

## Anticolitis Activity of Myrobalan Powder via Regulating Colonic Enterochromaffin Cells and Serotonin

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### Abstract

*Objective:* To investigate whether Myrobalan powder has an anti-inflammatory effect on colonic inflammation and to explore the mechanism involved.

*Materials and Methods:* Myrobalan powder was orally administered to trinitrobenzene sulfonic acid (TNBS)-induced colitis mice at the dose of 3, 6, and 12 g/kg/d for 7 consecutive days. Body weight, stool consistency, histopathological score, and myeloperoxidase (MPO) activity were tested to evaluate the effect of Myrobalan powder on colonic inflammation while colonic enterochromaffin (EC) cell density and serotonin 5-hydroxytryptamine (5-HT) content were investigated to identify the effect of Myrobalan powder on colonic 5-HT availability.

*Results:* The results showed that the body weight of colitis mice was markedly decreased by 10, 12, 14, and 17% at 1, 3, 5, and 7 days ( $P < 0.05$ ), whereas stool consistency score (3.6 vs. 0.4,  $P < 0.05$ ), histopathological score (3.6 vs. 0.3,  $P < 0.05$ ), and MPO activity (2.7 vs. 0.1,  $P < 0.05$ ) in colitis mice were significantly increased compared to that of the normal mice; Myrobalan powder treatment dose-dependently increased the body weight (7–13% increase) and decreased the stool consistency score (0.4–1.4 decrease), histopathological score (0.2–0.7 decrease), and MPO activity (0.1–0.9 decrease) in colitis mice. Colonic EC cell density (70% increase) and 5-HT content (40% increase) were markedly increased in colitis mice ( $P < 0.05$ ), Myrobalan powder treatment dose-dependently reduced EC cell density (20–50% decrease), and 5-HT content (5–27% decrease) in colitis mice.

*Conclusion:* The findings demonstrate that the anti-inflammatory effect of Myrobalan powder on TNBS-induced colitis may be mediated via reducing EC cell hyperplasia and 5-HT content. The important role of Myrobalan powder in regulating colonic EC cell number and 5-HT content may provide an alternative therapy for colonic inflammation.

**Keywords:** Colonic inflammation, enterochromaffin cell, serotonin, ulcerative colitis

### Introduction

Ulcerative colitis (UC), a chronic intestinal inflammatory disease, is characterized by severe diarrhea, pain, fatigue, and weight loss. Based on the statistical analysis data, the incidence of UC has been increasing throughout the world, especially in the developing countries, this increasing trend in UC epidemiology may increase the healthcare burden and hinder economic development. Although the causes of colonic inflammation have not been clearly identified, it is believed to have a correlation with genetic susceptibility, immune imbalance, and alterations in commensal microbiota. Increased intestinal immune cells, such as T-cells, macrophages, enterochromaffin (EC) cells, as well as the altered cytokines and chemokines are associated with immune imbalance of gut and has been considered as the therapeutic targets for the treatment of colonic inflammation.

It is well known that nearly 95% of the body's serotonin 5-hydroxytryptamine (5-HT) comes from the gut, with large amount stored in EC cells. As a neurotransmitter and intercellular signaling molecule, 5-HT activates both intrinsic and extrinsic primary afferent neurons, therefore, regulates the gastrointestinal

activity. Increased intestinal EC cell number and 5-HT bioavailability have long been reported to play an important role in intestinal symptoms generation, such as visceral pain, motility dysfunction, and the altered barrier permeability. Recently, several studies have shown that 5-HT may contribute to the initiation of intestinal inflammation. It is reported that increase of 5-HT content in intestinal tissue by knocking out 5-HT reuptake transporter can exaggerate the severity of trinitrobenzene sulfonic acid (TNBS)-induced colitis and spontaneous colitis that arises from interleukin-10 deletion while decrease in the production of mucosal 5-HT by selectively inhibiting or knocking out the rate-limiting enzyme responsible for 5-HT synthesis can markedly attenuate experimental colitis in mice. Given the important role of 5-HT in intestinal inflammation, it is proposed that strategies that aim at decreasing intestinal 5-HT bioavailability may provide an alternative therapeutic target to ameliorate symptoms of colonic inflammation.

