

Effect of Chang Run Tong on The Biomechanical and Morphometric Remodeling of Colon and Rectum in Streptozotocin-Induced Diabetic Rats

Hong Sha¹ Dong Zhao¹ Jingbo Zhao^{2,3,*} Guifang Liu⁴ Zhong Zhen⁴
Pengmin Chen¹ Xiaolin Tong⁴ Hans Gregersen⁵

¹Institute of Clinical Medicine, China-Japan Friendship Hospital, Beijing 100029, China

²Mech-Sense, Aalborg Hospital, DK 9000 Aalborg

³Institute of Clinical Medicine, Aarhus University, DK8200 Aarhus N, Denmark

⁴Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing 100053, China

⁵The College of Bioengineering, Chongqing University, Chongqing, China

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Abstract

The present study investigates the effect of Chang Run Tong (CRT) on the biomechanical and morphometrical remodeling of colon and rectum in streptozotocin-induced diabetic rats. The colonic and rectal segments were obtained from diabetic (DM), CRT-treated diabetic (T1, high dosage: 50 g/kg/day; T2, low dosage: 25 g/kg/day) and normal (Con) rats. The experimental period was 60 d. The blood glucose level and body weight were measured. The circumferential length, wall thickness, and opening angle were measured from the segments in the no-load and zero-stress states. The residual strain was computed from the morphometry data. The step-wise distension was done on the colonic segment (from 0 to 20 cmH₂O). The circumferential and longitudinal stresses and strains were computed. The blood glucose level was significantly higher and the body weight was significantly lower in the DM, T1, and T2 groups compared to those in the Con group ($p < 0.01$, $p < 0.001$). The glucose level did not differ among the DM, T1, and T2 groups. The wet weight per unit length to body weight ratio, wall thickness, cross-sectional wall area, opening angle, and absolute value of residual strain of colonic and rectal segments in the DM group were significantly higher than those in the Con group ($p < 0.05$ and $p < 0.01$), and those in the T1 group, but not those in the T2 group, were significantly lower than those in the DM group ($p < 0.05$, $p < 0.01$). Furthermore, the circumferential and longitudinal stiffness of the colonic wall in the DM group was higher than those in the Con group. T1, but not T2, treatment could significantly decrease the colonic wall stiffness in both directions ($p < 0.01$). CRT (high dose) treatment could partly restore the morphometric and biomechanical remodeling of the lower gastrointestinal tract in diabetic rats.

Keywords: Diabetic rats, Colon, Rectum, Chang Run Tong, Biomechanics, Morphometry, Remodeling

1. Introduction

Constipation is very common in the general population [1] and affects the quality of life [2]. Colonic and rectal sensory-motor function (sensation and motility) and biomechanical components (compliance and capacity) are now strongly implicated in the pathogenesis of constipation [3]. However, the pathophysiological mechanisms underlying chronic constipation remain to be explored [4,5]. Constipation is difficult to treat and has a high recurrence rate [6].

A large number of clinical data have shown that Chinese medicine has good clinical effects on constipation [7]. Through clinical observation, the authors have found that the Chang Run Tong (CRT) decoction produced by China-Japan Friendship Hospital can effectively treat senile constipation. From Chinese medicine point of view, CRT regulates qi, relieves stagnation and lubricates the bowel. However, from the western medicine point of view, the mechanism of CRT in the treatment of constipation is unclear.

The gastrointestinal (GI) tract is functionally subjected to dimensional changes. Hence, biomechanical properties such as the stress-strain relationship are of particularly importance [8]. The biomechanical properties are crucial for GI motor function because peristaltic motion that propels the food through the GI tract is a result of the interaction of the passive and active tissue

* Corresponding author: Jingbo Zhao
Tel: +45-99326907; Fax: +45-99326801
E-mail: jz@rn.dk

forces and the hydrodynamic forces in the food bolus. The remodeling of the mechanical properties reflects the changes in the tissue structure that determine a specific motor dysfunction. A previous study has demonstrated that experimental diabetes could induce colon morphological and biomechanical remodeling [9]. Following the development of diabetes, the colonic wall became thicker and the stiffness of the wall increased in a time-dependent manner. Diabetes-induced morphological and biomechanical remodeling plays an important role in diabetic GI complications, including constipation [10]. Therefore, the streptozotocin (STZ)-induced diabetic rat model is adopted to study the effect of drugs in the treatment of constipation on the morphological and biomechanical remodeling of the GI tract caused by diseases.

