Drug Design, Development and Therapy

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Therapeutic Effect of *Brucea Javanica* Oil Emulsion in Mice with Irinotecan-Induced Delayed Diarrhea

Zixuan Lai[1,2], Yong Zhang[1], Xiaoxia Hu[1], Li Chen[1], Weimu Huang[1], Juan Wang[1], Baoyi Chen[3], Mihong Ren[1,2], Bowen Yang[1], Ziren Su[1,2], Jiannan Chen[1,2], Jianhui Xie[4,5], Zhengquan Lai[6], Youliang Xie[1,2,5]

[1]School of Pharmaceutical Sciences, Guangzhou University of Chinese Medicine, Guangzhou, 510006, People's Republic of China; [2]Dongguan Institute of Guangzhou University of Chinese Medicine, Dongguan, 523808, People's Republic of China; [3]Dongguan Hospital, Guangzhou University of Chinese Medicine, Dongguan, 523127, People's Republic of China; [4]State Key Laboratory of Traditional Chinese Medicine Syndrome, State Key Laboratory of Dampness Syndrome of Chinese Medicine, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, 510120, People's Republic of China; [5]Guangdong Provincial Key Laboratory of Clinical Research on Traditional Chinese Medicine Syndrome, Guangzhou, 510120, People's Republic of China; [6]Department of Pharmacy, Shenzhen University General Hospital/Shenzhen University Clinical Medical Academy, Shenzhen University, Shenzhen, 518055, People's Republic of China

Correspondence: Youliang Xie, School of Pharmaceutical Sciences, Guangzhou University of Chinese Medicine, Guangzhou, 510006, People's Republic of China, Email xieyl@gzucm.edu.cn; Zhengquan Lai, Department of Pharmacy, Shenzhen University General Hospital/Shenzhen University Clinical Medical Academy, Shenzhen University, Shenzhen, 518055, People's Republic of China, Email cruise0303@163.com

Background: Chemotherapy-induced diarrhea (CID), particularly delayed diarrhea, often limits clinical use. *Brucea javanica* oil emulsion (BJOE), an adjuvant chemotherapy agent, has been shown to reduce irinotecan-related gastrointestinal side effects. However, its underlying molecular mechanism remains unclear. The cGAS-STING pathway, composed of the cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS) and the adaptor protein stimulator of interferon genes (STING), plays an essential role in delayed diarrhea. This work aimed to investigate the therapeutic potential and underlying mechanism of BJOE on irinotecan-induced delayed diarrhea.

Methods: Gas chromatography-mass spectrometry (GC-MS) was employed to explore the components of BJOE. Macro-observation, histology, PCR, immunohistochemistry, and Western blotting were performed to illuminate the potential mechanism of BJOE on irinotecan-induced delayed diarrhea mice model.

Results: GC-MS analysis identified linoleic acid (20.67%) as BJOE's main component. BJOE effectively mitigated irinotecan-induced delayed diarrhea in mice, as characterized by attenuation of weight loss, colon shortening, hematochezia, and histopathologic damage. It significantly inhibited the mRNA expression levels of inflammatory mediators TNF-α, IL-1β, IL-6, and iNOS, and upregulated barrier gene expression (ZO-1 and occludin). Furthermore, BJOE markedly enhanced mucin production, and increased PCNA protein expression. Concurrently, BJOE remarkably down-regulated the colonic mRNA levels of cGAS, STING, CXCL10, CCL5, and IFN-β. Activation of the cGAS-STING pathway with agonist DMXAA significantly reduced BJOE's therapeutic, anti-inflammatory, and barrier-protective effects. Similarly, stimulating STING substantially reversed BJOE's inhibition on cGAS-STING pathway.

Conclusion: BJOE effectively mitigated inflammation and preserved intestinal barrier function, at least partially, via inhibiting cGAS-STING pathway in irinotecan-induced delayed diarrhea. Active components, long-term safety and pharmacokinetics studies were warranted to facilitate translational application.

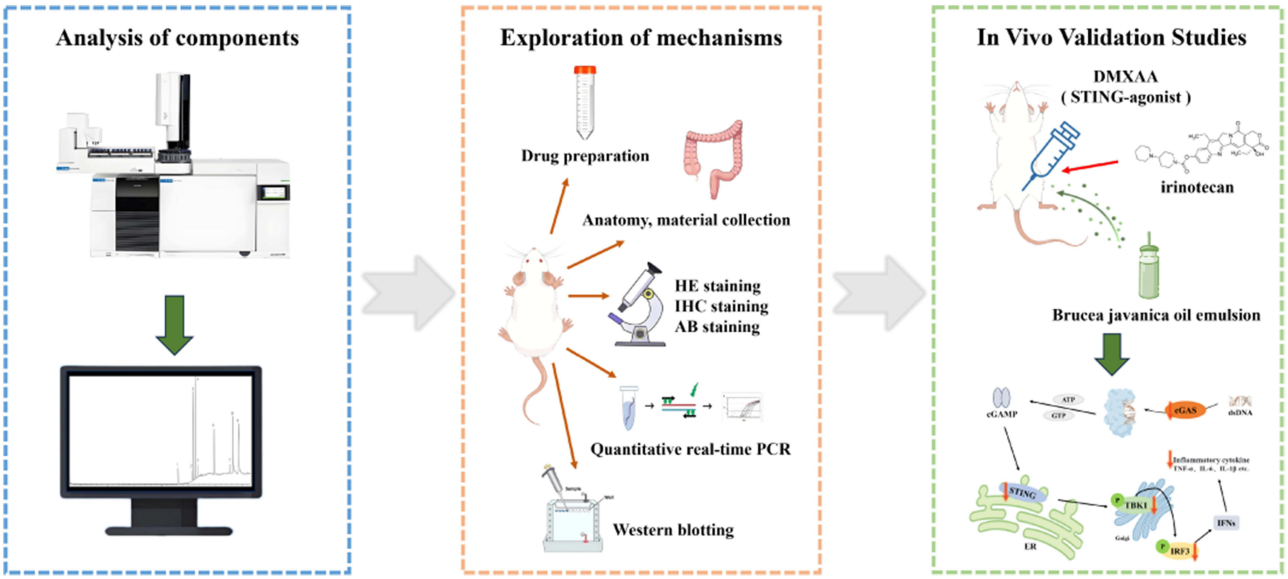
Keywords: *brucea javanica* oil emulsion, chemotherapy, irinotecan, delayed diarrhea, cGAS-STING pathway

Introduction

Chemotherapy can improve patient survival, and is currently essential for cancer treatment. However, its adverse effects, particularly chemotherapy-induced diarrhea (CID), can severely impact quality of life, often leading to premature discontinuation and reduced survival.[1] Clinically, irinotecan is an important chemotherapy drug commonly used for solid tumors such as lung, gastric, cervical, and ovarian cancers.[2] However, irinotecan severely damages the proliferative mucosal lining of the

Received: 23 February 2025
Accepted: 10 June 2025
Published: 24 June 2025

Graphical Abstract



digestive tract, leading to intestinal inflammation, ulceration, impaired absorption, mucosal barrier dysfunction, and heightened immune suppression.[3] Delayed diarrhea is a significant adverse reaction associated with irinotecan, with an incidence ranging from 60% to 90% and severe diarrhea (grade 3–4) occurring in 28% to 40% of cases.[4] The primary medications used clinically to treat irinotecan-induced diarrhea are loperamide and octreotide.[5] However, available therapies often come with various adverse effects. Consequently, developing drugs that do not compromise the anticancer efficacy of irinotecan while alleviating its major adverse effect delayed diarrhea, is a pressing concern.

Brucea javanica oil emulsion (BJOE), an approved and widely marketed formulation, represents the primary application of *Brucea javanica* as an anti-inflammatory and anti-dysentery[6,7] agent. Various studies have indicated that BJOE has low toxicity in clinical application and exhibits numerous bioactive activities, including anti-inflammatory, antimalarial, and antitumor effects.[8] In clinical practice, BJOE is primarily used in conjunction with chemotherapeutic agents such as irinotecan and docetaxel[9] to enhance their anticancer effects, improve safety and efficacy, and increase treatment compliance among oncology patients.[10] Notably, it has been shown to significantly increase the disease remission rate, protect immune function, and improve the quality of survival for patients with brain metastases, while also significantly reduce the incidence of gastrointestinal reactions.[11] Our preceding studies have shown that *Brucea javanica* and its active ingredient exhibit anti-inflammatory and anti-inflammatory bowel disease (IBD) effects,[12] supporting its traditional use in treating dysentery. Our previous work has also reported that *Brucea javanica* oil effectively ameliorated symptoms of weight loss and diarrhea, increased food intake, and alleviated intestinal mucosal damage in mice with 5-fluorouracil-induced intestinal mucosal injury.[13] Therefore, we hypothesized that BJOE might exert therapeutic effect on irinotecan-induced delayed diarrhea.

It has been shown that damaged double-stranded DNA (dsDNA) induced by irinotecan accumulates and releases in the cytoplasm, leading to abnormal activation of the cGAS-STING pathway. This subsequently triggers the activation and production of a series of downstream immune cells and inflammatory factors, thereby promoting the inflammatory response in the organism.[14] Irinotecan, derived from the plant alkaloid camptothecin, is a topoisomerase I (Topo1) inhibitor that forms a Topo1-drug-DNA complex, preventing DNA reattachment and resulting in double-strand breaks. Cyclic GMP-AMP synthase (cGAS) acts as both a biosynthetic enzyme and an intracellular DNA receptor, recognizing aberrant double-stranded DNA (dsDNA) and catalyzing the generation of second messengers to activate the adaptor molecule stimulator of interferon genes (STING). Activated STING subsequently triggers downstream signalling pathways, including TANK-binding kinase 1 (TBK1)

and interferon regulatory factor 3 (IRF3), leading to the activation and production of various immune cells and inflammatory factors, thereby promoting the inflammatory response in the body.

The properly functioning cGAS-STING signalling pathway is crucial for maintaining gut homeostasis. However, its overactivation is associated with irinotecan-induced delayed diarrhea.[15] This indicated that abnormal activation of the cGAS-STING signalling might constitute an important pathological basis for delayed diarrhea in the context of irinotecan treatment. Given its critical role in innate immunity, targeting this pathway presents a promising strategy to alleviate inflammation and maintain intestinal barrier integrity. To date, the therapeutic efficacy of BJOE in mitigating irinotecan-associated delayed diarrhea has not been systematically examined, nor has its mechanistic inhibition of the cGAS–STING pathway been characterized in this context. Therefore, in this study, we investigated the therapeutic effect and underlying mechanism of BJOE in irinotecan-induced delayed diarrhea mice model. Results indicated that BJOE demonstrated partial efficacy in alleviating irinotecan-induced delayed diarrhea by protecting the intestinal barrier and reducing inflammation via inhibition of aberrant cGAS-STING pathway activation.

This study was the pioneering endeavor to explore the therapeutic potential of BJOE in irinotecan-induced delayed diarrhea and also the first time to indicate the inhibitory effect of BJOE on the cGAS-STING pathway. These findings are expected to provide further support for the traditional use of *Brucea javanica* in the treatment of diarrhea, and add new therapeutic dimension to its current clinical application as an anti-cancer adjunct, simultaneously endowed with beneficial effect in alleviating irinotecan-associated delayed diarrhea.