Until now, no guaranteed curative therapeutic regimen has been developed for colonic inflammation. The currently used management, such as corticosteroids, anti-inflammatory drugs, as well as immunomodulators, primarily focus on promoting remission and preventing relapse. In view of the side effects of conventional therapeutic medicines, more and more colitis sufferers seek the help of Traditional Ayurvedic Medicine. Nowadays, Traditional Ayurvedic Medicine is not only used by colitis patients across Asia, but also by various proportions of Western patients with colitis, ranging from 23% to 49%. Myrobalan powder is a traditional Ayurvedic Medicine, which has been widely prescribed to treat bacterial infection, allergic rhinitis, as well as respiratory infection in India and Other countries surrounding India. According to the theory of Ayurvedic medicine, Myrobalan powder can invigorate and consolidate the body's defensive ability, which has been considered as an anti-inflammatory and immune-regulatory agent. Recently, results from one clinical study showed that Myrobalan powder had a therapeutic effect in children with persistent diarrhea, the underlying mechanism may have a correlation with its immunomodulatory function.

In this study, we hypothesized that Myrobalan powder can attenuate colonic inflammation in experimental UC induced by intracolonic TNBS instillation. While colonic EC cell density and 5-HT content were also investigated in order to identify whether the therapeutic effect of Myrobalan powder on colonic inflammation have a correlation with its effect on regulating colonic 5-HT availability.

## **Materials and methods**

### **Materials**

TNBS, hexadecyltrimethylammonium bromide, o-dianisidinedihydrochloride, and pentobarbital sodium were purchased from Kartikeya Chemicals, Telangana, India. Myrobalan powder was purchased from Priya Trading Company, Jharkhand, India. Methanol was of high-performance liquid chromatography (HPLC)-grade Alpha Chemika, Andheri, India. All other reagents and solvents were of analytical grade and were commercially available.

Animals' Male Swiss albino mice (aged 8 weeks with a body weight around 22 g) were obtained from the Mahaveera Enterprises, Telangana, India. All the mice were maintained at 25°C under 12 h-12 h alternating light-dark cycle with free access to food and water. Studies were carried out in accordance with the proposals of the Committee on the Ethics of Animal Experiments of Saraswati college of Pharmacy, Mumbai.

### **Development of colitis model**

Mice were fasted overnight and then anesthetized by pentobarbital sodium intraperitoneal administration (50 mg/kg). A plastic catheter was inserted into the colon at a depth of 4 cm from the anus. TNBS solution (2.5 mg in 50% ethanol, 100  $\mu$ l) was instilled slowly into the colon, after that the catheter

was gently removed. The mice in the control group were given with 100  $\mu$ l saline instead of TNBS solution. All the mice were left on a warm pad until they recovered from anesthesia. At 1 and 3 days post TNBS administration, body weight and stool consistency were evaluated and recorded, the mice that showed soft or diarrhea stool with body weight decrease were selected as the colitis mice.

### **Study design**

The colitis mice were randomly divided into five groups. Group 1 (n = 3) was set as the colitis model group, mice in this group were orally treated with water. Mice in group 2, 3, and 4 (n = 3 per group) were orally treated with Myrobalan powder at the dose of 3, 6, and 12 g/kg, respectively, the dosage was selected based on the previous study. Group 5 (n = 3) was set as the positive control, mice in this group were orally treated with sulfasalazine (SASP) at the dose of 500 mg/kg. Group 6 (n = 3) was set as a normal control, mice in this group were orally treated with water. The body weight change and stool consistency (0: Normal; 2: Soft; 4: Diarrhea) was scored according to previous methods at 1, 3, 5, and 7 days after drugs administration. All the drugs were administered for consecutive 7 days and after the final drugs administration, the mice were sacrificed. A 3 cm long proximal colon was collected and divided into 2 parts, one part was fixed in formalin and embedded in paraffin for EC cell counting, and the other part was frozen at  $-20^{\circ}\text{C}$  for 5-HT content determination. A 3 cm long distal colon was collected and divided into 2 parts; the proximal was fixed in formalin for inflammation evaluation; the distal part was frozen at  $-20^{\circ}\text{C}$  for myeloperoxidase (MPO) activity determination.

