APPENDIX

Appendix 1. Contents of crude drugs contained in each pack of BLWTG (5 g)

Each pack of BLWTG (5 g) contained 20 g crude drugs, and the proportion, pharmacological components, and efficacies of each TCM ingredient are shown in Supplementary Table 1.

Supplementary Table 1. Contents of crude drugs contained in each pack of BLWTG (5 g) and their pharmacological components

TCM ingredients	Crude drug	Proportion (%)	Pharmacological components and efficacies
Fructus Litseae	2.515	12.58	Limonene has analgesic effect and can inhibit visceral hypersensitivity [19]; it can also protect gastric mucosa and promote mucosal healing [20,21]. Citral can inhibit neurogenic and inflammatory pain, prevent and treat mechanical hyperalgesia and chronic regional pain, and protect gastric mucosa from ulcers caused by nonsteroidal anti-inflammatory drugs [22].
Rhizoma Corydalis	1.510	7.55	Tetrahydropalmatine has significant antihyperalgesic effects on neuralgia and inflammatory pain in animal models [23,24]. Also, it can effectively inhibit visceral hyperalgesia and thermal, mechanical, and inflammatory hyperalgesia [25].

Rhizoma Coptidis	0.755	3.78	Rhizoma Coptidis and berberine have certain inhibitory effects on *Staphylococcus aureus*, *Shigella* and *Vibrio cholerae*; berberine can remarkably inhibit various influenza viruses; Rhizoma Coptidis, berberine, coptisine, and jatrorrhizine have significant anti-inflammatory effects. Rhizoma Coptidis and berberine have antipyretic effects. Rhizoma Coptidis and berberine can be used to treat experimental gastric ulcer by inhibiting gastric acid secretion and protecting gastric mucosa. Rhizoma Coptidis decoction and berberine can lower blood glucose [26]
Fructus Evodiae	0.380	1.90	Evodiamine can induce the increase of the intracellular calcium and electronic current in dorsal root ganglion neurons, increase the number of transient receptor potential (TRP) V1-transfected HEK293 cells, and thus inhibit hyperalgesia by activating TRPV1 channels [27]. Evodiamine has anti-inflammatory activity. It has a strong inhibitory effect on the synthesis of prostaglandin E2 (PGE2), which is the main proinflammatory factor of prostaglandin, one of the key features of inflammation. Thus, it can inhibit inflammatory hyperalgesia [28].

Radix et Rhizoma Rhei	0.755	3.78	Emodin can inhibit inflammatory activity, mainly by inhibiting the expressions of receptors and the common inflammatory pathways (such as nuclear-κB activation and tumor necrosis factor-α production), reducing neutrophil infiltration, and suppressing cytokine generation [29]. Emodin enhances the small intestinal motility in mice through mechanisms such as promoting motilin secretion, reducing somatostatin content, and inhibiting Na^+-K^+-ATP enzyme activities in the small intestine mucosa [30].
Rhizoma Cyperi	2.515	12.58	α-Cyperone: volatile oil of Rhizoma Cyperi has sedative, gastric mucosa-protective, anti-inflammatory, and other pharmacological activities, especially for pain caused by physical and chemical stimuli. Volatile oil of Rhizoma Cyperi can increase gastrointestinal motility (as demonstrated by measuring the gastric residual rate and intestinal propulsive rate) and promote the proliferation of small intestinal smooth muscle cells. GC-MS analysis of the volatile components has identified 17 chemical constituents [31]
Concha Arcae	2.515	12.58	The calcium carbonate contained in Concha Arcae can neutralize gastric acid and thus alleviate peptic ulcers. The mucus glue can form a thin protective layer on the gastric and duodenal mucosa, promote the growth of granulation tissue and accelerate the healing of ulcers. It can also inhibit *H. pylori* infection [26].
Fructus Citri	2.515	12.58	*Fructus Citri* can promote gastrointestinal motility, invigorate the stomach, dispel phlegm, alleviate inflammation, and fight against viral infections [26].

