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Pediatric tuina treatment for spleen deficiency diarrhea regulated through the skin-brain-gut axis and mast cell degranulation

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A R T I C L E I N F O

Keywords:
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Specific acupoints
Mechanism

A B S T R A C T

Background: Pediatric tuina is an effective alternative therapy for managing spleen deficiency diarrhea. The application of spleen-meridian and large-intestine-meridian points has been shown to alleviate symptoms and has garnered some support from evidence-based medicine. Nonetheless, there remains a dearth of research elucidating the biological effects of these specific acupoints. This study aimed to explore their effects by focusing on the material basis involving mast cells (MCs).
Methods: The experimental design induced spleen-deficiency diarrhea in Kunming mice through a 7-day administration of *Rheum officinale* extract through gavage. Following this induction, treatment was initiated, employing a combination of spleen-meridian and large-intestine-meridian points over 6 days. Efficacy was assessed using fecal scoring. Colonic structure was assessed through hematoxylin-eosin staining, while toluidine blue staining was employed to observe MC degranulation within the skin-brain-gut axis. Immunohistochemistry was performed to detect tryptase release from MCs.
Results: The treatment combining spleen-meridian and large-intestine-meridian points markedly ameliorated diarrhea symptoms and improved fecal scores in Kunming mice exhibiting spleen deficiency. Pediatric tuina treatment facilitated the restoration of the colonic barrier and reduction in MC counts within the skin acupoint-brain-gut axis, consequently affecting the biologically active substance tryptase.
Conclusion: This study reveals the biological mechanism underlying the efficacy of specific acupoints in pediatric tuina, employing a holistic perspective encompassing the skin-brain-gut axis and MCs. Our findings substantiate the scientific basis for the effectiveness of tuina therapy in managing diarrhea and offer a new avenue for fundamental research on specific acupoints within pediatric tuina.

1. Introduction

Pediatric diarrhea is a common gastrointestinal disorder among children caused by multiple factors and is prevalent in pediatric care. Globally, the incidence of childhood diarrhea in low- and middle-income countries varies from less than once a year to more than four times annually in 2017.[1] In China, children under 5 years old experience an average annual incidence rate of 1.9 times per person.[2] According to a survey by the World Health Organization, diarrhea is the second leading cause of death in children aged below 5 years old, resulting in approximately 525,000 deaths annually,[3] posing a significant threat to children's health. The global approach to treating childhood diarrhea involves fluid replacement, medication, and nutritional support.[4] Moreover, alternative medical interventions such as traditional Chinese medicine (TCM), tuina, and acupuncture have shown effectiveness in alleviating clinical symptoms of childhood diarrhea.[5] Among these, pediatric tuina can be used for both preventing and treating diarrhea, aligning with the World Health Organization's three strategies to reduce the burden of diarrhea: protection, prevention, and treatment.[1]

Pediatric tuina is an exceptional external treatment method rooted in

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the fundamental principles of TCM. It employs specific techniques at designated acupoints on the body surface to both prevent and address various diseases. The comprehensive framework of pediatric tuina's theoretical system traces its origins back to the mid to late Ming Dynasty, spanning over 400 years of history. Particularly in the last four decades, pediatric tuina has gained widespread recognition and application in clinical practice. Its versatility encompasses a broad spectrum, effectively addressing approximately 170 diseases across 15 disease systems. These encompass psychobehavioral or neurodevelopmental disorders (attention-deficit/hyperactivity disorder, autism spectrum disorders),[6,7] respiratory system diseases (pediatric rhinitis),[8] digestive system diseases (anorexia and diarrhea),[9,10] among others. Among these, childhood diarrhea is the most frequently reported disease in the literature.[11] Clinical evidence underscores the efficacy of tuina in treating both infectious diarrhea (e.g., rotavirus diarrhea) and noninfectious diarrhea (such as spleen deficiency diarrhea).[12,13] Spleen deficiency diarrhea (SDD) commonly manifests in the later stages of acute or chronic diarrhea, representing a notable advantage in pediatric tuina treatment. The historical application of specific acupoints in pediatric tuina to address diarrhea dates back centuries. Records from the Ming Dynasty's "Pediatric Massage Classic" in 1601 documented the combined use of spleen-meridian and large-intestine-meridian points for diarrhea treatment.[14]

Tuina therapy demonstrates the ability to enhance the clinical cure rate and shorten the diarrhea duration in children afflicted with spleen deficiency.[15] Animal studies highlight tuina's potential in reducing diarrhea by modulating the structure of intestinal microbiota and diminishing the population of bacteria that harm the intestinal mucosal barrier.[16] Contemporary literature identifies the spleen meridian and large intestine acupoints as specific targets for treating childhood SDD.[17] Single or combined utilization of these acupoints exhibits substantial clinical efficacy and a credible evidence-based medical basis.[18,19] Nevertheless, research regarding the biological effects of specific acupoints remains significantly underexplored.