### **Histopathological evaluation**

The colon sections (5  $\mu$ m thick) were stained with hematoxylin and eosin. All sections were observed by a pathologist blinded to the group setting. The severity of colonic inflammation was recorded according to previous macroscopic and histological scoring criteria. Five random fields were selected in each slide; all the scoring data were analyzed using Image J NIH software.

### **Myeloperoxidase activity determination**

MPO activity was determined by the modified method described as full. Briefly, the colon tissues were homogenized in 0.5% hexadecyltrimethylammonium bromide 0.5 mL/50 mg of colon tissue; then the homogenates were centrifuged at 18,000 g at  $4^{\circ}\text{C}$  for 15 min. Aliquots of 40  $\mu$ L supernatant were mixed with 60  $\mu$ L potassium phosphate buffer (50 mmol, pH 6.0) with o-dianisidinedihydrochloride and hydrogen peroxide. MPO activity was obtained from the rate of absorbance alteration in 1 min at 460 nm.

### **Immunohistochemistry and enterochromaffin cell counting**

Tissue sections were de-paraffinized and rehydrated for immunostaining. Antiserotonin primary antibody (1:4000, Sigma) was incubated at  $4^{\circ}\text{C}$  overnight. After that, sections were labeled streptavidin biotin. The primary antibody was omitted as a negative control. Five fields at  $\times 20$  magnifications were captured for each section by a pathologist blinded to the group setting. The areas of colonic mucosa were measured using ImageJ NIH software, and EC cell density was expressed as the number of EC cells per  $\text{mm}^2$  of the mucosal area.

### **Statistical analysis**

Data are presented as a mean  $\pm$  standard error. Differences between two groups were analyzed by Student's t-test. Data were analyzed using one-way analysis of variance followed by the Student-Newman-Keuls test. Differences were considered significant when  $P < 0.05$ .

## Results

Effects of Myrobalan Powder on Body Weight and Stool Consistency in Colitis Mice: The body weight in colitis mice was decreased about 10, 12, 14, and 17% at 1, 3, 5, and 7 days, respectively, when compared to that of the control, and SASP treatment markedly elevated the body weight in colitis mice after 5 and 7 days' drug administration ( $P < 0.01$ ). After Myrobalan powder administration, the body weight in high (12 g/kg) and median (6 g/kg) dose treated mice were significantly elevated after 3, 5, and 7 days' drug treatment when compared to that of the colitis mice ( $P < 0.05$ ). Consistent with the findings from body weight change, the results from stool consistency score also found that colitis mice showed elevated score when compared to that of the control, SASP treatment, as well as Myrobalan powder treatment at high and median dose markedly decreased the stool consistency score when drugs were administered for 5 and 7 days ( $P < 0.05$ ).

### Effects of myrobalan powder on inflammation severity in colitis mice

The results from histopathological evaluation and MPO activity assay showed that TNBS colonic administration induced acute inflammation in the colon tissue of mice with markedly increased histological score and MPO activity ( $P < 0.01$ ), whereas SASP treatment significantly decreased both histological score and MPO activity when compared to that of the colitis mice ( $P < 0.01$ ), suggesting that SASP had anti-inflammatory effect on TNBS-induced colitis. Myrobalan powder treatment dose-dependently decreased histological score and MPO activity in colitis mice. There were significant difference ( $P < 0.05$ ) in both high (12 g/kg) and median (6 g/kg) dose of Myrobalan powder-treated mice when compared to that of colitis mice, indicating that Myrobalan powder can reduce the severity of colonic inflammation in TNBS-induced colitis.

### Effects of Myrobalan Powder on Colonic Enterochromaffin Cell Density and 5-hydroxytryptamine Content in Colitis Mice

The colonic EC cell density and 5-HT content were both significantly increased in colitis mice (about 70% increase in EC cell density, about 40% increase in 5-HT content) when compared to that of the control ( $P < 0.05$ ), suggesting the occurrence of EC cell hyperplasia in the colon of colitis mice. Compared with the colitis mice, SASP treatment slightly decreased the EC cell density and 5-HT content, but no significant difference was found between SASP treated mice and colitis mice. Myrobalan powder treatment dose-dependently decreased the colonic EC cell density (20–50%) and 5-HT content (5–27%) in colitis mice, but only the mice that treated with high (12 g/kg) and median (6 g/kg) dose of Myrobalan powder showed significant difference when compared to that of the colitis mice, suggesting that Myrobalan powder can reduce colonic EC cells hyperplasia and 5-HT content in colitis mice.