Materials and Methods

Chemicals and Reagents

BJOE was obtained from Tonghua Renmin Pharmaceutical Co., Ltd. (Jilin, China), which has been approved by National Medical Products Administration for marketing (230601036). Irinotecan (purity ≥ 98%) was purchased from Dalian Meilun Biotechnology Co., Ltd. (Liaoning, China), and loperamide was sourced from Xi'an Janssen Pharmaceutical Co., Ltd. (Shanxi, China). Vadimezan (DMXAA, purity ≥ 98%) was acquired from KKL MED (Ashland, USA). Rabbit anti-cGAS pAb (A8335) and rabbit anti-STING/TMEM173 mAb (A21051) were obtained from ABclonal (Wuhan, China). Rabbit anti-NAK/TBK1 (phospho-Ser172) mAb (14259) and rabbit anti-TBK1 mAb (52074) were obtained from Signalway Antibody (Maryland, USA). IRF3 recombinant rabbit monoclonal antibody (ET1612-14) was acquired from Huabio (Zhejiang, China). Rabbit anti-phospho-IRF3 (Ser396) (AF2436) was obtained from Affinity Biosciences (Jiangsu, China).

Gas Chromatography-Mass Spectrometry Analysis

The total composition of BJOE was analyzed by gas chromatography-mass spectrometry (GC-MS) after derivatization. Using helium as the carrier gas, the test solution was injected into a DB-WAX strong polarity column (30 cm × 250 μm, 0.25 μm, Agilent) at a flow rate of 1 mL/min with a split ratio of 1:10. The ion source temperature was set to 220°C, interface temperature to 250°C, and injection temperature to 250°C, with an injection volume of 1 μL. The column temperature was maintained at 100°C for 10 min, then ramped to 180°C at 10°C/min and held for 5 min, followed by an increase to 250°C at 20°C/min, where it was held for 10 min.

Animals and Treatments

BALB/c mice (weighed 22–25 g, male) were provided by Guangzhou Rigel Biotechnology Co., Ltd. (SCXK (Guangdong) 2023–0059). Mice were maintained under standard laboratory conditions (temperature: 22°C ± 2°C; humidity: 50% ± 10%; 12 h dark cycle). They were fed a standard diet and provided free access to water for one week to acclimatize to the environment before the experiment. All experimental procedures were approved by the Animal Ethics Committee of Guangzhou University of Chinese Medicine (Approval No. 20231211006). All procedures complied with international animal welfare guidelines, and all personnel received mandatory training to minimize animal suffering.

After one week of acclimatization, 96 BALB/c mice were randomly divided into eight groups (n = 12 for each group): normal group, model group (irinotecan, 45 mg/kg/day, days 1–4, administered intraperitoneally), positive control group treated with loperamide (LO, 4 mg/kg/day, days 1–7, orally), and three groups orally receiving low, medium, and high doses of BJOE (0.25, 0.5, and 1.0 g/kg/day, days 1–7). The dose was administered as previously described with minor modifications.[13,15] Additionally, there was a STING agonist group (DMXAA, 10 mg/kg/day, administered intraperitoneally every other day) and a DMXAA + BJOE combination group. Mice in the normal group received saline injection and blank emulsion orally. The remaining groups were treated with irinotecan to establish the delayed diarrhea model. Mice were allowed free access to water and food, while the corresponding doses of the positive control, BJOE, and agonist were administered via gavage for seven consecutive days. During this process, water and food intake, body weight, faecal output, and general behaviour of mice were recorded. After the final gavage, mice were fasted but watered for 12 h, followed by cervical dislocation for euthanasia and tissue and blood collection.

Disease Activity Index

Body weight, faecal pattern, and faecal occult blood were measured daily. The scoring criteria followed the previous report,[16] where the disease activity index (DAI) was calculated as follows: DAI = (weight change score + blood stool score + dilute stool score) / 3. The DAI score was classified according to the criteria outlined in Table 1.

Hematoxylin and Eosin (H&E) Staining

Immediately following euthanasia, colonic tissues were excised and placed in 4% paraformaldehyde fixative for 24 h. Subsequently, the tissues were embedded in paraffin, and sections (5 μm thick) were deparaffinized and stained according to standard operating protocols. Hematoxylin and eosin (H&E) staining of the colonic sections was then observed under a microscope and scored by two independent and blinded researchers.[17] The scoring was conducted according to the criteria specified in Table 2.

Alcian Blue Staining

The colon tissue was taken, fixed, dehydrated, and embedded in paraffin according to standard protocols. The resulting sections were sequentially placed in wax solution and ethanol before being rinsed with tap water. Subsequently, the

Table 1 Disease Activity Index (DAI)

Score	Weight loss (%)	Stool Consistency	Hemorrhage
0	0	Normal	None
1	1–5	Mild soft	Slight bleeding
2	5–10	Wet and soft	Moderate bleeding
3	10–20	Semi-loose stools	Severe bleeding
4	>20	Loose stools	Blood clot around the anus

Table 2 Histological Colitis Score

Score	Degree of Inflammatory Infiltration	Degree of Tissue Damage
0	No inflammatory cells	No damage
1	Infiltration around the base of the crypt	Goblet cell depletion
2	Infiltration into the mucosa	Extensive goblet cell depletion
3	Extensive infiltration into the mucosa	Crypts depletion
4	Infiltration within the submucosa	Extensive crypts depletion

sections were stained with alcian blue staining solution for 10–15 min, followed by another wash with tap water to facilitate nuclear staining. The slices were then dehydrated in anhydrous ethanol and xylene, sealed with neutral gum, and examined microscopically under a light microscope for imaging.

Immunohistochemical Staining

Paraffin-embedded sections of colon tissue were dewaxed and rehydrated, then incubated in citrate antigen retrieval buffer (pH 6.0) for antigen retrieval. Following this, endogenous peroxidase activity was quenched, and the sections were blocked with 3% BSA. Primary and secondary antibodies were then applied sequentially according to the experimental protocol. Subsequently, the sections were stained using the DAB (diaminobenzidine) method, followed by hematoxylin staining of the cell nuclei for 3 min. Immunohistochemical staining was performed to assess the PCNA expression, and images were captured under light microscopy by a blinded technician, with positive cells identified by the presence of brown granules.

RNA Extraction and Quantitative Real-Time PCR

Total RNA was extracted from mouse colon tissues using TRIzol reagent (Vazyme, R401-01). Complementary DNA (cDNA) was synthesized via reverse transcription of RNA using a reverse transcription kit (Vazyme, R223-01). The amplification procedure was subsequently performed according to the manufacturer's protocol. Primer design was conducted using Oligo 7 software (Molecular Biology Insights, Inc., Colorado Springs, USA) based on sequences from GenBank, with the synthesized primers provided by Sangon Bioengineering (Shanghai, China) Co., Ltd. as detailed in Table 3.

Western Blotting

Colon tissues were sheared and rinsed with PBS, followed by lysis using RIPA lysis buffer. The samples were centrifuged at 14,000 rpm for 20 min at 4°C, and the supernatant was collected for protein quantification using the BCA method. Each sample was prepared with 40 μg of protein and subjected to separation on a 10% SDS-PAGE gel, after which the proteins were transferred to a PVDF membrane. The membrane was blocked with 5% skim milk powder at room temperature for 1 h. Following washing with TBST, the diluted primary antibody was added and incubated

Table 3 Primer Sequences for Real-Time PCR

Gene	Forward (5'-3')	Reverse (5'-3')
TNF-α	GCCTCTTCTCATTCCTGCTTGTGG	GTGGTTTGTGAGTGTGAGGGTCTG
IL-1β	TCGCAGCAGCACATCAACAAGAG	AGGTCCACGGGAAAGACACAGG
IL-6	CTTCTTGGGACTGATGCTGGTGAC	TCTGTTGGGAGTGGTATCCTCTGTG
iNOS	ACTCAGCCAAGCCCTCACCTAC	TCCAATCTCTGCCTATCCGTCTCG
Occludin	TGGCTATGGAGGCGGCTATGG	AAGGAAGCGATGAAGCAGAAGGC
ZO-1	GCGAACAGAAGGAGCGAGAAGAG	GTGGGCTTTGCGGGCTGAC
cGAS	ACGGGGACACCACGGAGAAG	CCGAGGGCTGTGCATCTTTGG
STING	TGCCATGTCCAGTCCAGGTAC	AAGACGATAGTAGAATCATAGCCATACAG
CXCL10	TGCCTCATCCTGCTGGGTCTG	TCCCTATGGCCCTCATTCTCACTG
CCL5	GACACCACTCCCTGCTGCTTTG	CTCTGGGTTGGCACACACTTGG
IFN-β	GCGTTCCTGCTGTGCTTCTCC	TGAAGTCCGCCCTGTAGGTGAG
β-actin	GATGGTGGGAATGGGTCAGAAGG	TTGTAGAAGGTGTGGTGCCAGATC

https://doi.org/10.2147/DDDT.S517973

overnight at 4°C, using β-actin (1:5000) as the internal reference. The next day, the membrane was washed with TBST, and an HRP-conjugated secondary antibody (1:10,000) was applied, followed by 1 h incubation at room temperature. After further washing with TBST, ECL chemiluminescent solution was added for detection. Images were captured using a gel imaging system (Tanon 4200SF, China), and protein bands were analyzed with ImageJ (National Institutes of Health, Maryland, USA). The expression levels of cGAS, STING, p-TBK1, TBK1, p-IRF3, IRF3 were evaluated in the colonic tissues of each experimental group.

Statistical Analysis

All data were presented as mean ± standard deviation (SD) and statistically analyzed using SPSS software version 26.0 (Chicago, USA). To ensure reliability, each independent experiment was replicated a minimum of three times. The experimental data were tested for normal distribution. If they met the criteria, one-way ANOVA was employed to compare differences between groups. If the data did not conform to normal distribution, the Kruskal–Wallis test was utilized. For homogeneous variance ($P > 0.05$), post hoc comparisons were conducted using the Bonferroni method; conversely, if the variance was heterogeneous ($P < 0.05$), the Dunn's T3 test was applied. A P-value < 0.05 was considered statistically significant, while a P-value < 0.01 was considered very significant.

