# Mechanism of Dachaihu Decoction's Effect on Visceral Pain Behavior in a Rat Model of Ulcerative Colitis

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**Abstract** [**Objectives**] To investigate the mechanism by which Dachaihu Decoction affects visceral pain behavior in a rat model of ulcerative colitis (UC). [**Methods**] Twenty-one male Sprague-Dawley (SD) rats were randomly and equally divided into a control group, a UC group, and a Dachaihu Decoction group ($n=7$ per group). After treatment completion, visceral pain behavior and colonic motility indices were assessed using in vivo colorectal distension (*CRD*) and abdominal withdrawal reflex (*AWR*) tests. [**Results**] Compared to the UC group, the Dachaihu Decoction group exhibited increased colon length, elevated colonic basal tension, heightened peak contractile tension, and increased contraction frequency. These differences were statistically significant ($P<0.05$). [**Conclusions**] Dachaihu Decoction may ameliorate visceral hyperalgesia induced by rectal distension stimuli in the UC rat model by improving colonic motility.

**Key words** Rat, Dachaihu Decoction, Ulcerative colitis, Visceral pain behavior, Intestinal motility

## 1 Introduction

Ulcerative colitis (UC) is a chronic, non-specific inflammatory bowel disease of unknown etiology. Its primary clinical manifestations include fever, chronic anemia, mucopurulent bloody stool, abdominal pain, and diarrhea[1]. Pathologically, it is characterized by non-specific inflammation of the rectal and/or colonic mucosa and submucosa. In prolonged cases, inflammation typically involves the distal colon and may progress proximally to affect the entire colon in severe instances[2]. Dachaihu Decoction, a classical formula originating from Zhang Zhongjing's *Treatise on Cold Damage and Miscellaneous Diseases* (*Shang Han Za Bing Lun*), is renowned for its effects in promoting blood circulation to resolve stasis, clearing heat to stop diarrhea, regulating *qi* to relieve depression, alleviating pain, soothing the liver, and fortifying the spleen. Clinical application reports exist for its use[3]. Therefore, this study aimed to observe and analyze the effect of Dachaihu Decoction on intestinal sensitivity in a rat model of ulcerative colitis, thereby providing a theoretical basis for its clinical application in UC treatment.

## 2 Materials and methods

**2.1 Animals** Twenty-one specific pathogen-free (SPF) male Sprague-Dawley (SD) rats, aged 49 d and weighing 200 - 230 g, were used. The housing and experimental procedures adhered to the 3R principles (Replacement, Reduction, Refinement) to ensure animal welfare: rats had free access to food and water, and were maintained in both the animal facility and laboratory at a constant temperature of 24 ℃, relative humidity of 65% - 75%, and under a 12-hour light/dark cycle.

**2.2 Equipment and reagents** P-97 glass micropipette puller [MicroData Instrument (Wuhan) Co., Ltd.]; BL-420N biological signal recording system (Chengdu Taimeng Software Co., Ltd.). Crude herbs for Dachaihu Decoction were provided by the Traditional Chinese Medicine Pharmacy of Taihe Hospital (Affiliated Hospital of Hubei University of Medicine). The decoction was prepared according to the formula: *Bupleurum chinense* 15 g, *Pinellia ternata* 9 g, *Scutellaria baicalensis* 9 g, *Citrus aurantium* (immature bitter orange) 9 g, *Paeonia lactiflora* (white peony root) 9 g, *Rheum palmatum* (rhubarb) 6 g, *Ziziphus jujuba* 5 pieces, *Zingiber officinale* (fresh ginger) 15 g. The mixture was decocted and concentrated to a solution containing 2 g of crude herbs per 1 mL for use.

**2.3 Model establishment and grouping** Fourteen SD rats with similar body weights were selected. They were provided with 3% dextran sodium sulfate (DSS) in drinking water ad libitum for 7 d. On days 8 and 16, an 8% acetic acid solution was administered via colonic instillation. Successful model establishment was defined by the presence of dull fur, reduced activity, decreased food intake, failure to gain or loss of body weight, loose or bloody stool, and significant elevation of IL-6 or IL-1β levels in colonic tissue[4]. On day 8 post-modeling, rats that met the successful modeling criteria were randomly and equally divided into the UC group and the Dachaihu Decoction group. An additional seven non-modeled rats served as the control group.

**2.4 Treatment** The Dachaihu Decoction group received Dachaihu Decoction via enema (0.4 mL per rat, twice daily, morning and evening). Both the control group and the UC group received an equivalent volume of saline via enema. Treatment continued for 4 consecutive weeks.

**2.5 Assessments**

**2.5.1** Colorectal Distension (*CRD*) and Abdominal Withdrawal Reflex (*AWR*) test. After a 12-h fast, rats were anesthetized with isoflurane. A lubricated balloon catheter was gently inserted into the anus, positioning the balloon 2 cm proximal to the anal verge, and the catheter was secured. Following a 30-min adaptation period, the balloon was rapidly inflated sequentially with 0.04, 0.08, 0.12, and 0.16 mL of saline. Each distension lasted 20 sec, with a 2-min interval between distensions. Each pressure gradient was

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repeated 3 times, with a 5-min interval between gradients. The abdominal wall response to distension was observed and the *AWR* score was recorded: 0 = no response; 1 = slight head movement; 2 = abdominal contraction without lifting; 3 = abdominal contraction with lifting off the surface; 4 = body arching and lifting of the pelvis or scrotum. The pain threshold (PT) was defined as the minimal distension volume at which visible lifting of the lower abdomen off the chamber floor or significant abdominal contraction was observed. The experiment was conducted independently by two operators. The *AWR* score for each distension volume was calculated as the average of the three repetitions[5].