Pediatric tuina specific acupoints encompass the spleen-meridian point and the large-intestine-meridian point, which deviate from the 14 meridian routes commonly associated with acupuncture. The spleen-meridian point is a linear area along the lateral side of the thumb, spanning from its tip to its root, while the large-intestine-meridian point is a linear area along the lateral side of the index finger, extending from its tip to its root (Fig. 1A). Our research team has posited that the effective biological basis of these specific acupoints is closely linked to the activation effect of subcutaneous mast cells (MCs). MCs, characterized as widely mobile neuroimmunoendocrine cells abundant in alkaline granules, play significant roles in defense and initiating inflammation. The hypothesis of "MCs and meridian phenomena" was first proposed by Chinese scholar Song Jimei in 1977. Since then, investigating the effects of MCs has become a prominent focus within meridian phenomena and acupoint sensitization. The mechanism underlying acupuncture effects is intricately associated with MC degranulation and the activation of signal transduction pathways.[20,21] The distribution pattern of MCs in the human skin has been explored previously,[22–24] affirming that skin MCs primarily concentrate in the dermis, exhibiting a distribution pattern of "running to the end, gathering at the orifice," with a centrifugal distribution density. The highest concentration occurs in the extremities and head, aligning remarkably with the main distribution pattern of pediatric tuina specific acupoints located on the hands, head, and face. Additionally, MCs are prevalent in various brain regions and the gastrointestinal tract, playing crucial roles as effectors of the central nervous system on the enteric nervous system. An essential characteristic of MCs is their mechanical sensitivity.[25,26] They can be triggered by stimuli such as needling and tuina, subsequently releasing histamine, 5-hydroxytryptamine, and various enzymes upon activation.[27–29] The widespread distribution of MCs in the skin-brain-gut axis tissue and their mechanical sensitivity provide a foundational basis for exploring the mechanism of action of pediatric tuina specific acupoints on this pathway. However, in the field of tuina, research on MC effects is still in its nascent stages, limited to the effects of MCs on acupoints and lesion tissues under techniques such as spine kneading and abdomen rubbing.[30–32] Notably, no research has focused on the biological effects of MCs within the skin-brain-gut axis and their relationship with pediatric tuina specific acupoints.

This study aimed to investigate the biological effects and mechanisms of specific core acupoints in pediatric tuina therapy for treating SDD in mice. This study constructed a mouse model exhibiting SDD and observed MC degranulation within the acupoint skin-brain-gut axis. Additionally, we monitored the release of bioactive substances, such as tryptase. Sodium cromoglycate was utilized to pre-treat the large-intestine meridian acupoint, aiming to prevent MC degranulation. The therapeutic effect was evaluated using diarrhea scoring. The study results aim to offer scientific insight and guidance for the clinical application of specific acupoints in pediatric tuina therapy for SDD.

2. Methods

2.1. Experimental animals

A total of 35 male Kunming mice, aged 4–5 weeks and weighing 23–25 g, certified with specific pathogen-free validation, were obtained from Skerbes (Henan) Biotechnology Co., Ltd. (Animal Lot: SCXK(Yu) 2020–0005). These animals were provided with ad libitum access to food and water and were housed in a controlled environment

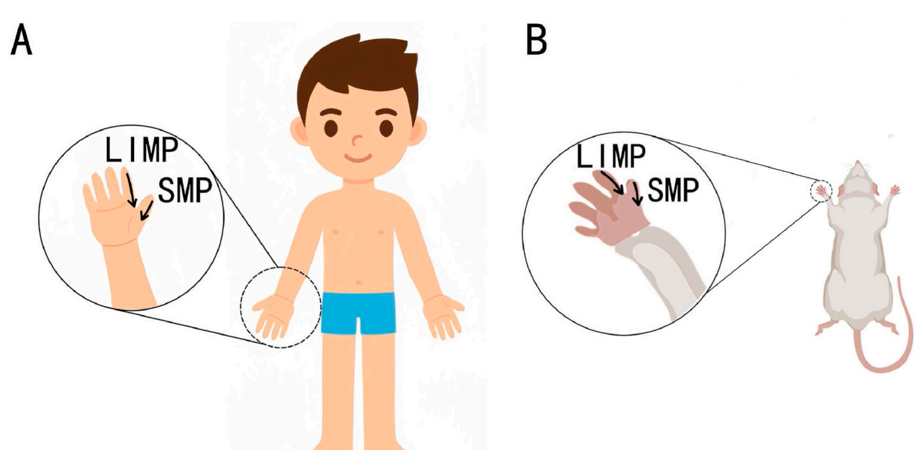

Fig. 1. Acupoint cartoon. A:Cartoon figure of acupoints on the spleen-meridian point and large-intestine-meridian point of children. B: Cartoon figure of acupoints on the spleen-meridian point and large-intestine-meridian point of mice.

maintaining a temperature range of 20–26 °C and relative humidity of 40%–70%. Following a 3-day acclimation period, the mice were randomly assigned to one of the following five groups, each comprising 7 animals: normal group (NG), model group (MG), spleen-meridian point treatment (SMPT), large-intestine-meridian point treatment (LIMPT), and sodium chromate group (SCG). The experimental procedures were approved by the Ethics Committee of the Animal Experimental Center of Tianjin University of Traditional Chinese Medicine (TCM-LAEC2022162). Efforts were made to minimize the number of animals used and reduce the suffering of the animals during the experimentation.