Fructus Citri Sarcodactylis	1.510	7.55	It can inhibit intestinal smooth muscle, alleviate stress, regulate immune system, and treat tumors [26].
Os Sepiellae seu Sepiae	2.515	12.58	The calcium carbonate contained in *Os Sepiellae seu Sepiae* can neutralize gastric acid, change the pH value of gastric contents, lower pepsin activity, and promote healing of ulcers. Its cavity contents can interact with the organic matter and gastric juice in the stomach, forming a protective film on the ulcer surface and thus promoting the clotting process. [26]
Fructus Meliae Toosendan	2.515	12.58	*Fructus Meliae Toosendan* relaxes Oedipal sphincter, shrinks the gallbladder, and promotes bile excretion. It can excite the intestinal smooth muscles and increase its tension and contraction. Toosendanin can expel intestinal parasites. *Fructus Meliae Toosendan* has inhibitory effect on *Staphylococcus aureus* and a variety of pathogenic fungi. Also, it has anti-inflammatory, analgesic, and anti-cancer effects [26].
Total	20	100	

Appendix 2. Fingerprint profile of BLWTG and quality control

1. Fingerprint of BLWTG

BLWTG granules were ground finely, 0.5 g BLWTG powder was collected and accurately weighed, placed in a conical flask with stopper, added with 25 mL 75% methanol and sonicated for 30 min, followed by high-speed centrifugation at 12 000 rpm for 5 min. The supernatant was harvested.

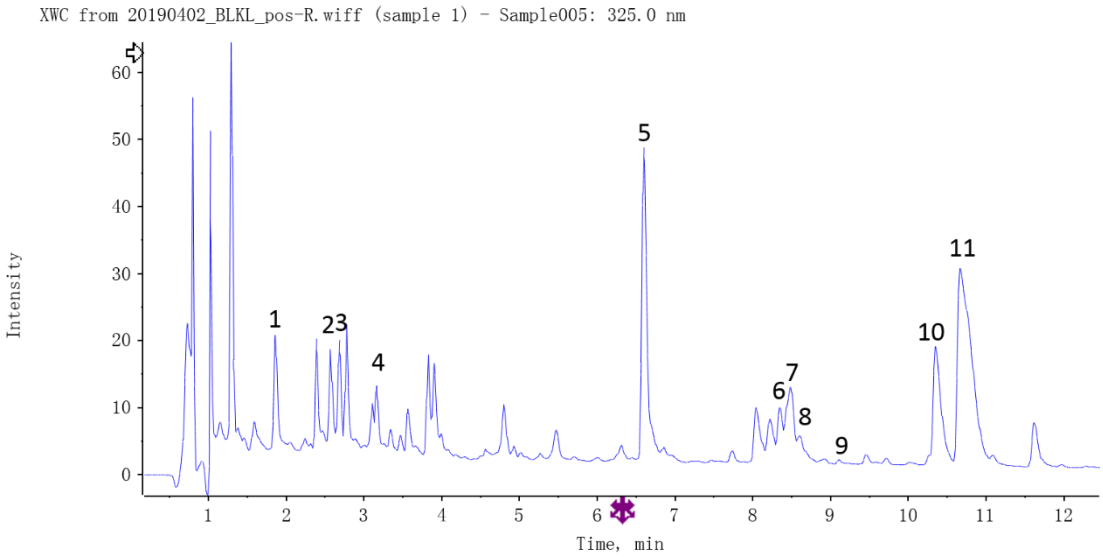
A total of 27 compounds were found by liquid chromatography-mass spectrometry, of which, 12 were confirmed by comparisons in high-resolution mass spectrometry database (Supplementary Table 2) and marked in red in Supplementary Figure 1. Compounds without a serial number were chromatographic peaks that were not detected in the customer spectrum.