**2.5.2** Disease Activity Index (DAI) scoring. Colonic tissue was collected. The disease activity index (*DAI*) was calculated as: *DAI* = (Weight loss index + Stool consistency score + Fecal occult blood score)/3.

**2.5.3** *Ex vivo* colonic motility test. Segments of colon were harvested. The BL-420N system was used to record basal tension, peak contractile tension, and contraction frequency. Basal tension was measured as the trough tension. Peak contractile tension was expressed in grams (g). Contraction frequency was expressed as contractions per minute.

**2.6 Statistical analysis** Statistical analysis was performed using SPSS 25.0 software. Measurement data are presented as mean ± standard deviation ($\bar{x} \pm s$). Comparisons among multiple groups were conducted using analysis of variance (ANOVA). For data conforming to a normal distribution, inter-group comparisons were made using the Student's *t*-test. For non-normally distributed data, the adjusted *LSD* *t*-test was used. A *P*-value of less than 0.05 ($P < 0.05$) was considered statistically significant.

## 3 Results and analysis

The effects of Dachaihu Decoction on visceral pain behavior and intestinal motility in rats were analyzed. As shown in Table 1, compared to the control group, rats in the UC group exhibited a shortened colon length, decreased colonic basal tension, reduced peak contractile tension, and diminished contraction frequency ($P < 0.05$). Compared to the UC group, the Dachaihu Decoction group demonstrated increased colon length, reduced *CRD* and *AWR* scores, elevated colonic basal tension, heightened peak contractile tension, and increased contraction frequency ($P < 0.05$).

**Table 1 Comparison of colon length, pain behavior, and intestinal motility results among groups** ($n = 7$, $\bar{x} \pm s$)

| Group | Colon length cm | CRD//mmHg | AWR//mmHg | PT//mmHg | Basal tension//g | Contraction frequency times/min | Peak contractile tension |
|---|---|---|---|---|---|---|---|
| Control | 7.85 ± 0.21 | 0.01 ± 0.01 | 25.36 ± 1.04 | 25.36 ± 1.04 | 2.74 ± 0.05 | 8.01 ± 0.09 | 4.36 ± 0.17 |
| UC | 6.07 ± 0.41# | 5.26 ± 0.54# | 19.81 ± 0.87# | 19.81 ± 0.87# | 1.22 ± 0.13# | 5.32 ± 0.22# | 3.02 ± 0.09# |
| Dachaihu Decoction | 7.74 ± 0.69* | 0.52 ± 0.01* | 25.61 ± 1.95* | 25.61 ± 1.95* | 2.69 ± 0.12* | 7.86 ± 0.14* | 4.25 ± 0.27* |

**NOTE** #$P < 0.05$ vs control group; *$P < 0.05$ vs UC group.

## 4 Discussion

Traditional Chinese Medicine (TCM) posits that dampness is the primary pathogenic factor in ulcerative colitis, arising from "dampness generating heat and congesting qi and blood". Prolonged or recalcitrant cases are particularly attributed to "yin deficiency complicated by dampness". Consequently, treatment primarily focuses on clearing heat, supplemented by nourishing the kidney to moisten dryness, and fortifying yin to support the spleen[6]. Dachaihu Decoction originates from the *Treatise on Cold Damage and Miscellaneous Diseases* (*Shang Han Za Bing Lun*). Composed of *S. baicalensis*, *B. chinense*, *P. lactiflora*, *C. aurantium*, *P. ternata*, *R. palmatum*, *Z. jujuba*, and *Z. officinale*, it is traditionally indicated for combined Shaoyang-Yangming syndrome, characterized by symptoms such as fullness and discomfort in the chest and hypochondrium, alternating chills and fever, epigastric fullness and stuffiness, and diarrhea or constipation[7]. Modern research has demonstrated that this formula possesses effects including modulating gut microbiota, balancing inflammatory cytokines, protecting the gastric mucosa, and promoting healing and functional recovery of the colonic mucosa[8]. Within the formula: *S. baicalensis* clears heat and dries dampness; *B. chinense* soothes the liver and promotes bile flow; *P. lactiflora* nourishes blood, softens the liver, and relieves spasm and pain; *P. ternata* directs rebellious qi downward and resolves phlegm; *R. palmatum* purges fire and promotes defecation; *Z. officinale* harmonizes the stomach and checks vomiting; *C. aurantium* moves qi and dissipates stagnation; *Z. jujuba* tonifies *qi* and harmonizes the middle *jiao*. The combined actions of these herbs synergistically achieve the effects of soothing the liver and fortifying the spleen, clearing heat and stopping diarrhea, and activating blood circulation to relieve pain[9].

*CRD* and *AWR* are established methods for assessing visceral sensitivity and pain behavior. This study demonstrated that, compared to the UC group, the Dachaihu Decoction group exhibited reduced *CRD* and *AWR* scores, increased colon length, and enhanced colonic basal tension, peak contractile tension, and contraction frequency. These findings indicate that Dachaihu Decoction alleviates diarrhea, improves stool consistency, and reverses the hyperalgesia induced by rectal distension stimuli.

In summary, Dachaihu Decoction possesses the therapeutic actions of promoting blood circulation to resolve stasis and clearing heat to stop diarrhea. From an electrophysiological perspective, it is hypothesized that Dachaihu Decoction may enhance colonic motility in the UC rat model and reverse distension-induced hyperalgesia by promoting the opening of L-type calcium channels, there-

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by increasing $Ca^{2+}$ influx. This mechanism likely contributes positively to maintaining intestinal motility and overall gastrointestinal physiological function.

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