2.2. Establishment of spleen deficiency diarrhea Kunming young mice

To induce the SDD model, researchers utilized a bitter-cold purgation method employing rhubarb decoction, as described in prior research.[33,34] Rhubarb, scientifically known as *Rheum palmatum* L. and belonging to the Polygonaceae family,[35] contains various chemical components, including anthraquinone, anthrone, tannins, flavonoids, and polysaccharides.[36,37] The preparation involved mixing 300 g of rhubarb with 300 mL of water to produce a crude drug solution with a concentration of 1 g/mL. This solution was stored in a 4 °C refrigerator, and the raw rhubarb herb was supplied by the Chinese Pharmacy of the Second Teaching Hospital of Tianjin University of Traditional Chinese Medicine. Subsequently, young mice received an oral administration of 0.2 mL/10 g of rhubarb decoction once daily for 7 consecutive days, except for NG, wherein the mice received an equivalent volume of normal saline. The successful induction of SDD was determined based on specific criteria, including (a) abnormal stools (loose stool, anal filth), (b) decreased eating, (c) weight loss or no gain, slow growth, (d) expression of listlessness, (e) curled-up posture, (f) struggling to grasp, reduced movement, or slow action, and (g) lack of luster, defecation abnormalities, and withered hair. The first two criteria were considered primary criteria, while the last five criteria were secondary. The successful establishment of the young mice model with SDD required the simultaneous fulfilment of two major and two minor criteria.

2.3. Interventions

In this particular tuina procedure, a well-coordinated team of two individuals worked seamlessly together. One person delicately lifted and held the young mice in the supine position while carefully securing their front paws to facilitate the tuina. Simultaneously, the other operator used a flocking swab for the tuina. Push directly on the thumb pad of the young mice to target the spleen-meridian point, while stimulation of the large-intestine-meridian Point involved pushing the fingertip toward the finger root along the radial edge of the index finger (Fig. 1B).

Pediatric tuina was administered at a frequency of 3–4 times per second, lasting for 5 min per session, over 6 consecutive days. The entire process was executed with great precision and care.

2.4. Observation indicators

2.4.1. General information

This information included changes in body weight, stool characteristics, spirit, diet, Fur, and exercise of young mice

2.4.2. Diarrhea grade score

The diarrhea grade of the observed mice was evaluated using a comprehensive scoring system.[38] Mice were assessed based on specific criteria: normal stool scored 0 points, mild diarrhea with wet and soft stool scored 1 point, moderate diarrhea with loose stools and an unclean perianal area scored 2 points, and severe diarrhea with watery stool and severe perianal staining scored 3 points. This scoring system was meticulously applied with attention to detail

2.5. Hematoxylin-eosin (HE) staining

For hematoxylin-eosin (HE) staining analysis, colon tissue was harvested and carefully fixed in 4% paraformaldehyde. Subsequently, the colon was paraffin-embedded and sectioned (5 μm thickness) using an automatic paraffin slicer (2M2255, Leica, Mannheim, Germany). Following this, the sections were diligently stained with HE and then scanned with a digital slide scanner to ensure the highest level of accuracy and precision.

2.6. Observation on mast cells of acupoints, colon and brain with toluidine blue staining

On day 14, subsequent to sacrificing the young mice, specific tissues at the large-intestine-meridian point and the spleen-meridian point, encompassing the skin, subcutaneous tissue, brain, and colon tissues, were carefully dissected. Each tissue fragment was precisely measured to be 1.5 × 1.5 × 1.5 mm in size. These tissues were then fixed in 10% neutral-buffered formalin, followed by dehydration and embedding in paraffin. Subsequently, they were sectioned using a 4-μm slicer. The sections were then subjected to dewaxing, dehydration, and stained with 1% toluidine blue for 40 min, followed by three washes with distilled water. MC counts were conducted at three areas per slice under a light microscope, and the averages were calculated. MCs exhibiting more than three granules outside the cell shape or showing empty cavities in the cytoplasm were categorized as degranulated. Representative photomicrographs were captured at a magnification of × 400 for morphological evaluation.

2.7. Immunohistochemical observation on anti–mast cell tryptase of acupoints, colon and brain

The specimens from acupoints, skin, colon, and brain underwent a delicate dewaxing process and subsequent hydration using 3% methanol hydrogen peroxide at room temperature for 10 min. After three washes with phosphate-buffered saline (PBS) for 5 min each, they were immersed in citrate buffer solution and microwaved for antigen retrieval. Following cooling, PBS washes were performed thrice for 5 min each. Mouse monoclonal antibody anti-MC tryptase (1:100 dilution) was cautiously applied to the specimens and left overnight at 4 °C. Subsequently, goat antimouse secondary antibody labeled with horseradish peroxidase was added and incubated at 37 °C for 30 min. After three additional washes with PBS for 5 min each, color development with DAB was executed, succeeded by Harris hematoxylin re-staining, dehydration, and transparency. Finally, the specimens were sealed with neutral gum.

Immunohistochemical staining revealed brownish-yellow staining particles in the nuclei, indicating a positive result, while the absence of brownish-yellow staining indicated a negative result. Image-Pro Plus software was used to measure the positive expression of optical density (OD) and conduct semi-quantitative analysis. This analysis was performed to determine the levels of anti-MC tryptase present in the acupoint skin, colon mucosa, and brain tissues. The intensity of the brownish-yellow staining directly correlated with both the OD value and the level of anti-MC tryptase, indicating a higher expression level.