In order to investigate the mechanism of CRT in the treatment of constipation, the present study investigates whether CRT treatment can improve the morphometric and biomechanical remodeling of the colon and rectum in STZ-induced diabetic rats.

2. Materials and methods

2.1 Animal model and groups

Forty male Sprague-Dawley rats weighing 220-250 g were included in this study. Thirty rats were made diabetic by a single tail-vein injection of 40 mg/kg STZ (Sigma-Aldrich, China). This dose of STZ resulted in a random blood glucose level (≥ 16.7 mmol/L) in 90% of the rats 7 d after injection. The remaining 10% of rats were excluded from this study. Twenty-seven STZ-induced diabetic rats were subdivided into three groups ($n = 9$ in each group), namely the diabetic control group (DM), the high-dose CRT group (T1), and the low-dose CRT group (T2). Another ten rats of similar age and body weight from the same vendor were used as the non-diabetic control group (Con).

2.2 Drugs and administration methods

CRT is composed of *Radix Angelicae Sinensis*, *Radix Cyathulae*, *Herba Cistanches*, *Rhizoma Alismatis*, *Rhizoma Cimicifugae*, *Fructus Aurantii Immaturus*, *Rhizoma Atractylodis Macrocephalae*, *Semen Arecae Preparata* and *hemp seed* provided by China-Japan Friendship Hospital, The Ministry of Health of the People's Republic of China. The medicine was directly injected into the stomach lumen by gastric gavage once daily from the beginning of the experiment. The dosage was 50 g/kg/day for T1 and 25 g/kg/day for T2. The rats of the DM and Con groups were only given physiological saline.

2.3 Experimental procedures

The body weight and blood glucose levels were measured at 2-week intervals after the start of the experiment. The experimental period was 60 d. At the ending of the experiment, the rats fasted overnight and then were anesthetized with 4% chloral hydrate (10 mL/kg, ip). Following laparotomy, the whole colon and rectum were harvested. After the lumen of the

segments was gently cleaned with saline, the length and the wet weight were measured. The colonic segment was divided into two parts: a proximal 1-cm-long part was used for the zero-stress state experiment and the remaining part was used for the distension test. The rectal segment was only used for the zero-stress state experiment.

2.4 Zero-stress state experiment

For obtaining data from the zero-stress state, three 1-2-mm-wide colonic and rectal rings were cut and placed in Krebs solution at room temperature. The composition of Krebs solution (mmol/L) is: NaCl, 118; KCl, 4.7; NaHCO₃, 25; NaH₂PO₄, 1.0; MgCl, 1.2; and ascorbic acid, 0.11. A photograph was taken of the cross-section of the rings using a camera (Canon PowerShot A2200, Japan), representing the no-load state. Each ring-shaped segment was then cut radially from the opposite mesentery site. Photographs were taken about 60 min after the radial cutting to allow viscoelastic creep to take place, representing the zero-stress state.

2.5 Distension test

The distal end of the remaining colonic segment was tied with a suture and the proximal end was cannulated with a tube for the distension experiment. After the segment was preconditioned (from 0 to 20 cmH₂O), it was inflated with Krebs solution using a step-wise distension protocol from 0 to 20 cmH₂O (0, 1, 2, 3, 5, 10, 15, and 20 cm H₂O). The segment conformed to a cylindrical geometry during the distensions. Each pressure application lasted 2 min. The outer diameter and length of the segment were then photographed using the camera.

2.6 Mechanical data analysis

The morphometric data were obtained from digitised images of the segments in the zero-stress, no-load, and pressurised states. Measurements were undertaken using image analysis software (SigmaScan ver. 4.0, Sigma Corp., San Rafael, CA, USA). The following data were measured from each specimen: the circumferential length (C), the wall thickness (h), the wall cross-sectional area (A), and the opening angle at zero-stress state (α). The subscripts i, o, n, z, and p refer to the inner (mucosal) surface, outer (serosal) surface, no-load state, zero-stress state, and pressurised state, respectively. The opening angle α was defined as the angle subtended by two radii drawn from the midpoint of the inner wall to the inner tips of the two ends of the specimen. The outer diameter (D) and length (L) were measured from images of the pressurised segments.