## Discussion

Myrobalan powder has been widely prescribed in India to treat bacterial and respiratory infection. As an anti-inflammatory and immune-regulatory agent, Myrobalan powder is recently found to have a therapeutic effect on chronic colitis in patients, but the underlying mechanism has not been clarified. This study investigated the anti-inflammatory effect of Myrobalan powder on colonic inflammation using the TNBS-induced, colitis model. Our study provides the first evidence that Myrobalan powder can effectively attenuate colonic histological score and MPO activity via reducing colonic EC cell density and 5-HT content in colitis mice. The findings from this study confirmed that Myrobalan powder can be used as an alternative herbal agent in attenuating intestinal inflammation.

Considering the large amount of body's 5-HT located in the gastrointestinal tract, the role of 5-HT in regulating gastrointestinal activity has been investigated widely. Nowadays, it is found that increased colonic 5-HT bioavailability play a vital role in gastrointestinal symptoms generation, i.e., visceral pain,

motility dysfunction, increased barrier permeability, and the initiation of intestinal inflammation. Evidence also showed that EC cell number and 5-HT content are elevated in inflamed intestinal mucosa from Crohn's disease patients and in animal models of inflammatory bowel disease. The discovery of the important role of 5-HT in intestinal inflammation has attracted more attention about intestinal 5-HT bioavailability. We all know that intestinal 5-HT is mainly stored EC cells, so the number and function of EC cells are considered to play a critical role in intestinal inflammation. Results from this present study showed that Myrobalan powder can dose-dependently attenuate colonic inflammation, suggesting Myrobalan powder has a potential therapeutic effect on colitis. Our results also found that Myrobalan powder can markedly and dose-dependently reduce colonic EC cell density and 5-HT content in colitis mice, indicating that the reduced EC cell number and intestinal 5-HT level in Myrobalan powder-treated mice may contribute to the anti-inflammatory effect of Myrobalan powder in colitis mice.

It is not very clear how does 5-HT signaling modulate the process of intestinal inflammation. 5-HT is well known for its immune-modulatory effect, for there are many 5-HT receptors expressed on lymphocytes, monocytes, macrophages, and dendritic cells. It is possible that the elevated 5-HT level may modulate overly active immune response or dysfunctional inflammatory process, which may partially explain the role of 5-HT in driving intestinal inflammation. Moreover, 5-HT is also known as a neurotransmitter, which activates intrinsic and extrinsic primary afferent neurons, and thus regulates the gastrointestinal function, such as sensitivity, motility, permeability, and secretion. It is possible that the increased intestinal 5-HT level during inflammation may also contribute to the symptoms exaggeration in colitis, such as visceral pain, diarrhea, and even urgency.

Myrobalan powder is an Indian Ayurvedic medicine commonly used to treat respiratory tract diseases. This study found that Myrobalan powder had anti-colitis activity in TNBS-induced colitis mice, this finding was consistent well with previous results which showed that Myrobalan powder could shorten the duration of disease and reduce diarrheal recurrence rate, and the underlying mechanism may have correlation with its immunomodulatory function. Myrobalan powder has been commonly considered as an anti-inflammatory and immune-regulatory agent, it has been found to promote proliferation of spleen cells and balance the ratio of helper (Th) 1/Th2 cells in allergic airway disease model in mice. Knowing that there are numerous components in Indian Ayurvedic medicine, even though the bioactive components that responsible for the anti-inflammatory and immune-regulatory effects of Myrobalan powder have not been identified, the pharmacological effects of many components of Myrobalan powder have been reported previously. To further explore the bioactive components and the mechanism of Myrobalan powder on colonic inflammation, as well as intestinal 5-HT bioavailability, more studies are still needed in the future.

## Conclusions

This study reveals that Indian Ayurvedic medicine Myrobalan can dose-dependently attenuate colonic inflammation in the mice model of colitis, and the underlying mechanism may be mediated via reducing colonic EC cell density and 5-HT content. These data support Myrobalan powder as a potential therapeutic formula for the treatment of colitis.

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