Supplementary Material
Content List

1. Supplemental Material 1 [SPIRIT checklist]

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	2-3
Protocol version	3	Date and version identifier	23
Funding	4	Sources and types of financial, material, and other support	25
Roles and	5a	Names, affiliations, and roles of protocol contributors	1-2

responsibilities	5b	Name and contact information for the trial sponsor	1-2
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	25
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	19-20
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	21
	6b	Explanation for choice of comparators	11-12
Objectives	7	Specific objectives or hypotheses	3
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	2
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6-7

Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7-11
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11-12
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	13
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	in "Supplemental Material[Test schedule]"
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	In "Supplemental Material[Informed Consent Form]" page 8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	14
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	7
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	11

Methods: Assignment of interventions (for controlled trials)

Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	11
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	22
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	enema will not be blinded

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14,20
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	20
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	19-20
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	21
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	21
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	21
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	19
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	19

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	19
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	24
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	24
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	24
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	24-25
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	In "Supplemental Material[Informed Consent Form]"
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	None declared

Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	in "Supplemental Material [Informed Consent Form]"
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	19
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	in "Supplemental Material [Informed Consent Form]"
	31b	Authorship eligibility guidelines and any intended use of professional writers	25
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	in "Supplemental Material [Informed Consent Form]"

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	_in "Supplemental Material [Informed Consent Form]_" _____
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	_in "Supplemental Material [Informed Consent Form]_" _____

2. Supplemental Material 2 [Investigational Schedule]

Visit Evaluation content	Screening period	Baseline period	Therapeutic period				Drug withdrawal observation period
		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5 /Premature Discontinuation	Visit 6
	Day 7-0	Day 0	Day 14 ±2 days	Day 28 ±2 days	Day 42 ±2 days	Day 56 ± 7 days	Within 28 days after treatment discontinuation
Informed consent and signature of informed consent form	■						
Demographic characteristics[1]	■						
Disease-related conditions[2]	■						

Visit Evaluation content	Screening period	Baseline period	Therapeutic period				Drug withdrawal observation period
		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5 /Premature Discontinuation	Visit 6
	Day 7-0	Day 0	Day 14 ±2 days	Day 28 ±2 days	Day 42 ±2 days	Day 56 ± 7 days	Within 28 days after treatment discontinuation
History of drug therapy[3]	■						
Past medical history[4]	■						
Personal history[5]	■						
Family history[6]	■						
Allergic history[7]	■						

Visit Evaluation content	Screening period	Baseline period	Therapeutic period				Drug withdrawal observation period
		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5 /Premature Discontinuation	Visit 6
	Day 7-0	Day 0	Day 14 ±2 days	Day 28 ±2 days	Day 42 ±2 days	Day 56 ± 7 days	Within 28 days after treatment discontinuation
Vital sign[8]	■	■	■	■	■	■	■
Physical examination[9]	■	■	■	■	■	■	■
Blood routine test[10]	■					■	△
Erythrocyte sedimentation rate (ESR)	■					■	

Visit Evaluation content	Screening period	Baseline period	Therapeutic period				Drug withdrawal observation period
		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5 /Premature Discontinuation	Visit 6
	Day 7-0	Day 0	Day 14 ±2 days	Day 28 ±2 days	Day 42 ±2 days	Day 56 ± 7 days	Within 28 days after treatment discontinuation
C-reactive protein (CRP)	■					■	
Urine routine test[11]	■					■	△
Stool routine test (with occult blood)[12]	■					■	△
Fecal calprotectin test	■					■	

Visit Evaluation content	Screening period	Baseline period	Therapeutic period				Drug withdrawal observation period
		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5 /Premature Discontinuation	Visit 6
	Day 7-0	Day 0	Day 14 ±2 days	Day 28 ±2 days	Day 42 ±2 days	Day 56 ± 7 days	Within 28 days after treatment discontinuation
Liver and kidney function[13]	■					■	△
Electrolyte[14]	■						
Urine pregnancy test[15]	■						
Colonoscopy	■					■	
Mucosal histological examination	■					■	