Results

The Components of BJOE

The total components of BJOE were analyzed using GC-MS. As shown in Figure 1, nine main components were identified as: hexadecanoic acid ($C_{17}H_{34}O_2$, 5.13%), methyl stearate ($C_{19}H_{38}O_2$, 2.27%), 11-octadecenoic acid ($C_{19}H_{36}O_2$, 19.16%), linoleic acid ($C_{19}H_{34}O_2$, 20.67%), 9,12,15-octadecatrienoic acid ($C_{19}H_{32}O_2$, 1.88%), n-hexadecanoic acid ($C_{16}H_{32}O_2$, 8.35%), octadecanoic acid ($C_{18}H_{36}O_2$, 19.16%), oleic acid ($C_{18}H_{34}O_2$, 8.35%), and 9,12-octadecadienoic acid ($C_{18}H_{32}O_2$, 18.79%) (Table 4). Among the nine components detected, linoleic acid was identified as the major component, which was in concert with previous investigation.[18]

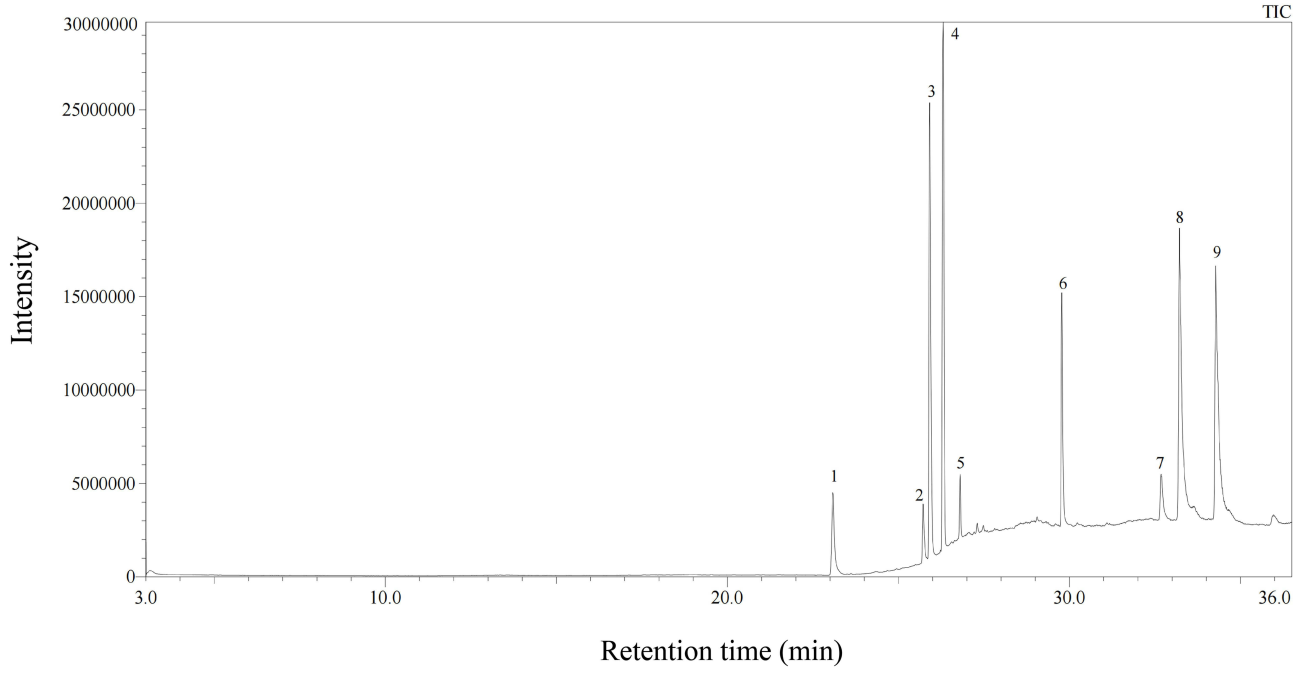


Figure 1 BJOE was subjected to GC-MS analysis to identify its major chemical constituents. The percentages of the nine ingredients in BJOE were hexadecanoic acid ($C_{17}H_{34}O_2$, 5.13%), methyl stearate ($C_{19}H_{38}O_2$, 2.27%), 11-octadecenoic acid ($C_{19}H_{36}O_2$, 19.16%), linoleic acid ($C_{19}H_{34}O_2$, 20.67%), 9,12,15-octadecatrienoic acid ($C_{19}H_{32}O_2$, 1.88%), n-hexadecanoic acid ($C_{16}H_{32}O_2$, 8.35%), octadecanoic acid ($C_{18}H_{36}O_2$, 19.16%), oleic acid ($C_{18}H_{34}O_2$, 8.35%), and 9,12-octadecadienoic acid ($C_{18}H_{32}O_2$, 18.79%), respectively.

Table 4 The Composition of BJOE

	Composition	Percentage (%)	Retention Time (min)
1	Hexadecanoic acid	5.13	23.085
2	Methyl stearate	2.27	25.721
3	11-Octadecenoic acid	19.16	25.913
4	Linoleic acid	20.67	26.306
5	9,12,15-Octadecatrienoic acid	1.88	26.805
6	N-Hexadecanoic acid	8.35	29.775
7	Octadecanoic acid	19.16	32.679
8	Oleic acid	8.35	33,216
9	9,12-Octadecadienoic acid	18.79	34.276

BJOE Mitigated Enterotoxicity in Mice Experiencing Delayed Diarrhea

Irinotecan was administered to BALB/c mice via intraperitoneal injection for 4 consecutive days, while BJOE and loperamide were administered in each dose group from days 1 to 7 (Figure 2a). As shown in Figure 2b, the rate of body weight change in the model group declined progressively, with a noticeable deceleration in this decline observed following the administration of BJOE and loperamide. The DAI scores for each group are depicted in Figure 2c Mice in the model group exhibited symptoms of perianal soiling and bloody stools. However, those treated with BJOE had significantly lower DAI scores compared to the model group ($P < 0.01$).

Furthermore, Figure 2d–e showed that a marked reduction was observed in colon length following modelling. While the high-dose BJOE group exhibited a notable recovery in colon length relative to the model group ($P < 0.01$). In the normal group, the colon maintained structural integrity, with intact mucosa, closely arranged and orderly crypts (glands) with no atrophy, and minimal inflammatory cell infiltration in the lamina propria. In contrast, the model group displayed pronounced necrosis in the intestinal tissues of both the mucosal and epithelial layers, along with significant cellular detachment, disrupted intestinal villi, disordered crypt arrangements, and extensive inflammatory cell infiltration within the lamina propria, indicative of successful establishment of delayed diarrhea mouse model ($P < 0.01$). Nevertheless, administration of BJOE and loperamide resulted in varying degrees of recovery, characterized by a reduction in inflammatory infiltration and a restoration of glandular architecture ($P < 0.01$). Notably, the high-dose BJOE group showed recovery levels comparable to those in the positive control group (Figure 2f–g). These findings collectively suggested that BJOE exerted beneficial effect in ameliorating irinotecan-induced delayed diarrhea.

BJOE Mitigated Inflammation and Preserved Intestinal Barrier Function in Mice with Delayed Diarrhea

To investigate whether BJOE could reduce intestinal inflammation and protect the intestinal barrier, we measured the mRNA expression levels of *TNF-α, IL-1β, IL-6*, and *iNOS* in mouse colon tissues. As shown in Figure 3a, the mRNA expression levels of *TNF-α, IL-1β, IL-6*, and *iNOS* were significantly elevated in the model group ($P < 0.01$). In contrast, administration of varying doses of BJOE and loperamide resulted in a significant reduction in these inflammatory markers ($P < 0.01$).

Intestinal mucin expression in mice with delayed diarrhea was assessed using alcian blue staining. Mucins were almost absent in the colons of the model group, while varying degrees of restoration were observed in both the BJOE-treated and positive control groups (Figure 3b). The expression of PCNA in colonic tissues, indicative of the level of intestinal proliferation, was also restored following drug administration (Figure 3c).

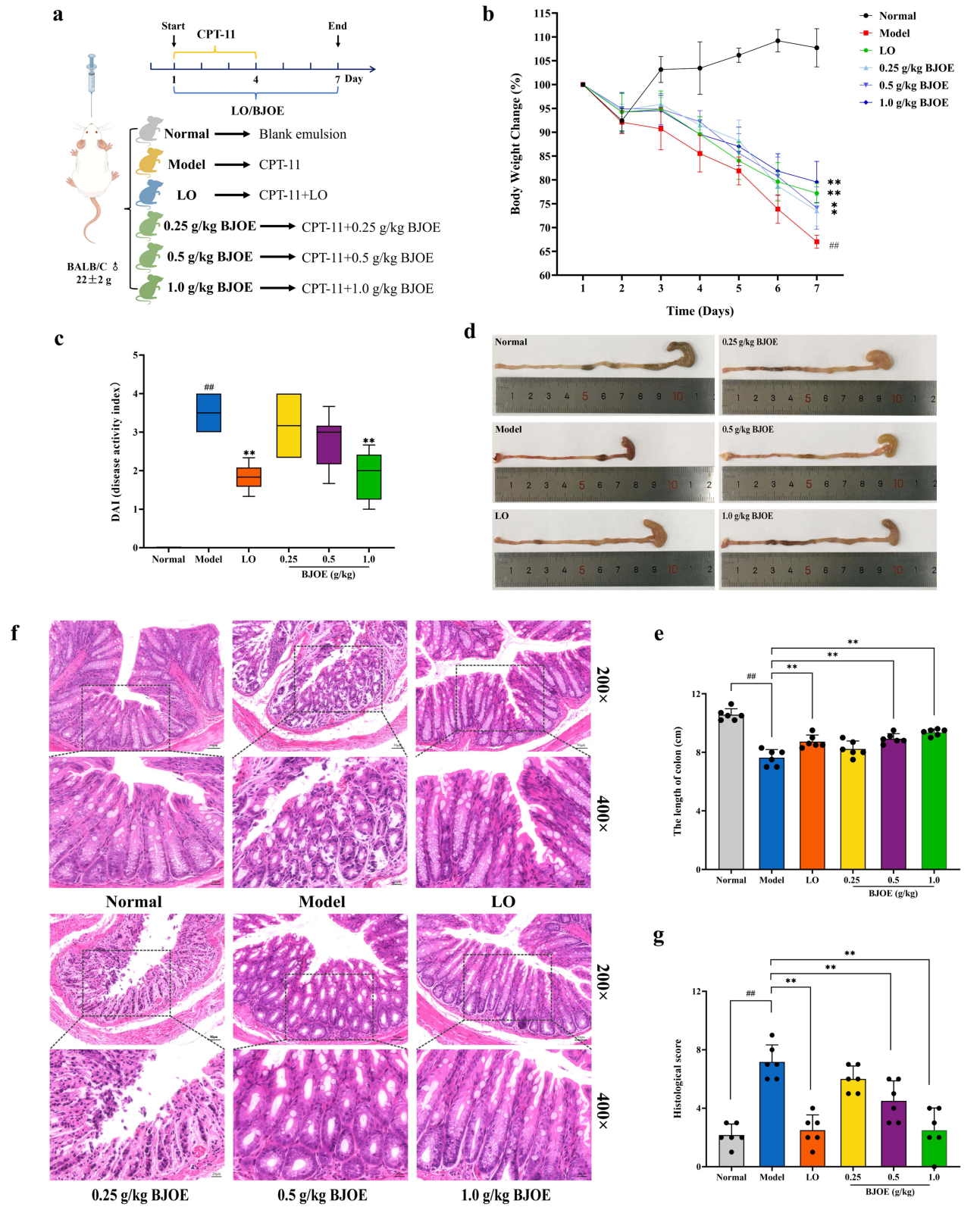


Figure 2 Effect of BJOE on symptoms of mice with delayed diarrhea. (**a**) Schematic of animal experimental protocol. (**b**) Rate of change in body weight of mice. (**c**) DAI score. (**d–e**) Length of mouse colon. (**f–g**) HE staining and scoring of mouse colon tissue (200× and 400×). Data are expressed as mean ± SD (n = 6). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with the normal group. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the model group.