The measured data was used for computation of the following biomechanical parameters:

Residual Green's strain at the mucosal surface:

$$E_i = ((C_{i-n}/C_{i-z}) * (C_{i-n}/C_{i-z}) - 1) / 2 \quad (1)$$

Residual Green's strain at the serosal surface:

$$E_o = ((C_{o-n}/C_{o-z}) * (C_{o-n}/C_{o-z}) - 1) / 2 \quad (2)$$

The stress and strain of the colonic segment in the pressurised state were determined under assumptions that the wall was homogenous and the organ shape was cylindrical. The calculation was done using the no-load state dimensions and the outer diameters and lengths of the specimen at varying pressures, with the assumption that the wall is incompressible. Series calculation was performed using previously reported methods [11] and the parameters in the pressurized state, namely the longitudinal stretch ratio λ_ϕ , the luminal radius r_{i-p} , the wall thickness h_p , the mucosal circumferential length C_{i-p} , the serosal circumferential length C_{o-p} , the mid-wall circumferential length C_{m-p} , and the circumferential stretch ratio λ_θ . Then, Kirchhoff's stress and Green's strain in a wall at a given pressure were computed as:

Circumferential Kirchhoff's stress:

$$S_\theta = (\Delta P * r_{i-p}) / (h_p * \lambda_\theta * \lambda_\theta) \quad (3)$$

Longitudinal Kirchhoff's stress:

$$S_\phi = (\Delta P * (r_{i-p}) * (r_{i-p})) / (h_p * \lambda_\phi * \lambda_\phi * (r_{o-p} + r_{i-p})) \quad (4)$$

Circumferential mid-wall Green's strain:

$$E_\theta = (\lambda_\theta * \lambda_\theta - 1) / 2 \quad (5)$$

Longitudinal Green's strain:

$$E_\phi = (\lambda_\phi * \lambda_\phi - 1) / 2 \quad (6)$$

where ΔP is the transmural pressure difference. The longitudinal mid-wall stretch ratio was referenced to the no-load state because tissue strips could not be cut for obtaining the zero-stress state in the longitudinal direction. The longitudinal mid-wall length in the rat intestine does not differ between the no-load and zero-stress states [10].

2.7. Statistical analysis

The data were representative of a normal distribution and accordingly the results were expressed as means \pm standard error of the mean (SEM). The stress-strain curve for each direction was fitted using the exponential function equation,

$$S = (S^* + b) * EXP(a * (E - E^*)) - b \quad (7)$$

where S^* and E^* are the stress and strain at a physiological reference level, respectively [8]. The constants a and b were used for the statistical evaluation of the stress-strain data. Analysis of variance was used to detect the differences of different parameters in different groups (Sigmastat 2.0TM). The results were regarded as significant when $p < 0.05$.

3. Results

3.1 Blood glucose and body weight

The blood glucose and body weight measured at the end of the experiment are shown in Fig. 1. The blood glucose level was about 4-fold higher in the DM group compared with that of the Con group (Fig. 1a, $p < 0.01$). The body weight in the DM group was nearly 50% lower than that in the Con Group

(Fig. 1b). Compared with the DM group, the blood glucose level in the T1 group (Fig. 1a, $p < 0.05$) was lower but that in the T2 group ($p > 0.05$) was no difference. The body weight did not differ among the DM, T1, and T2 groups (Fig. 1b, $p > 0.05$).