Visit Evaluation content	Screening period	Baseline period	Therapeutic period				Drug withdrawal observation period
		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5 /Premature Discontinuation	Visit 6
	Day 7-0	Day 0	Day 14 ±2 days	Day 28 ±2 days	Day 42 ±2 days	Day 56 ± 7 days	Within 28 days after treatment discontinuation
12 lead electrocardiogram	■					■	△
Modified Mayo score	■					■	
Modified Mayo sub-score of stool frequency and rectal bleeding		■	■	■	■		
UCEIS score	■					■	

Visit Evaluation content	Screening period	Baseline period	Therapeutic period				Drug withdrawal observation period
		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5 /Premature Discontinuation	Visit 6
	Day 7-0	Day 0	Day 14 ±2 days	Day 28 ±2 days	Day 42 ±2 days	Day 56 ± 7 days	Within 28 days after treatment discontinuation
Mucosal healing histological score	■					■	
Symptom quantification scale	■	■	■	■	■	■	■
IBDQ scale score	■					■	■
Randomization		■					
Drug dispensing		■		■			

Visit Evaluation content	Screening period	Baseline period	Therapeutic period				Drug withdrawal observation period
		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5 /Premature Discontinuation	Visit 6
	Day 7-0	Day 0	Day 14 ±2 days	Day 28 ±2 days	Day 42 ±2 days	Day 56 ± 7 days	Within 28 days after treatment discontinuation
Recycling of surplus drugs				■		■	
Instruct the patient on enema administration	■	■	■	■	■		
Record of adverse events		■	■	■	■	■	■
Comorbidities and treatments	■	■	■	■	■	■	■

1. Demographic characteristics: including gender, age, height and weight.
2. Disease-related conditions: including diagnosis (including lesion range, disease stage and severity), course of diarrhea and hematochezia.
3. History of drug therapy: including the history of nonsteroidal anti-inflammatory drugs, antibiotics and other types of drugs within the last 1 month.
4. Past medical history: including hepatitis (which type), schistosomiasis, liver and biliary diseases, gastrointestinal diseases and other systemic diseases.
5. Personal history: recent travel history, history of abdominal surgery and postoperative condition, smoking and alcohol consumption and duration.
6. Family history: whether there is a history of similar diseases and tumors, genetic diseases and infectious diseases such as hepatitis in the family.
7. Allergic history: whether allergic to the test drug, and whether allergic to other drugs or foods.
8. Vital sign: including temperature, pulse, respiration, blood pressure (sitting).
9. Physical examination: including general condition and nutritional status, skin and mucous membrane, abdomen, perianal and perineal examination. Attention should be paid to extraintestinal manifestations such as mouth, skin, joint and eye, and perianal condition.
10. Blood routine test: red blood cell count, white blood cell count, platelet, hemoglobin, neutrophil count, lymphocyte count, monocyte count.
11. Urine routine test: protein in urine, sugar in urine, white blood cells in urine, red blood cells in urine.
12. Stool routine test (with occult blood): color, shape, red blood cells, white blood cells, fecal parasites, fat globules, Charcot-Leyden crystals, occult blood, fungi.
13. Liver and kidney function: alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin (TBIL), gamma-glutamyl

transferase (GGT), blood urea nitrogen (BUN), creatinine (Cr).

14. Electrolyte: potassium (K+), sodium (Na+), chloride (Cl-).

15. Urine pregnancy test: only women of childbearing age should do urine pregnancy test.

16. For the relevant laboratory tests during the screening period, if the subject has undergone the required laboratory tests (or endoscopic examinations) for the screening period at our hospital within 7 days prior to the baseline period and provided the results, which have been approved by the investigator, then the subject does not need to undergo the corresponding screening period tests again. The specific test items shall be based on the laboratory tests conducted by the research center, including but not limited to the required items, and in this trial, only the measurement values of the required items will be recorded, with additional recording for any clinically significant abnormalities.

17. "△" indicated that the safety index (laboratory test and 12-lead electrocardiogram test) was abnormal and clinically significant at the visit 5 or the last visit, and the relevant index was followed up.

18. "■" indicated that the visit evaluation content must be completed within the designated time window.

19. Colonoscopy, mucosal histological examination, modified Mayo score, UCEIS score and mucosal healing histology score can be performed at either visit 5 or visit 6.