https://doi.org/10.2147/DDDT.S517973

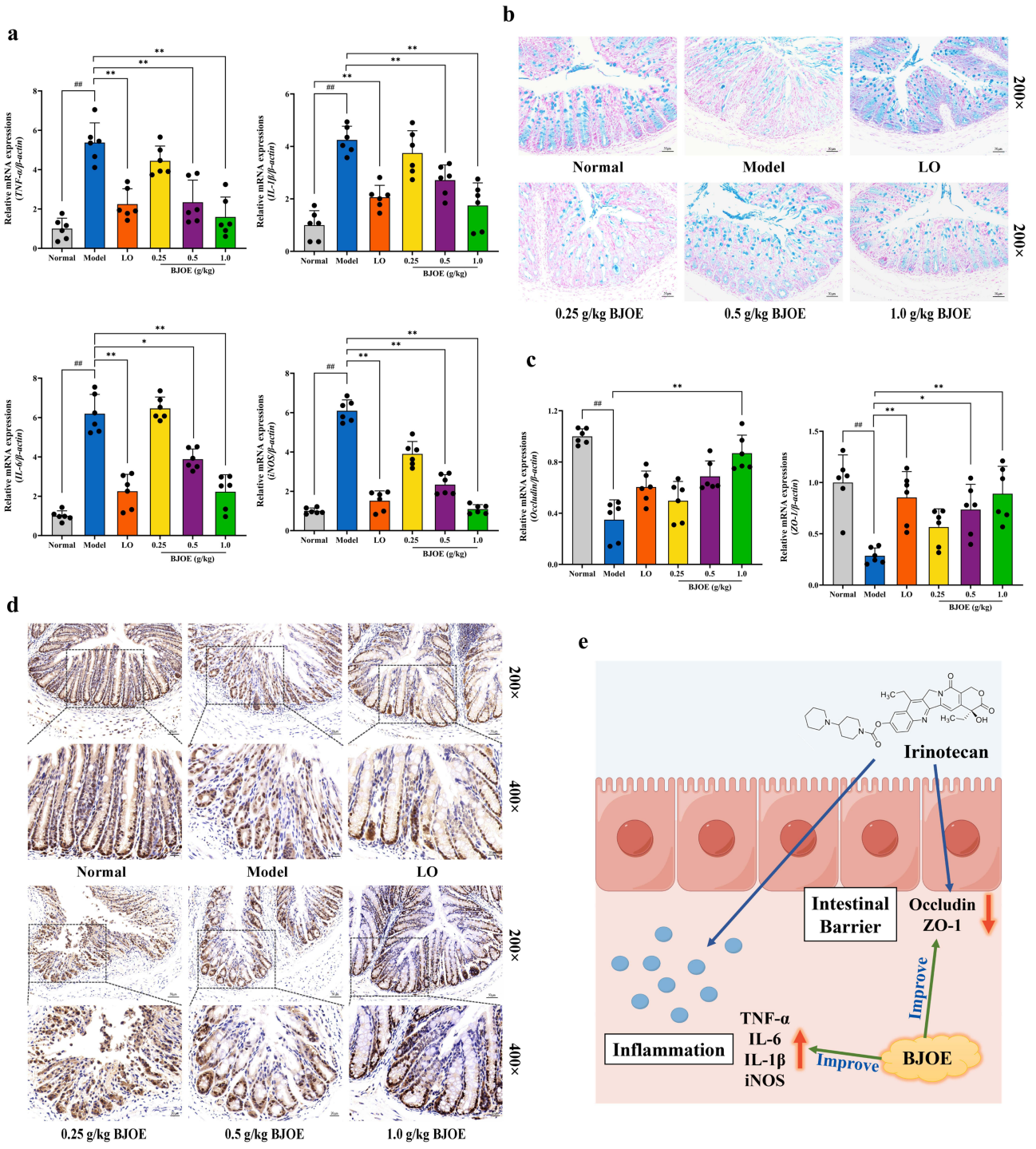


Figure 3 Effect of BJOE on inflammation level and intestinal barrier in mice with delayed diarrhea. (**a**) mRNA expression levels of *TNF-α*, *IL-1β*, *IL-6*, and *iNOS* by quantitative real-time PCR (n = 6). (**b**) Representative graphs of alcian blue staining in mice of each group (200×). (**c**) mRNA expression levels of *occludin* and *ZO-1* detected by quantitative real-time PCR (n = 6). (**d**) Immunohistochemical staining to detect PCNA expression (200× and 400×). (**e**) Disruption of the intestinal barrier and inflammatory response induced by irinotecan. Data are expressed as mean ± SD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with the normal group. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the model group.

Additionally, the key tight junction genes, *occludin* and *ZO-1*, were evaluated using quantitative PCR to further assess the ameliorative effect of BJOE on irinotecan-induced delayed diarrhea. As depicted in Figure 3d, the mRNA expression of *occludin* and *ZO-1* was significantly downregulated in irinotecan-treated mice compared to the normal group. Notably, the barrier protein expression was upregulated following administration of three doses of BJOE and loperamide. The high-dose BJOE group displayed significant increases in both *occludin* and *ZO-1* levels ($P < 0.01$). These results

https://doi.org/10.2147/DDDT.S517973

suggested that BJOE effectively downregulated the intestinal inflammation and protected the intestinal barrier in mice experiencing delayed diarrhea (Figure 3e).

Effect of BJOE on cGAS-STING Pathway in Mice with Delayed Diarrhea

RNA and protein expression levels of cGAS-STING pathway-related factors were assessed in the colonic tissues of mice across different groups using PCR and Western blotting. (Figure 4a). The mRNA expression levels of cGAS-STING pathway-related genes including *cGAS, STING, CXCL10, CCL5*, and *IFN-β* in the colon tissues across the experimental groups are depicted in Figure 4b. Compared to the model group, treatment with BJOE significantly downregulated the mRNA expression of *cGAS, STING, CXCL10, CCL5*, and *IFN-β* in colonic tissues ($P < 0.01$). Furthermore, while the levels of cGAS-STING pathway-related proteins were significantly elevated after modelling ($P < 0.05$). However, these proteins were significantly downregulated following BJOE administration ($P < 0.05$). These findings suggested that BJOE effectively inhibited the activation of the cGAS-STING signalling pathway (Figure 4c and d).

cGAS-STING Pathway Activation Aggravated Intestinal Toxicity in Mice with Delayed Diarrhea

Based on these experiments, we employed DMXAA, a STING agonist, to verify whether BJOE alleviated irinotecan-induced delayed diarrhea by modulating the cGAS-STING signalling pathway. The experimental design for each group of animals was depicted in Figure 5a. As illustrated in Figure 5b–g, both the DMXAA and model groups exhibited a continuous decline in body weight, significantly elevated DAI scores, and markedly shortening of colon length ($P < 0.01$). Histopathological analysis revealed the loss of crypts in the model group, accompanied by extensive inflammatory cell infiltration in the lamina propria. Notably, colonic tissue damage was exacerbated in the DMXAA group. However, the BJOE treatment group exhibited substantial recovery of colonic architecture ($P < 0.01$). In contrast, the therapeutic effect of BJOE was markedly diminished in the DMXAA+BJOE group ($P < 0.05$).

cGAS-STING Pathway Activation Influenced Inflammation and Intestinal Barrier in Mice with Delayed Diarrhea

We further assessed the levels of inflammatory factors, namely *TNF-α, IL-1β, IL-6*, and *iNOS*, in the colonic tissues of mice across different groups. The model and agonist groups exhibited a significant elevation in inflammation. However, the BJOE-treated group displayed a marked reduction in these inflammatory markers compared to the model group. Notably, this improvement was attenuated in the DMXAA+BJOE group (Figure 6a). The expression of intestinal mucins in mice was evaluated using alcian blue staining. Mucin levels were nearly absent in the model and DMXAA groups, while significant improvement was observed in the BJOE group. Improvement in mucin expression in the DMXAA + BJOE group was curtailed (Figure 6b).

The number of proliferating crypt cells in the colons of mice was significantly reduced in both the model and agonist groups. However, PCNA expression level was markedly restored in the BJOE group. Conversely, the DMXAA + BJOE group exhibited reduced PCNA level compared to the BJOE group (Figure 6c). Furthermore, mRNA levels of barrier genes *occludin* and *ZO-1* were significantly decreased in the model and agonist groups, while their expression was up-regulated following treatment with BJOE (Figure 6d). However, the mRNA levels of *occludin* ($P < 0.01$) and *ZO-1* were down-regulated in the DMXAA + BJOE group compared to the BJOE group. Collectively, these findings indicated that BJOE enhanced the gene and protein expression of intestinal barrier in mice with delayed diarrhea, and this effect was compromised by DMXAA.

BJOE Suppressed Aberrant Activation of the cGAS-STING Pathway

In comparison to the normal group, the mRNA expression levels of cGAS-STING pathway-related factors were significantly elevated in both the model and agonist groups ($P < 0.05$). Following the administration of BJOE, the mRNA expression levels of *cGAS, STING, CXCL10, CCL5*, and *IFN-β* were notably down-regulated ($P < 0.05$). Additionally, the expression levels of cGAS-STING pathway-related factors were evidently increased in the DMXAA +BJOE group compared to the BJOE group ($P < 0.05$) (Figure 7a).

