MAPK/NF-κB-dependent pathway. Biomed Pharmacother 93: 721-729, 2017.
24. Hu YR, Ma H, Zou ZY, He K, Xiao YB, Wang Y, Feng M, Ye XL and Li XG: Activation of Akt and JNK/Nrf2/NQO1 pathway contributes to the protective effect of coptisine against AAPH-induced oxidative stress. Biomed Pharmacother 85: 313-322, 2017.
25. He K, Ye X, Wu H, Wang Y, Zou Z, Ning N, Hu Y, Chen B, Fang X and Li X: The safety and anti-hypercholesterolemic effect of coptisine in Syrian golden hamsters. Lipids 50: 185-194, 2015.
26. Mangan MSJ, Olhava EJ, Roush WR, Seidel HM, Glick GD and Latz E: Targeting the NLRP3 inflammasome in inflammatory diseases. Nat Rev Drug Discov 17: 588-606, 2018.