20. Visit 6 acceptable colonoscopy results within 4 weeks.

3. Supplemental Material 3 [Informed Consent Form]

Informed Consent Form

Dear Participant:

We would like to invite you to participate in the study of "The efficacy of Huangkui Lianchang Decoction enema in the treatment of mild to moderate active distal UC: a multicentre prospective randomized controlled trial". This study will be conducted in 8 units, including Jiangsu Provincial Hospital of Traditional Chinese Medicine, Changshu Second People's Hospital, Huaian First People's Hospital, Lianyungang Hospital of Traditional Chinese Medicine, Lianyungang First People's Hospital, Changzhou Second People's Hospital, Yancheng First People's Hospital, and the Affiliated Hospital of Jiangnan University, etc. A total of 172 patients with UC will be voluntarily enrolled and randomly assigned to the experimental or control group in a 1:1 ratio. This study has been reviewed and approved by the Ethics Committee of Jiangsu Provincial Hospital of Traditional Chinese Medicine, an independent body composed of experts and lay members dedicated to protecting the rights of participants. It is important to note that participation in this study may still involve certain risks.

Why is this study being conducted?

UC is a chronic, non-specific inflammatory bowel disease of unknown etiology. Its rising incidence poses a significant disease burden and economic challenge. Achieving clinical symptom remission is a crucial treatment goal. However, current drug guidelines, primarily based on Western populations, are not universally effective. Chinese herbal enema, such as the Huang Kwai Astringent Formula for distal UC, represents a characteristic and advantageous approach in Traditional Chinese Medicine. It offers potential benefits in terms of safety, efficacy, and cost-effectiveness.

This project comprises a randomized controlled trial (RCT) in which 172 eligible patients with UC will be recruited and randomly allocated to an intervention group or a control group. The intervention group will be treated with Salofalk (mesalazine enteric-coated tablets) plus Huangkui Lianchang Decoction Enema (HKE), whereas the control group will receive Salofalk (mesalazine enteric-coated tablets) plus Salofalk (mesalazine enema).

Furthermore, anchored by this RCT, the project seeks to construct a multidisciplinary collaborative system for UC. This includes building an 8-hospital multicenter research network to boost collaborative capacity, completing the aforementioned clinical study to establish evidence-based TCM norms for UC, forming an expert pool and training talent, and ultimately strengthening the evidence-based research platform and collaborative network for TCM in UC treatment.

This study was supported by the National Natural Science Foundation of China (Grant No. 82341229). Funding was also received in part from the Evidence-Based Capacity Building Project of the National Administration of Traditional Chinese Medicine and from Jiangsu Jiuxu Pharmaceutical Co., Ltd.

What are the inclusion criteria for this study?

Only those who fulfill all of the following criteria will be enrolled:

（1）Meet UC medical diagnostic criteria1,11,14 : a. Disease stage: active phase; b. Disease severity is mild to moderate (refer to the modified Truelove and Witts disease severity classification criteria,15 see Table 1 for details); c. Lesion location is rectal or left-sided colonic type (recommended to use the Montreal classification,16 see Table 2 for details); d. Modified Mayo score17 of 3–10 points, and an endoscopic subscore of 2 or higher (modified Mayo score, see Table 3);

(2) Traditional Chinese medicine diagnosis of damp-heat accumulation in the intestines or spleen deficiency with dampness accumulation ;

(3) Age between 18 and 65 years;

(4) The patient voluntarily signs an informed consent form.

Who should not participate in the study?

Those who fulfill any of the following criteria need to be excluded:

(1) Patients with infectious colitis such as bacterial dysentery, amoebic dysentery, chronic schistosomiasis, intestinal tuberculosis, as well as Crohn's disease, ischemic colitis, and radiation colitis;

(2) Patients with severe complications such as local stricture, intestinal obstruction, intestinal perforation, toxic megacolon, massive bleeding, colorectal cancer, or rectal cancer;

(3) Patients with other primary or secondary infectious diseases, such as cholecystitis or pneumonia;

(4) Patients who have undergone enema therapy within the past two weeks;

(5) Patients currently receiving treatment with corticosteroids, thiopurine drugs, biologics, or probiotics;

(6) Patients with severe cardiovascular, hepatobiliary, pulmonary, renal, or hematological diseases;

(7) Patients with allergic constitutions, such as a history of allergy to two or more drugs or foods, or known allergy to the investigational drug;

(8) Patients with suspected or confirmed history of alcohol or drug abuse;

(9) Pregnant or breastfeeding women;

(10) Patients with disabilities as defined by law (blindness, deafness, mutism, intellectual disabilities, mental disorders, or physical disabilities);

(11) Other conditions that, in the investigator's judgment, may reduce eligibility or complicate enrollment, such as frequent changes in work environment that may lead to loss to follow-up;

(12) Patients currently participating in other clinical trials.