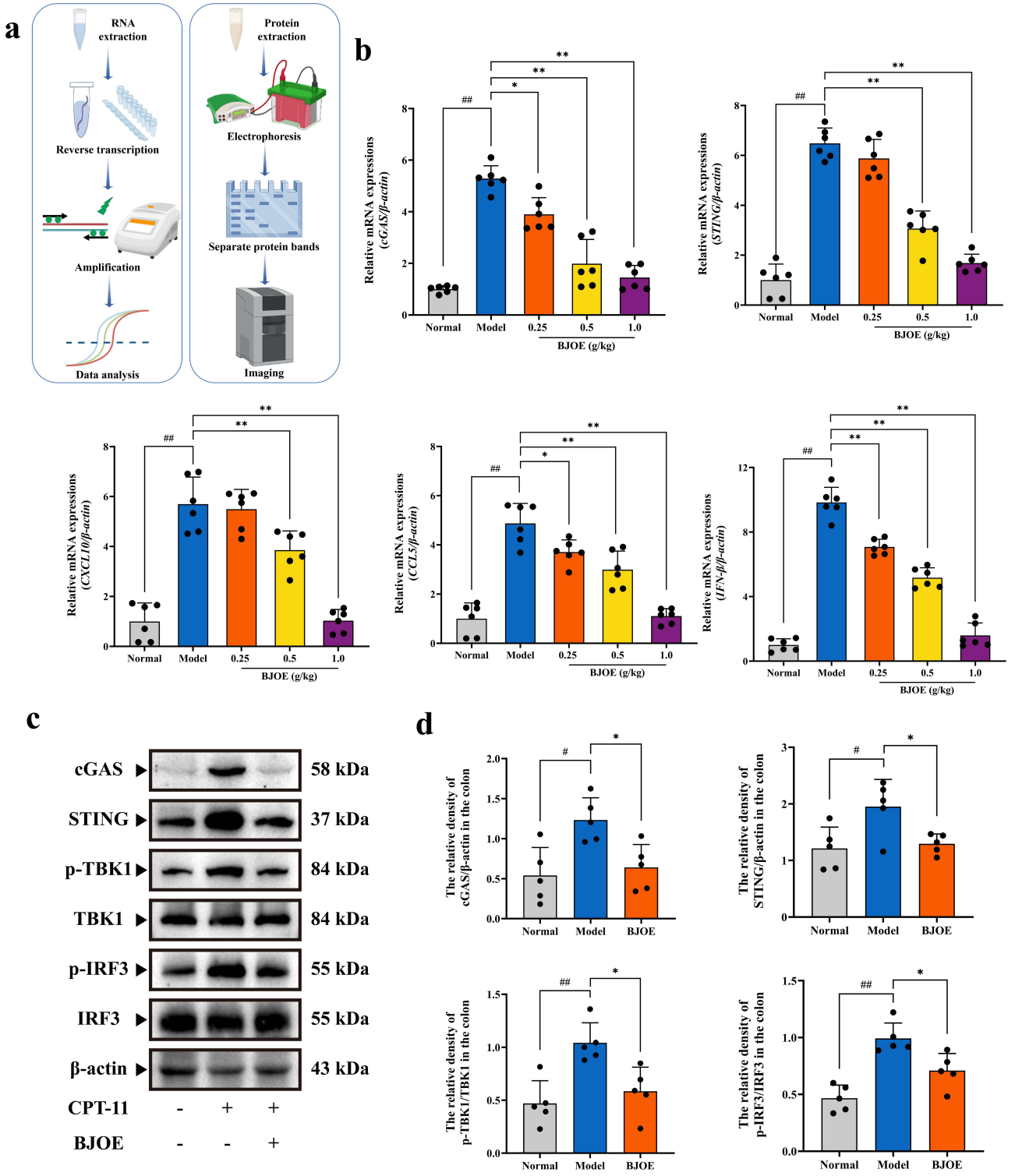


Figure 4 Effect of BJOE on cGAS-STING pathway-related factors in delayed diarrhea mice. (**a**) PCR and Western blotting workflow diagram. (**b**) mRNA expression levels of *cGAS, STING, CXCL10, CCL5*, and *IFN-β* measured by quantitative real-time PCR (n = 6). (**c**) Representative protein imprinted bands of cGAS, STING, p-TBK1, TBK1, p-IRF3, and IRF3. (**d**) Protein expression levels of cGAS, STING, p-TBK1, TBK1, p-IRF3, and IRF3 were measured by Western blot (n = 5). Data are expressed as mean ± SD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with the normal group. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the model group.

https://doi.org/10.2147/DDDT.S517973

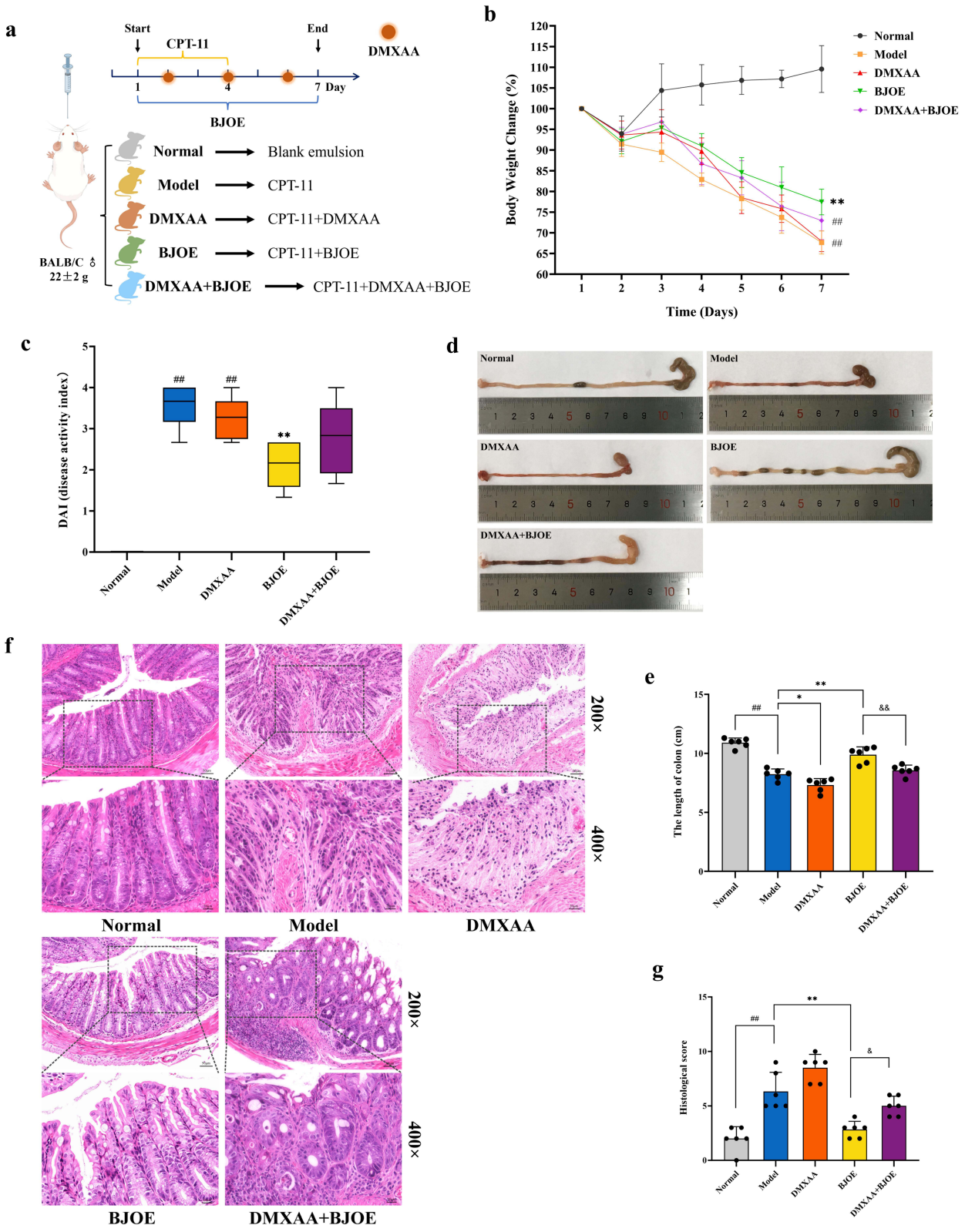


Figure 5 Enhancing STING secretion diminished the effect of BJOE on symptoms in mice with delayed diarrhea. STING agonist DMXAA was used to activate the cGAS-STING pathway. (**a**) Animal experimental design diagram. (**b**) Rate of change in body weight of mice. (**c**) DAI score. (**d–e**) Length of mouse colon. (**f–g**) HE staining and scoring of mouse colon tissue (200× and 400×). Data are expressed as mean ± SD (n = 6). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with the normal group. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the model group. $^{\&}P < 0.05$, $^{\&\&}P < 0.01$, compared with the BJOE group.

 https://doi.org/10.2147/DDDT.S517973

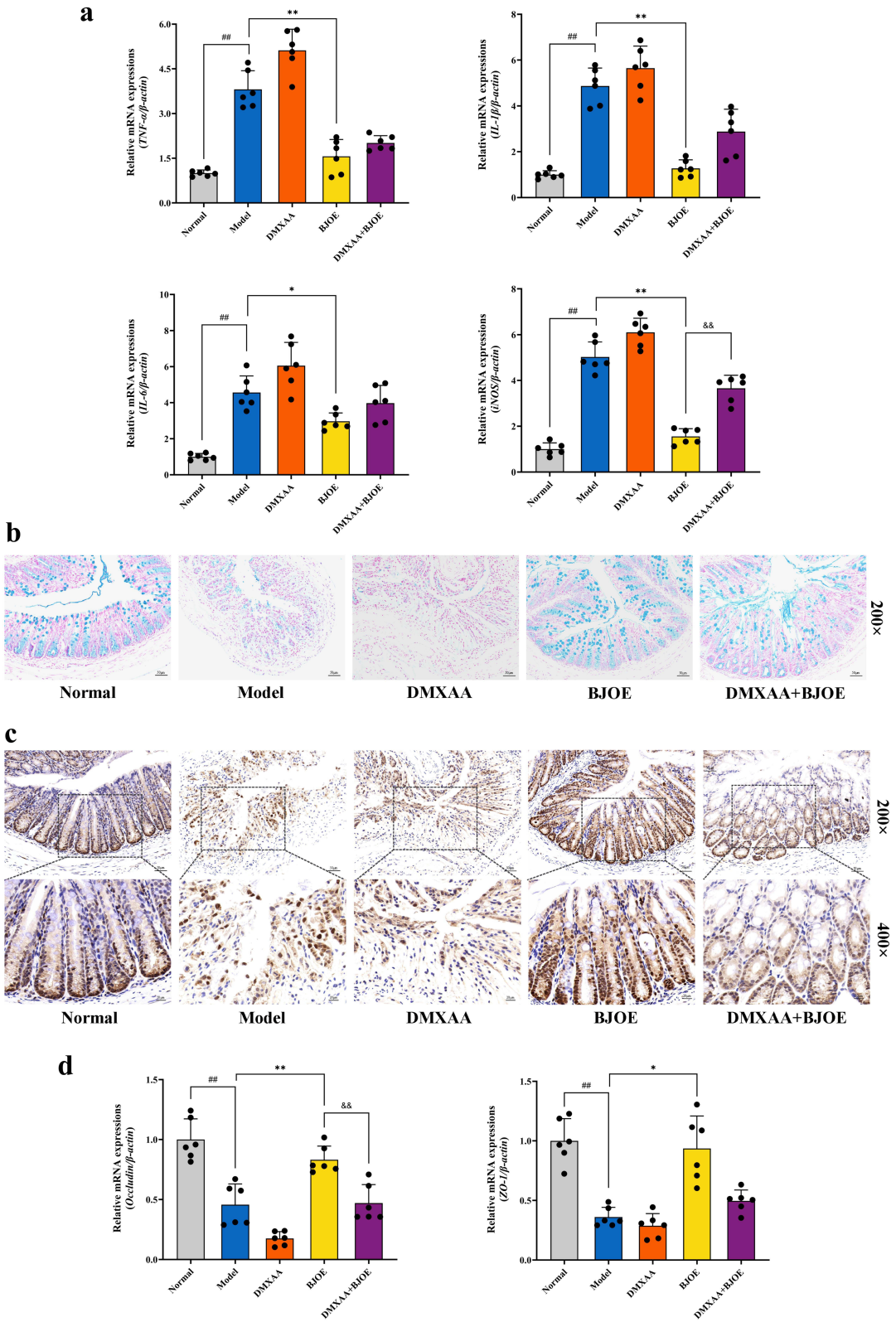


Figure 6 Promoting STING secretion mitigated BJOE's effects on inflammation and intestinal barrier in delayed diarrhea mice. STING agonist DMXAA was used to activate the cGAS-STING pathway. (**a**) mRNA expression levels of *TNF-α, IL-1β, IL-6*, and *iNOS* measured by quantitative real-time PCR (n = 6). (**b**) Representative graphs of mice stained with alcian blue in each group (200×). (**c**) Immunohistochemical staining to detect PCNA expression (200× and 400×). (**d**) mRNA expression levels of *occludin* and *ZO-1* detected by quantitative real-time PCR (n = 6). Data are expressed as mean ± SD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with the normal group. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the model group. $^{\&}P < 0.05$, $^{\&\&}P < 0.01$, compared with the BJOE group.