Supplementary Table 2 Identification results of BLWTG components

No	Retention time	Adduction ion	Actual value of m/z	Expected value of m/z	ppm	Molecular formula	Molecular weight	MS/MS data	Name of the identified compound	Database	Original attribute
1	1.86	[M-H]-	353.0878	353.0878	0	C16H18O9	354.0950	n/a	Neochlorogenic acid	√	Fructus Evodiae
2	2.57	[M-H]-	353.0878	353.0878	-0.3	C16H18O9	354.0950	352.9919;190.9960;178.9770;160.9714;134.9966	Chlorogenic acid	√	Fructus Evodiae
3	2.68	[M-H]-	353.0878	353.0878	0.3	C16H18O9	354.0950	352.938;178.9774;172.9895;134.9977;92.9974;	Cryptochlorogenic acid	√	Fructus Evodiae
4	2.16	[M-H]-	593.1523	593.1512	1.9	C27H30O15	594.1584	593.0171;603.0016;472.9948;382.9771;	Vicenin 2 or isomer	√	Fructus Citri

								352.9728	;			
								324.9820				
								279.0382	;			
5	6.60	[M-H]-	579.1729	579.1719	1.7	C27H32O14	580.1792	459.0016	;	Naringin	√	Fructus Citri
								270.9837	;			
								150.9523;				
								336.1247	;			
6	8.22	[M]+	336.1228	336.1230	-0.7	C20H18NO4	336.1235	320.0929	;	Epiberberine	√	Rhizoma Coptidis
								292.0973	;			
								234.0897;				
								338.1399	;			
								322.1081	;			
	8.34	[M]+	338.1386	338.1387	-0.3	C20H20NO4	338.1392	307.0849	;	Jatrorrhizine	√	Rhizoma Coptidis
								294.1130	;			
								279.0894	;			
								265.0731;				
								320.0928	;			
								292.0980	;			
7	8.47	[M]+	320.0922	320.0917	1.5	C19H14NO4	320.0922	262.0869	;	Coptisine	√	Rhizoma Coptidis
								234.0912	;			
								204.0811;				
										Dehydroevodiamine		
								302.1290	;			
8	8.60	[M]+	302.1292	302.1288	1.4	C19H16N3O	302.1293	286.0978	;		√	Rhizoma Coptidis
								258.1019;				

9	9.45	[M-H]+	370.2012	370.2013	-0.2	C22H27NO4	369.1940	370.1637; 352.1578; 275.0723; 190.0863;	Corydaline	√	Rhizoma Corydalis
10	10.36	[M]+	352.1545	352.1543	0.5	C21H22NO4	352.1548	352.1579; 336.1272; 308.1307; 294.1149; 278.0843; 250.0910;	Palmatine	√	Rhizoma Coptidis
11	10.70	[M]+	336.1239	336.123	2.6	C20H18N04	336.1235	336.1243; 320.0926; 292.0978; 278.0824;	Berberine hydrochloride	√	Rhizoma Coptidis



Supplementary Figure 1. Schematic diagram of DAD identification of a serial number of chromatographic peaks of Biling Weitong granules (UV 325nm).

3. Chromatographic conditions

Instrument: Agilent 1290 UPLC

Column: Agilent SB-C18; 2.1×100nm, 1.8μm;

Column temperature: 25℃

Injection volume: 1μL

Detection wavelength: 450nm

Mobile phase proportions and flow rate: Mobile phase A: 0.1% phosphoric acid aqueous solution; Mobile phase B: methanol. The gradient is shown in Supplementary Table 3.