2.8. Statistical analysis

Statistical analyses were conducted using SPSS 25.0 (SPSS Inc., USA). Experimental data were presented as mean ± standard deviation. Differences in mean were compared using one-way analysis of variance. A significance level of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Establishment of the mouse model

In this study, a total of 35 mice were included, and none of them died during the experiment. Initially, all groups of young mice had similar weights ($P > 0.05$). Throughout the study period, the young mice in the NG exhibited light yellow-brown stools without any signs of diarrhea. Their skin appeared healthy, displaying a plump texture. Conversely, young mice in each model group developed diarrhea within 12–24 h after consuming the rhubarb solution. Detailed stool conditions and scores are presented in Fig. 2. These mice experienced slow or no weight gain and exhibited symptoms such as mental fatigue, skin wrinkling, and reduced vitality compared with NG.

3.2. Pediatric tuina is highly effective in ameliorating diarrhea symptoms and improving fecal score in spleen deficiency diarrhea mice

Diarrhea severity was evaluated using the fecal index scoring criteria. As depicted in Fig. 3, young mice in MG displayed the most severe diarrhea, consistently showing higher fecal scores than those in NG from days 1–6, indicating a significant difference ($P < 0.01$). Subsequent tuina application on the large-intestine-meridian point and the spleen-meridian point led to a gradual improvement in fecal scores among the afflicted young mice, transitioning their stools from watery to normal. After 6 days of treatment, the fecal scores returned to normal levels, indicating a significant difference ($P < 0.05$) when compared with MG.

3.3. Colon barrier integrity in young mice with spleen deficiency diarrhea can be effectively restored by pediatric tuina

The results from HE staining shown in Fig. 4 indicate that the colon mucosa of normal young mice remained well preserved, exhibiting neatly arranged colon glands without signs of inflammatory cell infiltration. Conversely, MG displayed significant colon mucosal damage, with noticeable MC and monocyte infiltration in the mucosa or submucosa. Following treatment targeting acupoint groups on the spleen-meridian point and the large-intestine-meridian point, the arrangement of colonic glands tended toward normalcy, showing regularity. In the group treated with the addition of sodium cromoglycate, the colonic mucosa displayed slight congestion, fibrous tissue proliferation in the submucosa, and mild inflammatory cell infiltration, albeit less severe than in the MG.

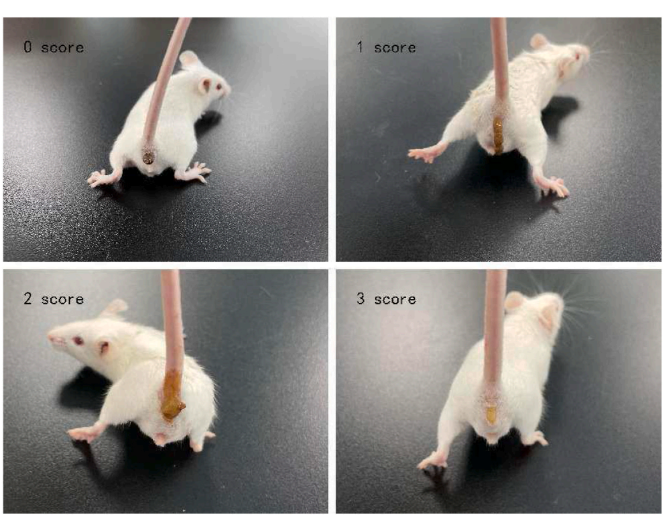

Fig. 2. Stool conditions and scores of the mice. A:normal stool was rated as 0 points, B:mild diarrhea with wet and soft stool was rated as 1 points, C: moderate diarrhea with loose stools and unclean perianal area were rated as 2 points, D: severe diarrhea with watery stool and severe perianal staining were rated as 3 points.

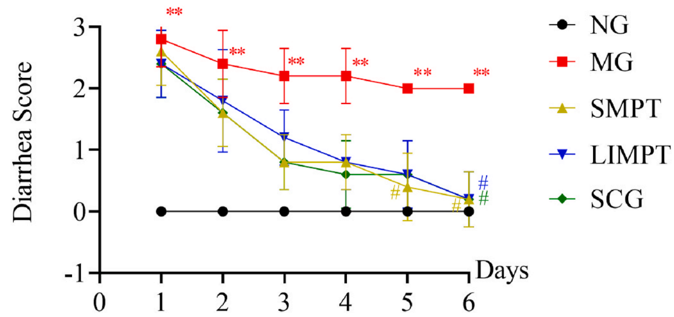

Fig. 3. Fecal Index Scores of Kunming Mice in Each Group During the treatment period, the diarrhea score in the NG remained at 0, while the model group had the highest diarrhea score. After treatment with SMPT and LIMPT, the diarrhea score of the mice gradually returned to normal. The diarrhea score in the SCG gradually decreased and became normal. #$P < 0.05$,**$P < 0.01$. (NG:normal group, MG:model group, SMPT:the spleen-meridian point treatment group, LIMPT: the large-intestine-meridian point treatment, SCG:sodium chromate group).

3.4. Regulation of mast cell numbers in the acupoint skin-brain-gut axis and release of tryptase

Regulation of MC numbers in the acupoint skin-brain-gut axis and release of tryptase are important mechanisms by which pediatric tuina applied on the spleen-meridian point and large-intestine-meridian point exerts therapeutic effects against SDD in Kunming mice.