https://doi.org/10.2147/DDDT.S517973

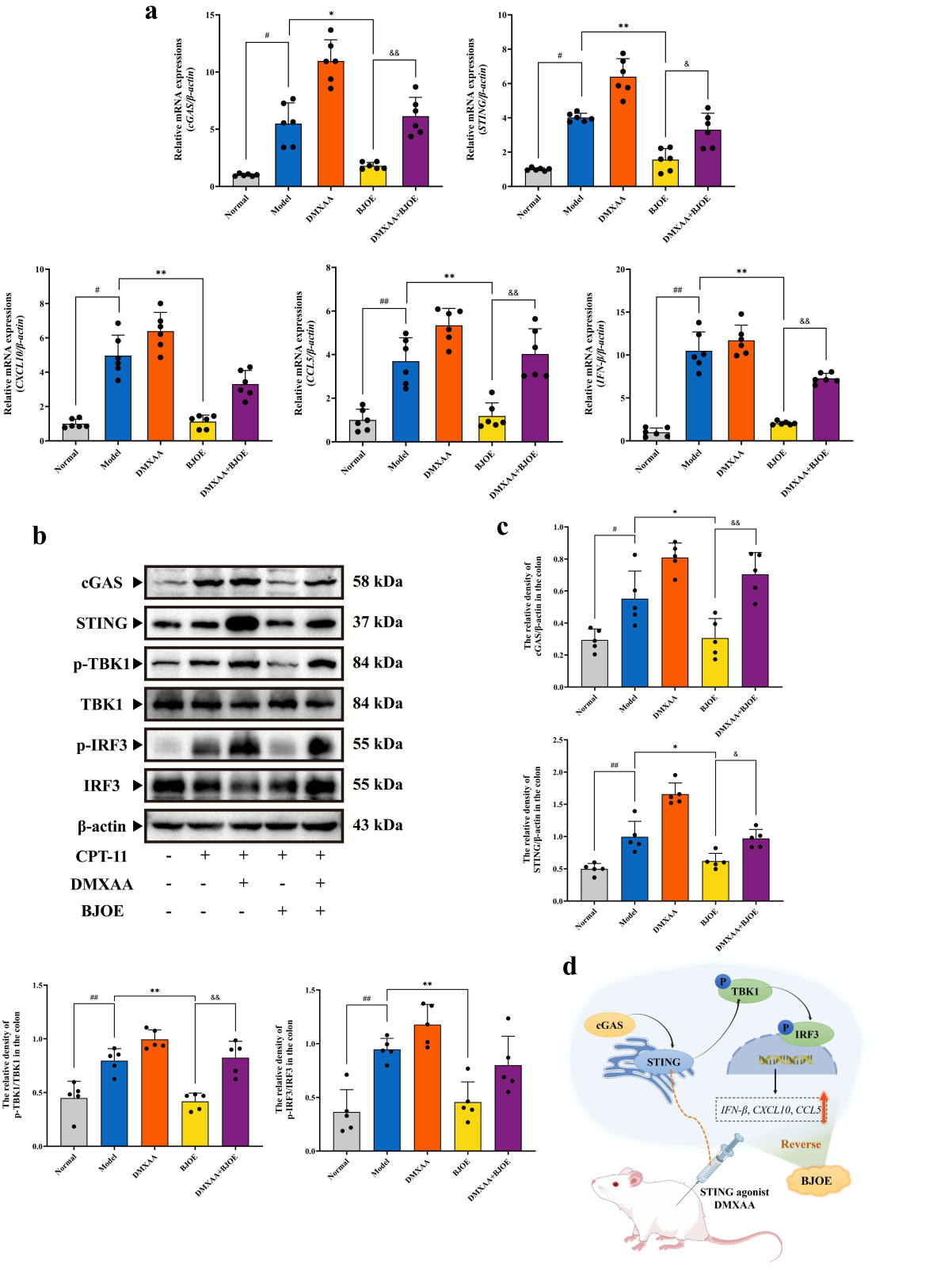


Figure 7 Promoting STING secretion attenuated BJOE's effects on cGAS-STING pathway-related factors in delayed diarrhea mice. STING agonist DMXAA was used to activate the cGAS-STING pathway. (**a**) mRNA expression levels of *cGAS*, *STING*, *CXCL10*, *CCL5*, and *IFN-β* measured by quantitative real-time PCR (n = 6). (**b**) Representative protein imprinted bands of cGAS, STING, p-TBK1, TBK1, p-IRF3, and IRF3. (**c**) Protein expression of cGAS, STING, p-TBK1, TBK1, p-IRF3, and IRF3 were measured by Western blot (n = 5). (**d**) BJOE suppressed aberrant activation of the cGAS-STING pathway. Data are expressed as mean ± SD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with the normal group. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the model group. $^{\&}P < 0.05$, $^{\&\&}P < 0.01$, compared with the BJOE group.

https://doi.org/10.2147/DDDT.S517973

Western blot analysis (Figure 7b and 7c) indicated that BJOE treatment significantly downregulated the protein expression levels of cGAS, STING, p-TBK1, and p-IRF3 when compared to the model group ($P < 0.05$). While the expression levels of these cGAS-STING pathway-related proteins were remarkably elevated in the DMXAA+BJOE group ($P < 0.05$). These results suggested that BJOE might alleviate irinotecan-induced delayed diarrhea in mice by inhibiting the aberrant activation of the cGAS-STING pathway (Figure 7d).

Discussion

Recent advancements in cancer treatment, especially in immunotherapy and targeted therapy, have achieved significant breakthroughs, yet adverse effects continue to limit their efficacy.[19] Irinotecan, a widely used chemotherapeutic agent for solid tumors like colorectal, pancreatic, and lung cancers,[20,21] is limited by the adverse effect of delayed diarrhea, which restricted its dosage and completion of chemotherapy regimens.[22] This side effect profoundly impacts patients' performance status and their ability to engage in daily activities, leading to decreased work capacity, strained interpersonal relationships, and psychological distress.

Currently, several Western medications are available to address irinotecan-induced diarrhea, including the opioid receptor agonist loperamide and the long-acting growth hormone analogue octreotide.[23] However, the incidence of adverse effects, such as paralytic intestinal obstruction, rises with escalating doses of loperamide.[24] A Phase III randomized clinical trial demonstrated that long-acting octreotide neither prevented nor alleviated the severity of diarrhea in colorectal cancer patients undergoing chemotherapy-induced diarrhea.[25] Currently, there is no standardized and effective clinical treatment for delayed diarrhea induced by irinotecan. Modern pharmacology has confirmed that traditional Chinese medicine effectively alleviates various adverse effects associated with chemotherapy, demonstrating a favorable safety profile.[26,27] Furthermore, several clinical trials[28–32] have demonstrated that adjuvant therapy with Chinese herbal medicine can significantly prevent and treat chemotherapy-associated diarrhea without compromising the anti-tumor activity of chemotherapy. This approach offers a promising and effective strategy for managing delayed diarrhea induced by irinotecan.

Brucea javanica is recognized as a pivotal treatment for dysentery. It is primarily used in clinical settings as a traditional Chinese medicine preparation, specifically BJOE, as a monotherapy or an adjunct to anti-tumor therapies in modern practice. Research has indicated that this emulsion possessed the capability to inhibit cell proliferation, induce apoptosis in tumor cells, and mitigate resistance to chemotherapy agents.[33] For example, the combination of BJOE with conventional chemotherapeutic drugs has been shown to enhance the quality of life for patients suffering from malignant pleural effusion.[34] Furthermore, the administration of BJOE has been shown to mitigate the myelotoxicity and gastrointestinal reactions associated with conventional chemotherapeutic agents. Existing research has reported that BJOE possessed significant anti-ulcer activity, exhibiting protective effects against gastric ulcers in both mice and rats.[35]

GC-MS analysis showed that linoleic acid was the main component of BJOE, which was consistent with previous report.[36] Linoleic acid reduced the production of pro-inflammatory mediators via the nuclear hormone receptor PPARγ in macrophages.[37] Thus, the anti-inflammatory effect of BJOE may be linked to its component linoleic acid. Our previous studies have confirmed that BJOE effectively alleviated ulcerative colitis (UC) induced by dextran sulfate sodium salt in mice. This effect was attributed to its ability to improve intestinal inflammation and promote the repair of intestinal mucosal damage, thereby exerting notable anti-UC effects.[36,38] The aforementioned studies indicated that BJOE enhanced the efficacy of chemotherapeutic agents and exhibited significant anti-inflammatory properties. Given that delayed diarrhea induced by the chemotherapeutic agent irinotecan was closely associated with inflammation, we hypothesized that BJOE could alleviate the intestinal adverse effects associated with irinotecan. Our results indicated that BJOE significantly improved the physical condition of mice, including reductions in weight loss, colon shortening, and DAI scores.

Chemotherapy-induced intestinal adverse effects frequently lead to mucositis, where inflammatory factors play a critical role in inflammation and tissue damage resulting from cytotoxic treatments. Subsequent studies have demonstrated that chemotherapeutic agents stimulated the activation of NF-κB, leading to the production of pro-inflammatory cytokines, including TNF-α, IL-6, and IL-1β, which contributed to mucosal injury.[39] In addition to enhancing the physical condition of mice with delayed diarrhea, BJOE significantly reduced the elevation of inflammatory factors associated with delayed diarrhea, as well as the mRNA expression levels of *TNF-α, IL-1β, IL-6*, and *iNOS*.

The dynamic regulation of tight junction proteins is fundamental to various physiological processes. Disruption of this regulation significantly alters mucosal barrier function and intestinal permeability, establishing tight junction proteins

as critical markers of numerous pathological states. Irinotecan has been shown to induce defects in tight junction proteins, resulting in mucosal barrier dysfunction and diarrhea.[40] Consequently, comparison of mRNA levels of the tight junction proteins occludin and ZO-1 across groups revealed a significant increase in the colon of mice treated with BJOE compared to the model group. The mucus barrier served as the primary defense layer separating the microbiota from the epithelial cells, comprising mucus secreted by goblet cells that coated the intestinal epithelium.[41]

Irinotecan has been reported to decrease the number of mucin-producing goblet cells in the colon.[42] Our study corroborated with the existing literature, indicating that goblet cells were significantly reduced in the model group. The mucin expression in the BJOE treatment group was comparable to that in the positive control group, suggesting that BJOE enhanced mucin secretion and played a protective role in maintaining the intestinal barrier. PCNA is essential for DNA replication, DNA repair, cell cycle regulation, and apoptosis.[43] PCNA is involved in DNA damage repair and promotes the proliferation of intestinal epithelial cells in the mouse intestine. Maintaining a balance between cell proliferation and apoptosis is essential for preserving the integrity of the intestinal barrier.[44] Our results indicated that irinotecan administration led to a significant loss of proliferating cells in the colons of mice. In contrast, treatment with BJOE restored cell proliferation across all dosage groups.