Grouping method of this study

A total of 172 eligible patients with UC were randomly assigned to either the experimental group or the control group. The experimental group received treatment with Salofalk (mesalazine enteric-coated tablets) in combination with HKE, while the control group received Salofalk (mesalazine enteric-coated tablets) combined with Salofalk (mesalazine enema solution).

The treatment program of this study

1.Standard Therapy

All participants will receive conventional oral therapy with Salofalk® (mesalazine enteric-coated tablets, 0.5g per tablet; Shenzhen Kangzhe Pharmaceutical Co., Ltd.) at a dose of 2g daily.

Lifestyle Guidance: In addition to pharmacotherapy, patients will be advised to adhere to the following lifestyle modifications throughout the study period:Maintain regular bowel habits and ensure thorough perianal hygiene after defecation. Adopt a bland diet, limiting high-fiber intake and avoiding spicy, greasy, or irritating foods, as well as tobacco and alcohol. Keep a regular daily schedule, balance work and rest, engage in moderate physical activity to improve overall fitness, and maintain emotional well-being.

2. Interventions and Administration Procedures

(1) Experimental Group (HKE Enema)

Participants in the experimental group received Huangkui Lianchang Enema (HKE) at a dose of 60 mL, administered via rectal instillation once daily prior to bedtime.

Administration Procedure: Preparation: Participants were instructed to evacuate their bowels beforehand. The enema apparatus was prepared, and the solution temperature was maintained at 37 – 39 ° C.Equipment Setup: The tip of the enema tube was lubricated with petroleum jelly. Air was expelled from the tube, which was then clamped. Patient Positioning: Participants assumed the left lateral decubitus position. Clothing was adjusted to expose the perianal area. A protective pad was placed beneath the hips, and a pillow was used to elevate the buttocks by approximately 10 cm. Administration: The lubricated tube tip was gently inserted into the rectum to a depth of about 10 cm. Subsequently, 60 mL of the solution was instilled slowly. Post-administration Care: The tube was carefully withdrawn, and the perianal area was dried. Participants were required to remain in a supine position for at least 30 minutes before resuming a comfortable posture. Safety Monitoring: The procedure was to be discontinued immediately upon the occurrence of severe abdominal pain, diaphoresis, or any other distressing symptoms.

(2) Control Group (Mesalazine Enema)

Participants in the control group received a mesalazine enema solution (60 mL containing 4 g mesalazine) once nightly before bedtime. Administration Procedure: Preparation: The medication bottle was shaken for 30 seconds before use, and the protective cap was removed. Patient Positioning: Participants assumed the left lateral decubitus position. Administration: After lubricating the applicator tip, the tube was inserted into the rectum. The entire contents of the bottle (60 mL) were instilled slowly to ensure complete delivery. Post-administration Care: Participants remained in a supine position for at least 30 minutes before assuming a comfortable posture.

(3) Treatment Duration

The total treatment duration for both groups was 8 weeks.

(4) Visit Schedule and Procedures

Screening Period (Days -7 to 0)

During the screening visit, participants will undergo a comprehensive eligibility assessment conducted by the study physician. This includes: Medical History: Collection of demographic data, disease history, concomitant/past medications, personal/family history, and allergy history. Clinical Assessments: Vital signs (pulse, respiration, seated blood pressure, temperature), physical examination, and administration of the Modified Mayo Score, UCEIS, histology score, symptom quantification questionnaire, and IBDQ scale. Laboratory Tests: Complete blood count, ESR, CRP, urinalysis, stool routine (including occult blood), fecal calprotectin, liver/kidney function, electrolytes, and a urine pregnancy test for women of childbearing potential. Diagnostic Procedures: 12-lead electrocardiogram and colonoscopy with mucosal biopsy for histology. Study Procedures: Verification of recent participation in other clinical trials and instruction on the proper enema administration technique.

Note: To avoid duplication, results from laboratory tests performed at this institution within 7 days prior to baseline, or endoscopy within 1 month prior to baseline, may be used if approved by the investigator.