To explore the effect of pediatric tuina on MCs within the skin-brain-gut axis, we assessed MC quantities and tryptase release in skin acupoints, colon tissue, and the brain. Typically, MCs are scattered in the subcutaneous loose connective tissue, appearing blue-purple when stained with toluidine blue and exhibiting a circular or oval shape. Tryptase comprises more than 50% of the total protein mass of MC secretory granules and serves as a marker for MCs and their degranulation. In immunohistochemical staining, tryptase appears as brown-yellow or brown. As depicted in Fig. 5, compared with the NG, both the number of subcutaneous MCs and released tryptase increased significantly in MG ($P < 0.01$). However, after treatment at the spleen-meridian point, the number of MCs and released tryptase in the acupoint area decreased significantly.

Similar to the spleen-meridian point treatment, applying pressure to the large-intestine-meridian point effectively decreased the count of MCs and tryptase in the skin acupoints (Fig. 6). Additionally, administering sodium cromoglycate into the large-intestine-meridian point could suppress MC quantities.

MCs have been associated with intestinal permeability and diarrhea. Pediatric tuina appears effective in addressing diarrhea by modulating MCs in the colon tissue. As shown in Fig. 7, MC infiltration in the colon mucosa of the MG increased, accompanied by a significant increase in released tryptase, indicating an intestinal inflammatory reaction in young mice experiencing SDD. However, these alterations were mitigated after treatments at the spleen-meridian and large-intestine meridian points. The number of intestinal MCs in the SMPT and LIMPT groups markedly decreased compared with the MG, along with a corresponding decrease in tryptase release, displaying a similar effect to sodium cromoglycate, a MC stabilizer.

Pediatric tuina may regulate diarrhea by exerting an influence on the entire central nervous system. In young mice with diarrhea, MCs in the brain were primarily situated near the dentate gyrus of the hippocampus, as illustrated in Fig. 8. The number of activated MCs in MG was significantly higher than that in NG. Nonetheless, subsequent treatment

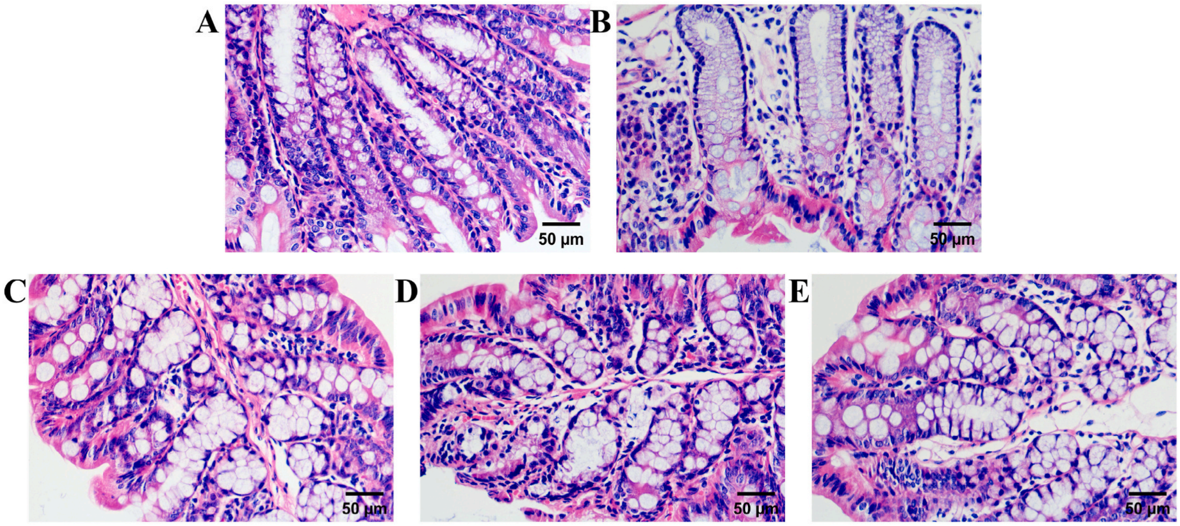

Fig. 4. Colon HE staining (× 400) A:NG:The colonic mucosa of the mice was intact, with well-arranged colonic glands and no apparent infiltration of inflammatory cells; B:MG: The colonic mucosa showed significant damage, with enlarged mast cells, mononuclear cells, and a large number of infiltrating inflammatory cells in the mucosal or submucosal layers; C:SMPT and D:LIMPT: the arrangement of colonic glands tended towards normal and exhibited regularity. E:SCG: the colonic mucosa showed slight congestion, fibrous tissue proliferation in the submucosa (NG:normal group, MG:model group, SMPT: the spleen-meridian point treatment group, LIMPT: the large-intestine-meridian point treatment, SCG: sodium chromate group).