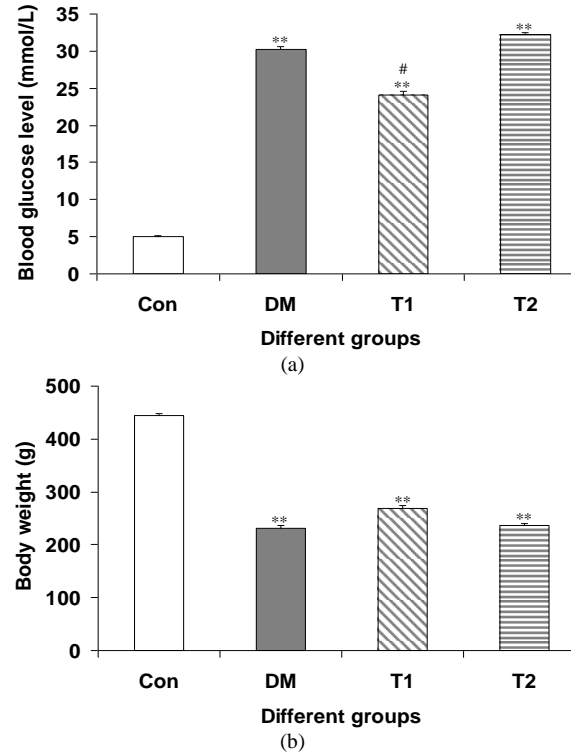


Figure 1. (a) Blood glucose levels and (b) body weight in various groups. Compared with Con group: ** $p < 0.01$; compared with DM group: # $p < 0.05$.

3.2 Weight/cm to body weight ratio, wall thickness, and wall area

The wet weight per unit length to body weight ratio (Fig. 2a), no-load wall thickness (Fig. 2b), and cross-section wall area (Fig. 2c) of the colonic and rectal segments were significantly higher in the diabetic group compared with those of the Con group ($p < 0.01$). After treatment with T1, these parameters significantly decreased in the two segments (Fig. 2, $p < 0.05$ and $p < 0.01$); however, they did not significantly change in the T2 group ($P > 0.05$) with the exception of the wet weight of the rectum (Fig. 2a, $p < 0.05$) and the wall thickness of the colon (Fig. 2b, $p < 0.05$).

3.3. Opening angle and residual strain

At the end of the experiment, the opening angles of the colonic and rectal segments were significantly higher in the DM group compared with those in the Con group (Fig. 3a, $p < 0.05$ for rectum and $p < 0.01$ for colon). Treatment with a high dosage of CRT decreased the opening angle significantly (Fig. 3a, $p < 0.05$ for colon and $p < 0.01$ for rectum); the opening angle did not change in the T2 group ($p > 0.05$).

A similar trend was found for the inner and outer residual strains in the two segments (Figs. 3b and 3c); i.e., the absolute values of the residual strain of the colonic and rectal segments

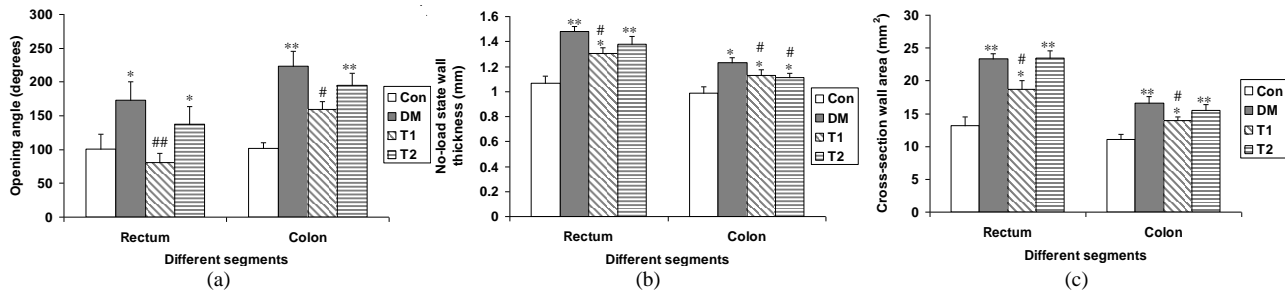


Figure 2. (a) Wet weight per unit length to body weight ratio, (b) wall thickness, and (c) wall area in different segments of various groups. Compared with Con group: * $p < 0.05$, ** $p < 0.01$; compared with DM group: # $p < 0.05$, ## $p < 0.01$.