Baseline / Visit 1 (Day 0)

Eligible participants will proceed to the baseline visit, which includes:

Clinical Assessments: Measurement of vital signs, physical examination, and completion of the stool frequency/blood subscales of the Modified Mayo Score and the symptom quantification form.

Randomization & Dispensing: Participants who meet all eligibility criteria will be formally enrolled, randomly assigned to a treatment group, and receive detailed enema administration instructions. Study medication for 28 days (+2 days allowance) will be dispensed.

Safety Monitoring: Recording of concomitant medications and assessment of any adverse events.

Treatment Period / Visit 2 (Day 14 ± 2 days)

Participants will return for the first on-treatment assessment, which involves:

Clinical Assessments: Vital signs, physical examination, and completion of the relevant Modified Mayo subscales (stool frequency and rectal bleeding) and the symptom quantification form.

Procedure Review: Re-institution on enema administration to ensure protocol adherence.

Safety Monitoring: Update on concomitant medications and assessment of any adverse events.

Treatment Period / Visit 3 (Day 28 ± 2 days)

Participants will return for the mid-point treatment assessment. The visit procedures are as follows:

Clinical Assessments: Measurement of vital signs (pulse, respiration, seated blood pressure, temperature), physical examination, and completion of the Modified Mayo subscales for stool frequency and rectal bleeding, along with the symptom quantification form.

Medication Management: Participants will return any unused study medication from the previous period. The next supply of trial medication (covering Days 29 to 56, with a +7-day allowance) will be dispensed.

Procedure Adherence: The study physician will reinforce enema administration instructions.

Safety Monitoring: Concomitant medications will be reviewed, and any adverse events or other safety concerns will be recorded.

Treatment Period / Visit 4 (Day 42 ± 2 days)

Participants will attend the final on-treatment assessment, which includes:

Clinical Assessments: Measurement of vital signs, physical examination, and completion of the relevant Modified Mayo subscales (stool frequency and rectal bleeding) and the symptom quantification form.

Procedure Review: The study physician will confirm the correct enema administration technique.

Safety Monitoring: An update on concomitant medication use and a systematic assessment for the occurrence of any adverse events.

End-of-Treatment / Visit 5 or Early Withdrawal (Day 56 ± 7 days)

Participants will attend the end-of-treatment visit. The procedures include:

Clinical & Laboratory Assessments: Measurement of vital signs (pulse, respiration, seated blood pressure, temperature), physical examination, and the following tests: complete blood count, ESR, CRP, urinalysis, stool routine (including occult blood), fecal calprotectin, liver/kidney function tests, and a 12-lead ECG.

Endoscopic & Histologic Evaluation: Colonoscopy with mucosal biopsy for histology examination.

Questionnaire Administration: Completion of the Modified Mayo Score, UCEIS, mucosal healing histology score, symptom quantification questionnaire, and the IBDQ scale together with the study physician.

Study Procedures: Return of any unused study medication.

Safety Monitoring: Review of concomitant medications and assessment of any adverse events.

Note: Colonoscopy with mucosal histology, and the associated endoscopic/histologic scores (Modified Mayo, UCEIS, mucosal healing), may be performed either at Visit 5 or at the follow-up Visit 6, based on clinical scheduling.

Post-Treatment Follow-up / Visit 6 (Within 28 days after treatment discontinuation)

Participants will return for a final safety and efficacy follow-up assessment. The visit comprises:

Clinical Assessments: Measurement of vital signs and physical examination.

Endoscopic & Questionnaire Evaluation: Colonoscopy (results from a procedure performed within the prior 4 weeks may be accepted), mucosal histology examination, and completion of the Modified Mayo Score, UCEIS, mucosal healing histology score, symptom quantification scale, and IBDQ scale.

Safety Follow-up Tests: If any clinically significant abnormalities were identified in the complete blood count, urinalysis, stool routine, liver/kidney function tests, or 12-lead ECG at Visit 5, the corresponding tests will be repeated during this visit.

Safety Monitoring: Final review of concomitant medications and assessment for any adverse events or other safety information.

Note: As indicated for Visit 5, the colonoscopy, mucosal histology, and related scoring may be conducted at either Visit 5 or Visit 6.