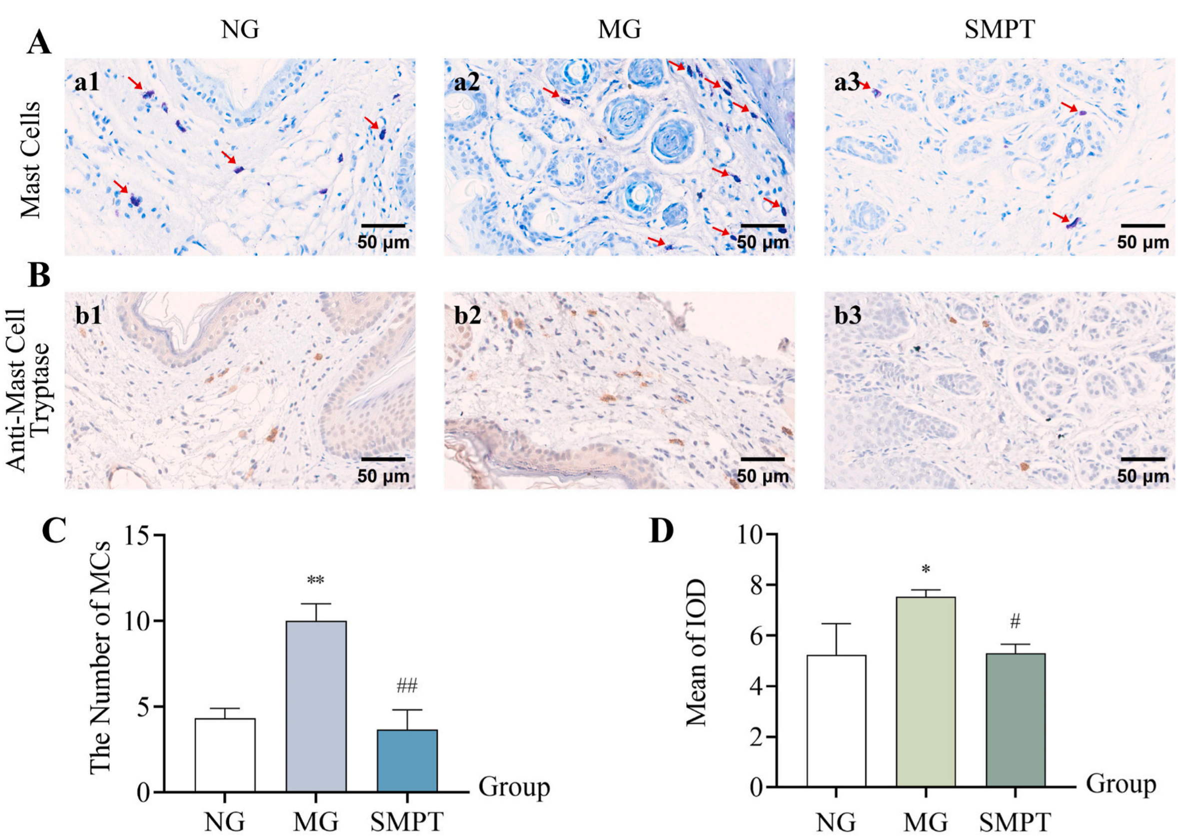

Fig. 5. Enhancement of mast cell activity and tryptase release through activation of the spleen-meridian point Detection of expression of mast cells and their release of tryptase after spleen-meridian point treatment. A:Expression of mast cells at acupoints of the spleen meridian. B:Release of tryptase from mast cells at acupoints of the spleen meridian. C: Statistical graph of mast cells in three groups. D:Statistical graph of tryptase IOD. $^{\#}P < 0.05$,$^{\#\#}P < 0.01$,$^{*}P < 0.05$,$^{**}P < 0.01$. Scale bar is 50 μm. (NG:normal group, MG:model group, SMPT: the spleen-meridian point treatment group).

at the spleen-meridian and large-intestine-meridian points notably reduced MC release and tryptase levels in the brain tissue ($P < 0.01$).

4. Discussion

Pediatric tuina therapy is rooted in TCM principles of syndrome differentiation and treatment. Diarrhea syndrome types, including