The innate immune system is crucial for maintaining mucosal homeostasis. Dysregulated innate immune responses are linked to gastrointestinal inflammation and cancer. Dysfunction in intestinal immune defense and imbalances in tolerance regulatory mechanisms can result in mucosal injury and gastrointestinal disorders. The cGAS-STING signaling pathway plays a crucial role in maintaining intestinal homeostasis by detecting both self and non-self DNA. However, excessive activation of this pathway can lead to gastrointestinal inflammation. Therefore, the cGAS-STING signaling pathway may provide enlightening insights for developing new therapeutic strategies.[45]

Irinotecan, a selective DNA topoisomerase I (ToPoI) inhibitor, covalently binds to the DNA-topoisomerase complex,[46] leading to the accumulation of damaged dsDNA in the cytoplasm that activates the cGAS-STING pathway in intestinal epithelial cells. Upon activation, STING initiates downstream signaling cascades involving TBK1 and IRF3.[47] Phosphorylated IRF3, upon dimerization and translocation to the nucleus, activates gene transcription of factors such as IFN-β, CXCL10, and CCL5.[48,49] In our work, the mRNA levels of *cGAS, STING, CXCL10, CCL5*, and *IFN-β*, as well as the protein expression levels of cGAS, STING, p-TBK1, and p-IRF3 in the cGAS-STING pathway were significantly upregulated in the model group. In contrast, the expression of cGAS-STING pathway-related factors in the BJOE group was significantly ameliorated in parallel to the irinotecan model group.

STING knockout mice demonstrated a reduced inflammatory response and decreased intestinal permeability. Conversely, mice treated with the STING agonist DMXAA exhibited increased intestinal apoptosis and exacerbated systemic inflammation, indicating that the activation of the STING pathway contributed to intestinal inflammatory responses.[50] Therefore, we employed the agonist DMXAA to investigate whether BJOE alleviated irinotecan-induced delayed diarrhea by inhibiting aberrant activation of the cGAS-STING pathway. As anticipated, our experimental results indicated a significant increase in the mRNA levels of *cGAS, STING, CXCL10, CCL5* and *IFN-β*, as well as in the protein expression levels of cGAS, STING, p-TBK1, and p-IRF3 in the DMXAA+BJOE group compared to those of the BJOE group. Irinotecan induced delayed diarrhea by disrupting the balance of the cGAS-STING pathway, resulting in hyperactivation, while BJOE inhibited this activation, thereby exerting a therapeutic effect.

Nevertheless, there were also some limitations in this study. Firstly, the absence of clinical validation challenged translational relevance. While species differences may affect the pharmacokinetics and pharmacodynamics of BJOE, highlighting the need for further evaluation in human trials. Secondly, potential off-target or dose-related adverse effects also warrant dedicated safety assessments, particularly when combined with irinotecan. Thirdly, a relatively small sample size may reduce power to detect subtle effects.

Further in-depth investigation was warranted. For example, future research should focus on identifying the active compounds in BJOE responsible for its therapeutic effects, particularly in modulating the cGAS-STING pathway. Advanced preclinical platforms, such as human intestinal organoids and STING-humanized mouse models, should be used to better understand BJOE's therapeutic mechanism. Furthermore, long-term safety and pharmacokinetics studies should be performed before clinical applications. These efforts are envisaged to further define BJOE's clinical potential.

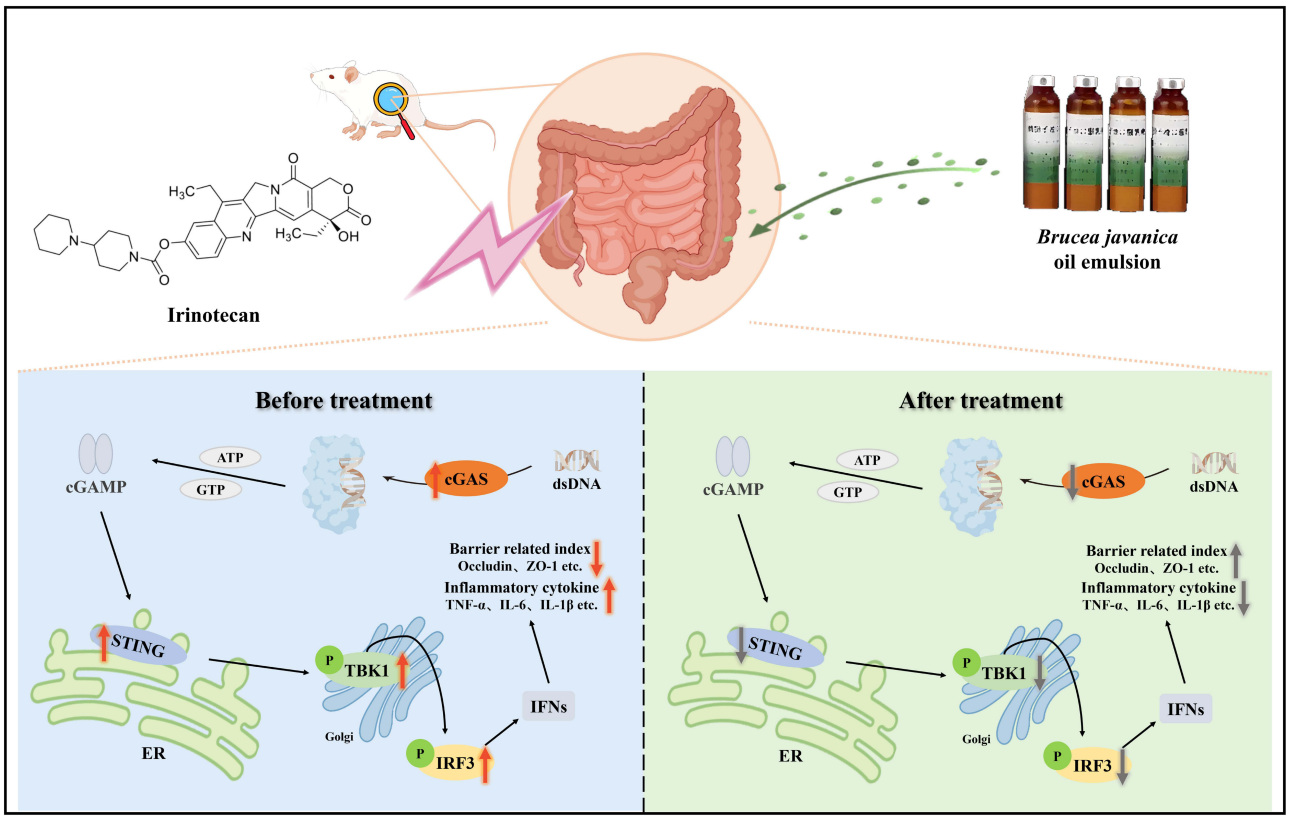


Figure 8 Schematic illustration of the potential protective mechanism of BJOE in irinotecan-induced delayed diarrhea mice. BJOE attenuated intestinal inflammation, as indicated by decreased levels of TNF-α, IL-1β, IL-6, and iNOS. It also preserved intestinal barrier integrity by upregulating the expression of mucins and tight junction proteins, including ZO-1 and Occludin. These protective effects may be associated with the inhibition of aberrant activation of the cGAS-STING signaling pathway.

Conclusion

In conclusion, BJOE significantly alleviated irinotecan-induced delayed diarrhea in mice, at least in part, via inhibiting the aberrant activation of the cGAS-STING signaling pathway and subsequent intestinal homeostasis (Figure 8). BJOE may serve as a promising adjuvant strategy to mitigate chemotherapy-associated gastrointestinal toxicity. Future studies should aim to isolate its active constituents and evaluate their safety and efficacy in clinically relevant models to advance translational application.

Abbreviations

ANOVA, analysis of variance; BJOE, Brucea javanica oil emulsion; CCL5, CeC motif chemokine 5; CXCL10, chemokine ligand 10; cGAS, cyclic GMP-AMP synthase; CID, chemotherapy-induced diarrhea; CTD, the Comparative Toxicogenomics Database; DAB, diaminobenzidine; Vadimezan, DMXAA; dsDNA, double-stranded DNA; GC-MS, gas chromatography-mass spectrometry; HE, hematoxylin and eosin; HRP, horseradish peroxidase; IFN-β, interferon-β; IL-1β, interleukin-1β; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; IRF3, interferon regulatory factor 3; PCNA, proliferating cell nuclear antigen; PCR, polymerase chain reaction; STING, stimulator of interferon genes; TBK1, TANK-binding kinase 1; TBST, Tris buffered saline with Tween-20; TCM, traditional Chinese medicine; TCMSP, traditional Chinese medicine systems; TNF-α, tumor necrosis factor-α; Topo1, topoisomerase I pharmacology; ZO-1, zona occludens 1.

Ethical Approval and Consent to Participate

All animal experimental procedures were performed in accordance with the guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal study was reviewed and approved by the Institutional Ethics Committee of Guangzhou University of Chinese Medicine (Approval No. 20231211006).