3. Concomitant Medications

(1) Restrictions on Concomitant Therapy

Throughout the study observation period, the use of any other pharmacological treatments (Chinese or Western medicine) or non-pharmacological therapies specifically for UC is not permitted, with the exception of the investigational interventions outlined in this protocol.

(2) Documentation of Permitted Medications

Participants will be instructed to bring all medications they are currently taking to each study visit for review. The use of medications necessary for the treatment of stable comorbid conditions, or any other therapies, must be documented in the case report form. Documentation will include the drug (or therapy) name, dosage, frequency, timing of administration, and indication, to facilitate subsequent analysis and reporting.

What are the potential risks or side effects of participating?

The investigational product, Huangkui Lianchang Enema (HKE), has shown a favorable safety profile in prior clinical use. Commonly reported reactions are mild and may include transient intestinal discomfort or a sensation of rectal irritation. In the rare event of such symptoms occurring during the study, the enema administration may be temporarily paused, and the study physician should be contacted for further guidance. There are currently no known significant adverse reactions associated with its use. Beyond these considerations, participation in this study is not expected to pose significant risks to your treatment outcome, recovery, or daily activities.

What are the potential benefits of participating?

Your participation will contribute to determining which treatment approach is safer and more effective for patients with UC. Throughout the study, you will receive close monitoring and standardized medical care from the research team. Additionally, you will be helping to advance medical knowledge in this field, which may benefit future patients.

Is there any cost to participate in this study?

No, you will not incur any costs for participating in this study. All study-related expenses will be covered. This includes: The full course of oral Salofalk (mesalazine enteric-coated tablets) and the

assigned enema medication (either Huangkui Lianchang Enema or Salofalk mesalazine enema). All scheduled tests and procedures, such as vital signs measurements, physical examinations, laboratory tests, colonoscopies (except for the initial diagnostic colonoscopy performed to confirm your eligibility), mucosal biopsies, and electrocardiograms (ECG). Upon completion of all required study visits, you will receive a stipend of 500 Chinese Yuan (CNY) to compensate for your time and travel expenses.

What tests and examinations are involved in the study?

Your health and treatment response will be carefully monitored throughout the study using a series of standardized assessments. These include: Regular checks of vital signs (pulse, respiration, blood pressure, temperature) and physical examinations. Blood Tests: Complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), liver and kidney function panels, and electrolyte analysis. Urinalysis, stool routine examination (including occult blood test), and fecal calprotectin test. Urine pregnancy test for women of childbearing potential. Colonoscopy with mucosal biopsy for histological examination, and 12-lead electrocardiogram (ECG).

Is my personal information confidential?

Your medical records will be stored at the hospital. Researchers, the research administration department, and the ethics committee will be permitted to access your medical records. Any public reports on the results of this study will not disclose your personal identity. We will make every effort to protect the privacy of your personal medical information within the scope permitted by law.

Will I be informed of the study results?

If you wish to receive the study results, please inform us. We will register your request and provide you with the findings upon completion of the study. In accordance with international ethical guidelines, data concerning major health issues, information with direct clinical applicability, or details demonstrating direct clinical efficacy will be communicated to participants both during and after the study. Conversely, information lacking scientific validity or clinical significance is not suitable for sharing with participants.

Will my medical records and biological specimens be used in studies other than this one?

In accordance with national regulations, the continued storage of remaining specimens after a clinical trial concludes, or their potential future use, requires participants to sign an informed consent form. This form must specify the storage period, data confidentiality, and the circumstances under which data and samples may be shared with other researchers. Therefore, we will not use your medical records or biological specimens for other research without your informed consent.

Do I have to participate in the study?

Participation in this study is entirely voluntary. You may decline to participate or withdraw at any time during the study without affecting your medical treatment. If you decide to withdraw, please contact your doctor.

Subject Declaration: I have read the above information about this study and fully understand the potential risks and benefits of participation. I voluntarily agree to participate in this study.

Participant's Signature：_____ Date：__ __ __ __, __ __ ,__ __

Contact Details of the Participant:_____

Investigator's Statement: I confirm that I have explained the detailed nature of this study to the participant, particularly the potential risks and benefits associated with participation..

Investigator's Signature ： _____ Date：__ __ __ __, __ __ , __ __

Contact Details of the Investigator:_____

Affiliated Hospital of Nanjing University of Chinese Medicine Ethics Committee Office
Contact Tel: 025-86560515