Supplementary Table 3. Gradient of mobile phases

Time (min)	Flow rate (ml/min)	%B
0	0.3	10
2	0.3	16
15	0.3	35

15.1	0.3	95
17	0.3	95
17.1	0.3	10
20	0.3	10

4. Mass spectrometry (MS) conditions

Instrument: Sciex Triple TOF 4600 LC/MS

Detection mode: Positive/negative ion mode

Mass spectrometry parameters: see Supplementary Tables 4 and 5

Supplementary Table 4. Mass spectrometry parameters

Parameters	Parameter value
TOF mass range	50-100
Ion source gas 1	50
Ion source gas 2	050
Curtain gas	35
IonSpray voltage floating(kv)	-4500/5000
Ion source temperature (℃)	500
Declustering potential	100
Collision energy	10

Supplementary Table 5. MS/MS parameters

Parameters	Parameter value
Declustering potential	100
Collision energy	±40
Collision energy spread	20
Ion release	30

Ion release width	15

5. Quality control of BLWTG

The contents of four compounds (berberine hydrochloride, corydaline, chlorogenic acid, and naringin) were identified by fingerprinting, and the samples were the final products of three BLWT batches (batch numbers: 19030522, 19093022, and 19102122) produced by Jiangsu Pharmaceutical Co., Ltd., Yangzijiang Pharmaceutical Group.

(1) Detection results

Supplementary Table 6. Detection results of three BLWT batches

Batch	Compound	Content
19030522	Berberine hydrochloride	0.189%
	Corydaline	0.0011%
	Chlorogenic acid	0.0263%
	Naringin	1.007%
19093022	Berberine hydrochloride	0.167%
	Corydaline	0.00126%
	Chlorogenic acid	0.0182%
	Naringin	1.013%
19102122	Berberine hydrochloride	0.171%
	Corydaline	0.00099%
	Chlorogenic acid	0.0146%
	Naringin	1.021%

(2) Experimental methods

1) Preparation of reference solutions:

The appropriate amounts of berberine hydrochloride, corydaline, chlorogenic acid, and naringin were accurately weighed, added with 70% methanol to produce the reference solutions containing berberine hydrochloride, corydaline, chlorogenic acid, and naringin, respectively. The concentration was 20μg/mg in each solution.

2) Preparation of test solution:

BLWTG was harvested and ground into fine powder. We accurately weighed 0.5 g of the powder and placed it in a conical flask with a stopper, followed by addition of 25 mL 70% methanol, sonication for 30 min, and cooling. The mixture was shaken and filtered and the filtrate was used as the test solution.

3) Chromatographic conditions

Mobile phase A: acetonitrile.

Mobile phase B: 0.1% phosphoric acid aqueous solution

Supplementary Table 7. Elution chromatography

Duration (minutes)	Mobile phase A (%)	Mobile phase B (%)
0	10	90
10	18	82
18	30	70
26	36	64
26.1	95	5
31	95	5
31.1	10	90
40	10	90

Chromatography column: Agilent, SB-C18, 5 μm, 4.6 × 250 nm.

Detection wavelength: 325/204 nm; column temperature: 35°C.

Flow: 1.0 mL/min; run time: 40 min.

Appendix 3. Evaluation of the simulation effect of placebo

1. Placebo ingredients

The ingredients in each placebo pack (5 g) are shown in Supplementary Table 8.

Supplementary Table 8. Ingredients and their contents in each placebo pack (5 g)

Ingredients	Content (g)	Proportion (%)	Role(s)
Dextrin	3.19	63.8%	Pharmaceutic adjuvant
Sucrose	1.0	20.0%	Pharmaceutic adjuvant
BLWT fine powder	0.375	7.5%	Equivalent to 7.5% of the original formula
Povidone K30	0.25	5.0%	Pharmaceutic adjuvant
Caramel	0.125	2.5%	Pharmaceutical excipient / food additive
Low-substituted hydroxypropylcellulose	0.06	1.2%	Pharmaceutic adjuvant

2. Evaluation of the simulation effect of placebo

2.1 Time points of evaluation

The simulation effect of the prepared placebo was evaluated before the blinding of the test drug and the placebo to determine whether the placebo in this trial could mimic the drug being tested (i.e. BLWTG).