https://doi.org/10.2147/DDDT.S517973

Funding

This study was supported by the National Natural Science Foundation of China (No. 82174045), Natural Science Foundation of Guangdong Province (Nos. 2021A1515010160 & 2025A1515011810), Science and Technology Planning Project of Shenzhen City (No. JCYJ20220531103010022), and Science and Technology Planning Project of Guangdong Province (No. 2023B1212060063).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Escalante J, McQuade RM, Stojanovska V, et al. Impact of chemotherapy on gastrointestinal functions and the enteric nervous system. *Maturitas*. 2017;105:23–29. doi:10.1016/j.maturitas.2017.04.021
2. Kciuk M, Marciniak B, Kontek R. Irinotecan—still an important player in cancer chemotherapy: a comprehensive overview. *Int J Mol Sci*. 2020;21(14):4919. doi:10.3390/ijms21144919
3. He Y, Zheng J, Ye B, et al. Chemotherapy-induced gastrointestinal toxicity: pathogenesis and current management. *Biochem Pharmacol*. 2023;216:115787. doi:10.1016/j.bcp.2023.115787
4. Lin X, Fang Y, Cheng Y, et al. Chinese herbal medicine for irinotecan-induced diarrhea: a systematic review and meta-analysis. *Explore*. 2024;20(2):158–167. doi:10.1016/j.explore.2023.08.003
5. Sharma R, Tobin P, Clarke SJ. Management of chemotherapy-induced nausea, vomiting, oral mucositis, and diarrhoea. *Lancet Oncol*. 2005;6(2):93–102. doi:10.1016/S1470-2045(05)01735-3
6. Wang D, Yao X, Xie B, et al. Anti-inflammatory effects of brucea javanica oil via inhibition of NF-κB activation. *Am J Transl Res*. 2021;13(11):12786–12796.
7. Xu S, Zhuang Y, Zhan Z, et al. Research progress in chemical constituents, pharmacological activity, and quality markers of bruceae fructus. *China J Chin Mater Med*. 2022;47(19):5158–5170. doi:10.19540/j.cnki.cjcmm.20220604.201
8. Pan L, Chin YW, Chai HB, et al. Bioactivity-guided isolation of cytotoxic constituents of brucea javanica collected in Vietnam. *Bioorg Med Chem*. 2009;17(6):2219–2224. doi:10.1016/j.bmc.2008.10.076
9. Song J, Zhou H, Sun C, et al. Combination of brucea javanica oil emulsion and aidi injection associated with the long-term survival of a patient with colon cancer and lung metastases post-chemotherapy: a case report. *Oncol Lett*. 2024;28(2):361. doi:10.3892/ol.2024.14494
10. Wang X, Wang H, Cao L, et al. Efficacy and safety of brucea javanica oil emulsion injection in the treatment of gastric cancer: a systematic review and meta-analysis. *Front Nutr*. 2021;8:784164. doi:10.3389/fnut.2021.784164
11. Shi R, Wu Z, Wang H, et al. Investigation on the efficiency of brucea javanica oil emulsion injection with chemotherapy for treating malignant pleural effusion: a meta-analysis of randomized controlled trials. *Front Pharmacol*. 2022;13:998218. doi:10.3389/fphar.2022.998218
12. Zhou J, Wang T, Dou Y, et al. Brusatol ameliorates 2, 4, 6-trinitrobenzenesulfonic acid-induced experimental colitis in rats: involvement of NF-κB pathway and NLRP3 inflammasome. *Int Immunopharmacol*. 2018;64:264–274. doi:10.1016/j.intimp.2018.09.008
13. Zheng X, Mai L, Xu Y, et al. Brucea javanica oil alleviates intestinal mucosal injury induced by chemotherapeutic agent 5-fluorouracil in mice. *Front Pharmacol*. 2023;14:1136076. doi:10.3389/fphar.2023.1136076
14. Yang Y, Wang L, Peugnet-González I, et al. cGAS-STING signaling pathway in intestinal homeostasis and diseases. *Front Immunol*. 2023;14:1239142. doi:10.3389/fimmu.2023.1239142
15. Wang Y, Wei B, Wang D, et al. DNA damage repair promotion in colonic epithelial cells by andrographolide downregulated cGAS–STING pathway activation and contributed to the relief of CPT-11-induced intestinal mucositis. *Acta Pharm Sin B*. 2022;12(1):262–273. doi:10.1016/j.apsb.2021.03.043
16. Qi M, Chu S, Wang W, et al. Safflower polysaccharide ameliorates acute ulcerative colitis by regulating STAT3/NF-κB signaling pathways and repairing intestinal barrier function. *Biomed Pharmacother*. 2024;174:116553. doi:10.1016/j.biopha.2024.116553
17. Qiu L, Yan C, Yang Y, et al. Morin alleviates DSS-induced ulcerative colitis in mice via inhibition of inflammation and modulation of intestinal microbiota. *Int Immunopharmacol*. 2024;140:112846. doi:10.1016/j.intimp.2024.112846
18. Kuanyun Z, Shuqing C, Xia Y, et al. Study on oleic acid in brucea javanica oil with GC. *Guangdong Chem Ind*. 2018;45(22):109–110.
19. Singh NK, Beckett JM, Kalpurath K, et al. Synbiotics as supplemental therapy for the alleviation of chemotherapy-associated symptoms in patients with solid tumours. *Nutrients*. 2023;15(7):1759. doi:10.3390/nu15071759
20. Peeters SL, Deenen MJ, Thijs AM, et al. *UGT1A1* genotype-guided dosing of irinotecan: time to prioritize patient safety. *Pharmacogenomics*. 2023;24(8):435–439. doi:10.2217/pgs-2023-0096
21. Basade M, Mane A. Optimum patient selection for irinotecan-containing regimens in metastatic colorectal cancer: literature review and lessons from clinical practice. *Indian J Cancer*. 2021;58(1):5. doi:10.4103/ijc.IJC_507_19
22. De Man FM, Goey AKL, Van Schaik RHN, et al. Individualization of irinotecan treatment: a review of pharmacokinetics, pharmacodynamics, and pharmacogenetics. *Clin Pharmacokinet*. 2018;57(10):1229–1254. doi:10.1007/s40262-018-0644-7
23. Zidan J, Haim N, Beny A, et al. Octreotide in the treatment of severe chemotherapy-induced diarrhea. *Ann Oncol*. 2001;12(2):227–230. doi:10.1023/A:1008372228462
24. Wu PE, Juurlink DN. Clinical review: loperamide toxicity. *Ann Emerg Med*. 2017;70(2):245–252. doi:10.1016/j.annemergmed.2017.04.008
25. Hoff PM, Saragiotto DF, Barrios CH, et al. Randomized phase III trial exploring the use of long-acting release octreotide in the prevention of chemotherapy-induced diarrhea in patients with colorectal cancer: the LARCID trial. *J Clin Oncol*. 2014;32(10):1006–1011. doi:10.1200/JCO.2013.50.8077
26. Qi F, Zhao L, Zhou A, et al. The advantages of using traditional Chinese medicine as an adjunctive therapy in the whole course of cancer treatment instead of only terminal stage of cancer. *Biosci Trends*. 2015;9(1):16–34. doi:10.5582/bst.2015.01019
27. Deng C, Deng B, Jia L, et al. Preventive effects of a Chinese herbal formula, shengjiang xiexin decoction, on irinotecan-induced delayed-onset diarrhea in rats. *Evid-Based Compl Alt*. 2017;2017(1):7350251. doi:10.1155/2017/7350251

28. Zou Y, Wang Y, Zhou W, et al. Banxia xiexin decoction combined with 5-ASA protects against CPT-11-induced intestinal dysfunction in rats via inhibiting TLR4/NF-κB signaling pathway. *Immun Inflammation Dis*. 2024;12(6):e1208. doi:10.1002/iid3.1208
29. Sun R, Basu S, Zeng M, et al. Xiao-chai-hu-tang (XCHT) intervening irinotecan's disposition: the potential of XCHT in alleviating irinotecan-induced diarrhea. *Curr Cancer Drug Targets*. 2019;19(7):551–560. doi:10.2174/1568009618666181029153255
30. Lu DX, Liu F, Wu H, et al. Wumei pills attenuates 5-fluorouracil-induced intestinal mucositis through toll-like receptor 4/myeloid differentiation factor 88/nuclear factor-κB pathway and microbiota regulation. *World J Gastroenterol*. 2022;28(32):4574–4599. doi:10.3748/wjg.v28.i32.4574
31. Wu Y, Wang D, Yang X, et al. Traditional Chinese medicine gegen qinlian decoction ameliorates irinotecan chemotherapy-induced gut toxicity in mice. *Biomed Pharmacother*. 2019;109:2252–2261. doi:10.1016/j.biopha.2018.11.095
32. Lam W, Bussom S, Guan F, et al. The four-herb Chinese medicine PHY906 reduces chemotherapy-induced gastrointestinal toxicity. *Sci Transl Med*. 2010;2(45). doi:10.1126/scitranslmed.3001270
33. Wu Y, Liu Z, Xu P, et al. Brucea javanica oil emulsion injection inhibits proliferation of pancreatic cancer via regulating apoptosis-related genes. *Am J Cancer Res*. 2023;13(11):5047–5064.
34. Fuhong D, Xiang G, Haiying L, et al. Evaluation of efficacy and safety for brucea javanica oil emulsion in the control of the malignant pleural effusions via thoracic perfusion. *BMC Cancer*. 2018;18(1):411. doi:10.1186/s12885-018-4328-3
35. Li Q, Yang L, Fan L, et al. Activity of brucea javanica oil emulsion against gastric ulcers in rodents. *Asian J Pharm Sci*. 2018;13(3):279–288. doi:10.1016/j.ajps.2017.12.005
36. Huang YF, Zhou JT, Qu C, et al. Anti-inflammatory effects of brucea javanica oil emulsion by suppressing NF-κB activation on dextran sulfate sodium-induced ulcerative colitis in mice. *J Ethnopharmacol*. 2017;198:389–398. doi:10.1016/j.jep.2017.01.042
37. Yu YL, Lu Y, Tang X, et al. Formulation, preparation and evaluation of an intravenous emulsion containing brucea javanica oil and coix seed oil for anti-tumor application. *Biol Pharm Bull*. 2008;31(4):673–680. doi:10.1248/bpb.31.673
38. Huang YF, Li QP, Dou YX, et al. Therapeutic effect of brucea javanica oil emulsion on experimental crohn's disease in rats: involvement of TLR4/NF-κB signaling pathway. *Biomed Pharmacother*. 2019;114:108766. doi:10.1016/j.biopha.2019.108766
39. Sultani M, Stringer AM, Bowen JM, et al. Anti-inflammatory cytokines: important immunoregulatory factors contributing to chemotherapy-induced gastrointestinal mucositis. *Chemother Res Pract*. 2012;2012(4):490804. doi:10.1155/2012/490804
40. Wardill HR, Bowen JM, Al-Dasooqi N, et al. Irinotecan disrupts tight junction proteins within the gut: implications for chemotherapy-induced gut toxicity. *Cancer Biol Ther*. 2014;15(2):236–244. doi:10.4161/cbt.27222
41. Sang X, Wang Q, Ning Y, et al. Age-related mucus barrier dysfunction in mice is related to the changes in Muc2 mucin in the colon. *Nutrients*. 2023;15(8):1830. doi:10.3390/nu15081830
42. Stringer AM, Gibson RJ, Logan RM, et al. Irinotecan-induced mucositis is associated with changes in intestinal mucins. *Cancer Chemother Pharmacol*. 2009;64(1):123–132. doi:10.1007/s00280-008-0855-y
43. Park SY, Jeong MS, Han CW, et al. Structural and functional insight into proliferating cell nuclear antigen. *J Microbiol Biotechnol*. 2016;26(4):637–647. doi:10.4014/jmb.1509.09051
44. Zhang H, Wang X, Zhao L, et al. Biochanin a ameliorates DSS-induced ulcerative colitis by improving colonic barrier function and protects against the development of spontaneous colitis in the Muc2 deficient mice. *Chem-Biol Interact*. 2024;395:111014. doi:10.1016/j.cbi.2024.111014
45. Ke X, Hu T, Jiang M. cGAS–STING signaling pathway in gastrointestinal inflammatory disease and cancers. *FASEB J*. 2022;36(1):e22029. doi:10.1096/fj.202101199R
46. Zhang X, chen BX, Chen ZJ. Structures and Mechanisms in the cGAS-STING Innate Immunity Pathway. *Immunity*. 2020;53(1):43–53. doi:10.1016/j.immuni.2020.05.013
47. Hopfner KP, Hornung V. Molecular mechanisms and cellular functions of cGAS–STING signalling. *Nat Rev Mol Cell Biol*. 2020;21(9):501–521. doi:10.1038/s41580-020-0244-x
48. Li M, Bai G, Cen Y, et al. Silencing HOXC13 exerts anti-prostate cancer effects by inducing DNA damage and activating cGAS/STING/IRF3 pathway. *J Transl Med*. 2023;21(1):884. doi:10.1186/s12967-023-04743-x
49. Hu X, Zhao M, Bai M, et al. PARP inhibitor plus radiotherapy reshape the immune suppressive microenvironment and potentiate the efficacy of immune checkpoint inhibitors in tumors with IDH1 mutation. *Cancer Lett*. 2024;586:216676. doi:10.1016/j.canlet.2024.216676
50. Hu Q, Ren H, Li G, et al. STING-mediated intestinal barrier dysfunction contributes to lethal sepsis. *EBioMedicine*. 2019;41:497–508. doi:10.1016/j.ebiom.2019.02.055