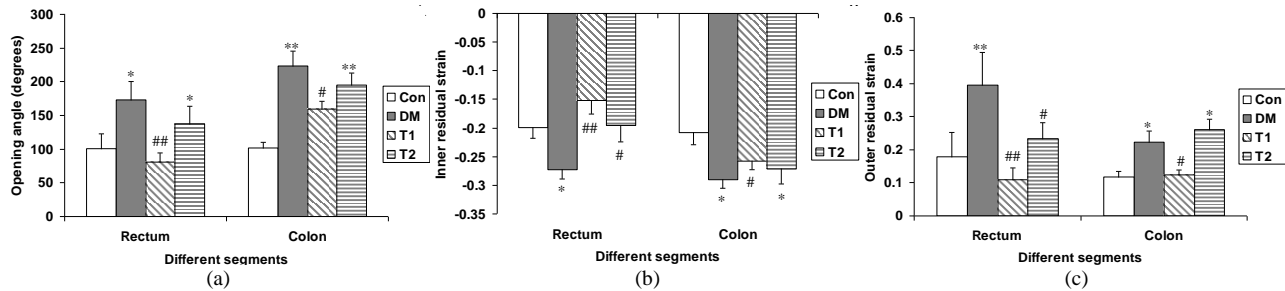


Figure 3. (a) Opening angle, (b) inner residual strain, and (c) outer residual strain in different segments of various groups. Compared with Con group: * $p < 0.05$, ** $p < 0.01$; compared with DM group: # $p < 0.05$, ## $p < 0.01$.

were significantly higher in the DM group compared with those in the Con group (Figs. 3b and 3c, $p < 0.05$, $p < 0.01$). Treatment with a high dosage of CRT (T1 group) partially reversed the changes of residual strain (Figs. 3b and 3c, $p < 0.05$, $p < 0.01$), whereas a low dosage of CRT (T2 group) only partially reversed the changes of residual strain in the rectal segment ($p < 0.05$).

3.4. Stress-strain distribution

At the end of the experiment, the stress-strain analysis showed that both the circumferential and longitudinal stress-strain curves of the colonic segment (Figs. 4a and 4b) in the DM group shifted to the left compared with those in the Con group. This indicates that the colonic wall became stiffer due to diabetes. Computation of constant a showed a significant difference between the DM group and the Con group (Figs. 5a and 5b, $p < 0.05$). High-dosage CRT (T1) treatment significantly decreased the stiffness of the colonic wall in both circumferential (Figs. 4a and 5a, $p < 0.05$) and longitudinal (Figs. 4b and 5b, $p < 0.05$) directions. Low-dosage CRT treatment (T2) did not show improvement in the stiffening of the colonic wall caused by diabetes (Figs. 4 and 5, $p > 0.05$).

4. Discussion

The global prevalence of constipation in the general population is about 16% and it seems that prevalence increases with age [1]. Constipation is caused either by a primary disorder of colonic and anorectal function or by many secondary conditions such as constipating drugs and metabolic disorders, such as diabetes and other colorectal problems [12]. Although the exact mechanism related to constipation is unknown, the biomechanical properties of the bowel seem important [3,12]. Patients with constipation are characterized

by impaired rectal sensitivity and decreased anal sphincter contractile pressure [13]. Increased anorectal compliance is seen in most patients with constipation [14]. Fasting colonic tone and/or postprandial colonic tone are reduced in patients with chronic constipation [15]. Therefore, in order to treat constipation, it is important to develop drugs to treat colonic and rectal biomechanical disorders.

In traditional Chinese medicine, diabetic GI disorder is characterized by satiety, epigastric pain, vomiting, diarrhea, and constipation. Constipation is a GI complication in diabetes [16]. Diabetic patients with constipation showed longer total, left, and recto-sigmoid colonic transit times than those of patients without constipation [17]. Furthermore, our previous study demonstrated that prominent proliferation and biomechanical remodeling of the colonic wall occurred in experimental diabetes [9]. This was confirmed in the present study. It is well known that the biomechanical properties of the wall are important for the function of the colon and rectum [18]. Therefore, it is likely that the biomechanical remodeling of the colonic and rectal wall with diabetes is related to constipation [10].

A large number of clinical data have shown that Chinese medicine treatment of constipation has a good clinical effect [7]. In our clinical study, it was found that CRT decoction was very effective in treating senile constipation. The composition of CRT includes *Radix Angelicae Sinensis*, *Radix Cyathulae*, *Herba Cistanches*, *Rhizoma Alismatis*, *Rhizoma Cimicifugae*, *Fructus Aurantii Immaturus*, *Rhizoma Atractylodis Macrocephalae*, *Semen Arecae Preparata*, and hemp seed. From the traditional Chinese medicine point of view, different herbs have different effects [19]. Modern pharmacological studies have shown that *Rhizoma Atractylodis Macrocephalae* affects intestinal activity, citrus increases GI contraction [20], immature bitter orange strengthens intestinal contraction,