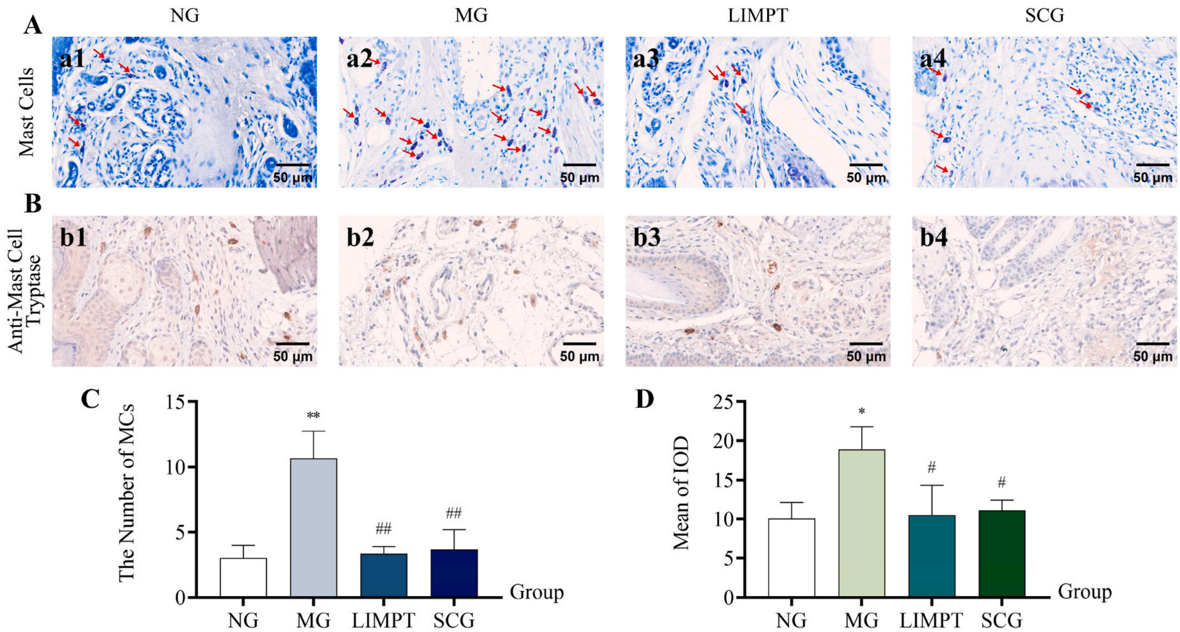

Fig. 6. Enhancing mast cell activation and tryptase release by stimulating the large intestine meridian point Detection of expression of mast cells and their release of tryptase after large-intestine-meridian point treatment. A:Expression of mast cells at acupoints of the large-intestine-meridian point. B:Release of tryptase from mast cells at acupoints of large-intestine-meridian point. C: Statistical graph of mast cells in four groups. D:Statistical graph of tryptase IOD. $^{\#}P < 0.05$,$^{\#\#}P < 0.01$,$^{*}P < 0.05$,$^{**}P < 0.01$. Scale bar is 50 μm. (NG:normal group, MG:model group, LIMPT: the large-intestine-meridian point treatment, SCG: sodium chromate group).

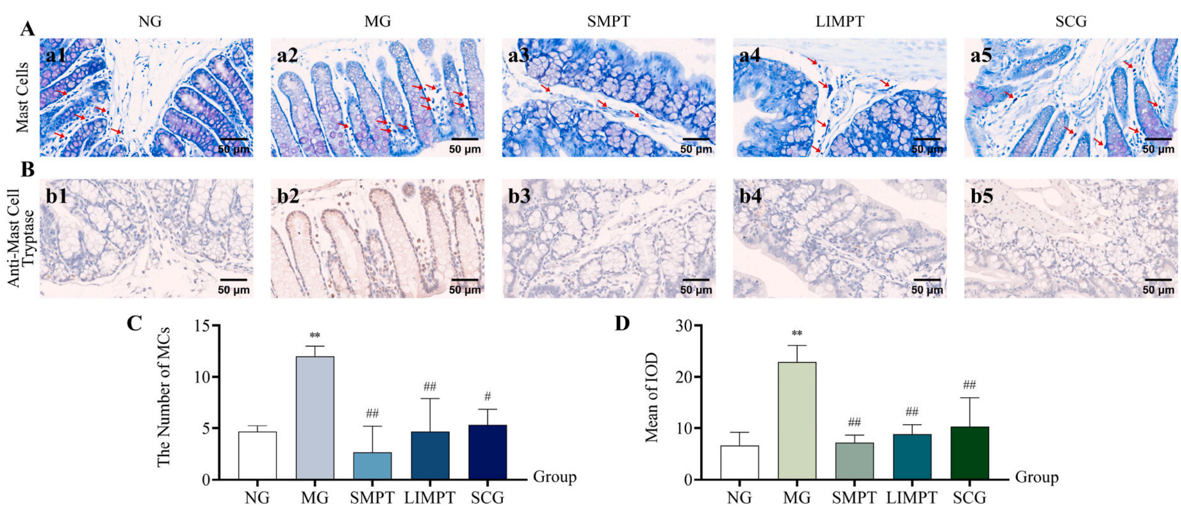

Fig. 7. Enhancement of mast cell activation and tryptase release in colonic tissue Expression of mast cells and their release of tryptase in colon tissue after spleen-meridian point and large-intestine-meridian point treatment. A:Expression of mast cells in colon tissue after spleen-meridian point and large-intestine-meridian point treatment. B:Expression of tryptase released from mast cells in colon tissue. C: Statistical graph of mast cells in five groups. D:Statistical graph of tryptase IOD. $^{\#}P < 0.05$,$^{\#\#}P < 0.01$,$^{*}P < 0.05$,$^{**}P < 0.01$. Scale bar is 50 μm. (NG:normal group, MG:model group, SMPT: the spleen-meridian point treatment group, LIMPT: the large-intestine-meridian point treatment, SCG: sodium chromate group).

spleen deficiency syndrome, damp-heat syndrome, and cold dampness syndrome, can be addressed through pediatric tuina.[39] Pediatric tuina therapy demonstrates positive therapeutic effects on noninfectious diarrhea and rotavirus-induced diarrhea.[40–43] This study confirms the efficacy of pediatric tuina therapy specifically for SDD.

One distinguishing aspect of pediatric tuina is its utilization of specific acupoints, predominantly located in the upper limbs, head, and face. These acupoints possess diverse shapes, mainly comprising facial and linear patterns, which markedly differ from the traditional acupuncture system's 14 meridians. In the clinical application of tuina therapy for childhood diarrhea, the spleen-meridian point and large-intestine-meridian acupoints are frequently used due to their substantial therapeutic effects, employing pushing manipulation techniques. Our study results demonstrate that utilizing these acupoints significantly ameliorates diarrhea symptoms and fecal scores in Kunming young mice displaying spleen deficiency, providing substantial evidence for the efficacy of specific acupoints in pediatric tuina therapy.