2.2 Patients and methods

A total of 36 patients with gastrointestinal diseases were enrolled. When the similarity of two samples was evaluated, four test bags were delivered to each patient. Each bag contained three numbered packs of preparations under the following two conditions: (1) two packs of placebo plus one pack of BLWTG; or (2) two BLWTG plus one pack of placebo. Each bag was randomly distributed to the evaluators (i.e., the subjects), and the samples in three packs were sequentially evaluated for similarity in terms of shape, texture, color, odor, and taste. One different sample or two identical samples were selected from each bag. During the three-point assay, the number of evaluators who judged correctly the shape, texture, color, and comprehensive evaluation should be smaller than half of the number of evaluators. (Supplementary Table 9)

Supplementary Table 9. Comparative Evaluation Form

Item	Scoring method	Criteria for a qualified placebo
A. Shape	A= Number of evaluators making a correct judgment	A < Number of evaluators/2
B. Texture	B= Number of evaluators making a correct judgment	B < Number of evaluators/2
C. Color	C= Number of evaluators making a correct judgment	C < Number of evaluators/2
D. Odor*	D= Number of evaluators making a correct judgment	
E. Taste*	E= Number of evaluators making a correct judgment	
S. Comprehensive evaluation	S= A+B+C+D+E/5	S < Number of evaluators/2

* In the comparative evaluation, Oder and taste were the most two difficult items. Therefore, individual evaluations were required only for shape, texture,

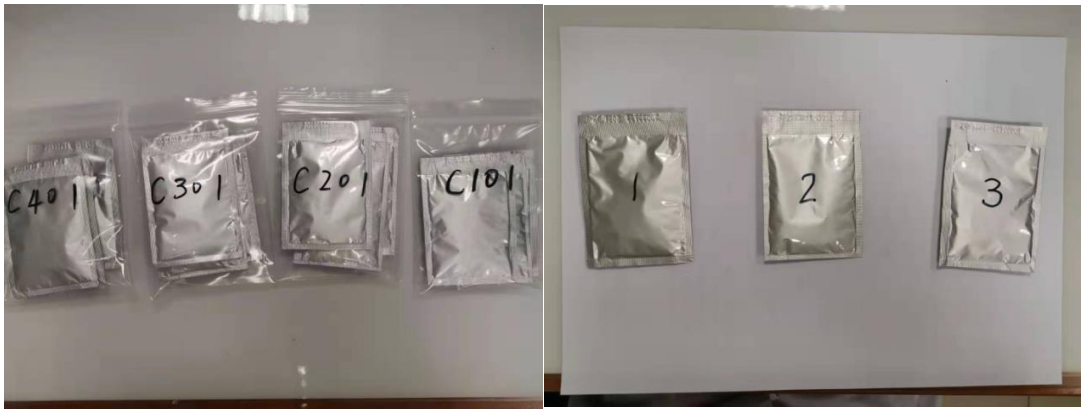
and color. Nevertheless, odor and taste were included in the comprehensive evaluation.

Supplementary Table 10. Results of comparative evaluation

Content of a comparative evaluation	Number of evaluators making a correct judgment	Criteria for a qualified placebo	Qualified or not
A. Shape	A= 4	A ＜ 18	Qualified
B. Texture	B= 3	B ＜ 18	Qualified
C. Color	C= 12	C ＜ 18	Qualified
D. Odor	D= 12	D ＜ 18	Qualified
E. Taste	E= 16	E ＜ 18	Qualified
S. Comprehensive evaluation	S= 9.4	S ＜ 18	Qualified

3. Conclusions

Evaluation of the simulation effect of the placebo showed that the placebo was consistent with BLWTG in terms of shape, texture, color, odor, and taste and during comprehensive evaluation (The results are shown in Supplementary Table 10). The subjects could not distinguish the placebo from BLWTG. Thus, the simulation effect of the placebo was qualified (Supplementary Figure 2).



Supplementary Figure 2. Comparisons between BLWTG and placebo.