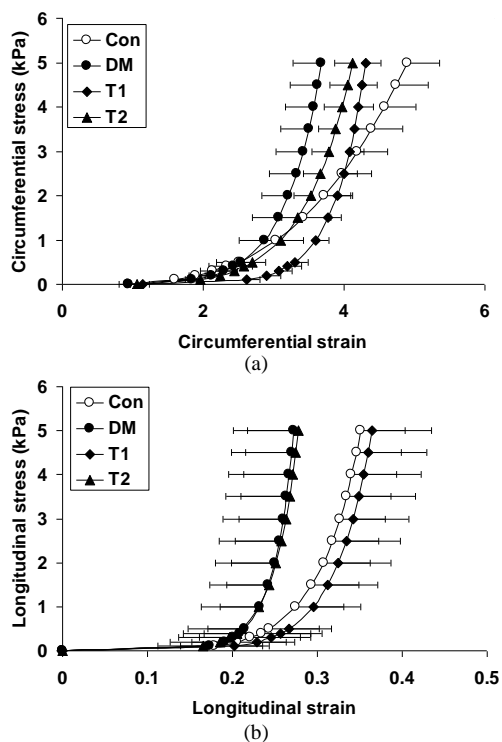


Figure 4. Circumferential and longitudinal stress-strain curves of colonic and rectal segments among various groups.

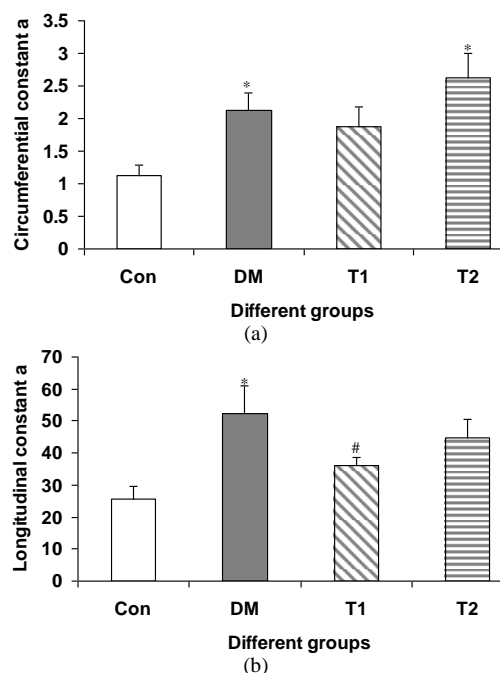


Figure 5. (a) Circumferential and (b) longitudinal constant a of colonic segments in various groups. Compared with Con group: * $p < 0.05$; compared with DM group: # $p < 0.05$.

Rhizoma Cimicifugae reduces intestinal spasms, *Semen Arecae Prepareta* increases intestinal peristalsis and leads to notable contraction of colonic smooth muscle cells in rats. Also, hemp seed stimulates the intestinal wall when it makes contact with the alkali intestine fluid in the intestinal tract, increasing peristalsis. CRT can thus regulate GI motility and improve the symptoms in patients with constipation. However, the

mechanism that improves constipation is still not well understood. The present study demonstrated that high-dosage CRT could partially reverse the morphological and biomechanical changes of the colon and rectum caused by diabetes. One mechanism of the CRT effect on constipation is likely through this pathway.

Morphological changes, such as increasing wall thickness caused by diabetes change the relative location of mechanosensitive afferents in the GI tract [21,22]. The changes in residual stress and wall stiffness caused by diabetes change the tension and stress distribution at the location of mechanosensitive afferents. Therefore, the morphometric and biomechanical remodeling of the GI wall in the diabetes can indirectly affect GI sensory and motor function. High-dosage CRT could partially correct the morphometric and biomechanical remodeling caused by diabetes and thus improves the motor and sensory dysfunction of a diabetic GI tract. However, it is important to further investigate the molecular mechanisms of the CRT effect on the morphological and biomechanical properties of the lower GI tract in diabetic rats. This important issue will be considered in future studies.

5. Conclusion

Morphological and biomechanical remodeling of the colonic and rectal wall was found in experimental diabetes. High-dosage CRT treatment can partly improve the biomechanical and morphometric remodeling of the colonic and rectal wall caused by diabetes, and thus can improve the symptoms of constipation. It is important to develop some Chinese herbs, such as CRT, to improve morphometric and biomechanical remodeling to further improve GI dysfunction caused by diabetes.

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