An examination of the mechanical aspects concerning the spleen meridian point and large-intestine-meridian points reveals that tuina therapy operates at the skin level, employing linear pushing motions across the body surface coupled with vertical pressure. This technique generates tactile and mechanical stimulation on the skin. The primary

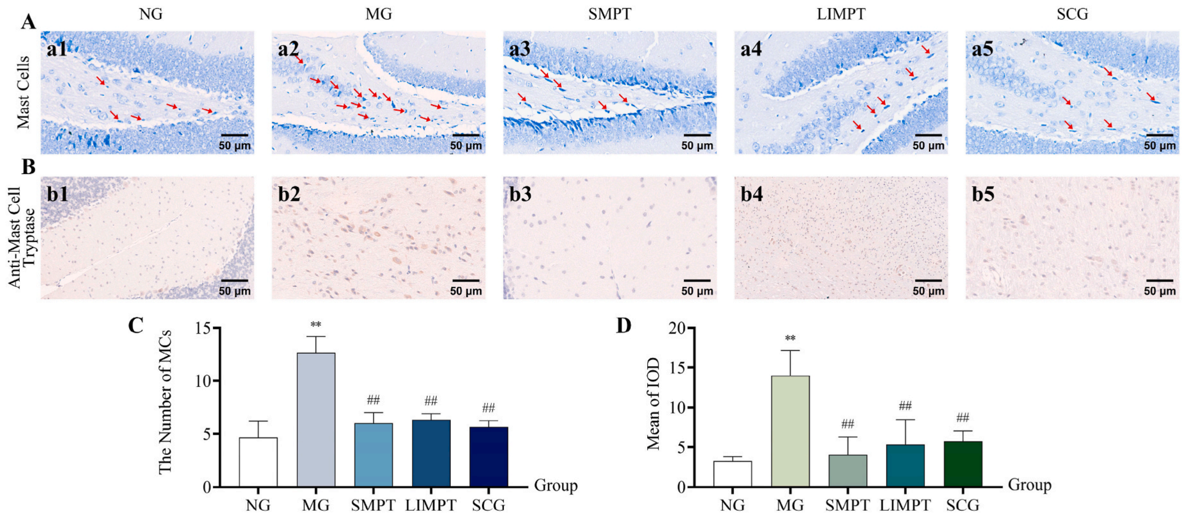

Fig. 8. Enhancement of mast cell activation and tryptase release in brain tissue Expression of mast cells and their release of tryptase in brain tissue after spleen-meridian point and large-intestine-meridian point treatment. A:Expression of mast cells in brain tissue after spleen-meridian point and large-intestine-meridian point treatment. B:Expression of tryptase released from mast cells in brain tissue. C: Statistical graph of mast cells in five groups. D:Statistical graph of tryptase IOD. $^{\#}P < 0.05$,$^{\#\#}P < 0.01$,$^{*}P < 0.05$,$^{**}P < 0.01$. Scale bar is 50 μm. (NG:normal group, MG:model group, SMPT: the spleen-meridian point treatment group, LIMPT: the large-intestine-meridian point treatment, SCG: sodium chromate group).

target structures are the organizational spaces and cellular composition of MCs dispersed in the dermal layer, specifically in contact with the fingers. The outcomes of this investigation validate the activation and clustering of MCs at acupuncture points in mice experiencing diarrhea. Following pediatric tuina therapy, there was a significant decrease observed in both MCs and released tryptase at the acupuncture points, suggesting that MCs constitute the material foundation for the specific biological effects and dynamic alterations at specific acupuncture points. Consequently, similar to acupuncture and moxibustion, the activation of MCs triggers the release of chemical substances, initiating signal transduction to manifest the effects of acupuncture and moxibustion.[44,45]

The main effect of addressing SDD through the push spleen meridian point and the large intestine meridian point is the promotion of intestinal health. Under normal conditions, the interaction between MCs and intestinal cells upholds the stability and barrier functionality of the intestine. In cases of external invasions or anomalies, MCs swiftly respond, fostering local inflammation.[46,47] Notably, this study observed severe damage to the colon mucosa in diarrheal mice, accompanied by MC aggregation around the intestinal mucosa and a substantial infiltration of inflammatory cells. Moreover, increased tryptase release from MCs led to heightened intestinal mucosal permeability. Clinical investigations have indicated a substantial rise in colonic MC numbers and activation rates in patients with diarrhea-predominant ulcerative colitis, with the severity of symptoms positively correlating with the degree of MC activation.[48] After pediatric tuina treatment, a marked reduction in MCs and tryptase in colon tissue was evident, implying that pediatric tuina can regulate colonic MCs and aid in clearing effects to alleviate diarrhea. Abdominal massage has also demonstrated the capacity to decrease MC infiltration in colon tissue, thereby improving irritable bowel syndrome.[30] Moreover, the use of Si–Ni-San in treating chronic stress-induced diarrhea can inhibit MC numbers and tryptase release,[49] aligning with the findings of this study.

Pediatric tuina therapy, distinguished by its emphasis on five meridian acupoints, is a treatment method exclusively tailored for children. The application of tuina therapy in treating adult diarrhea differs significantly from its use in pediatric cases, with a primary focus on abdominal tuina techniques. Abdominal tuina has been shown to reduce the hypertrophy of MCs in the colon tissue of rats with diarrhea-predominant irritable bowel syndrome.[50] Its mechanism of action involves direct intervention in the intestinal tract, in contrast to the holistic regulatory mechanism achieved through specific massage points in children via the skin-brain-gut axis.

The occurrence of diarrhea is influenced by various intricate factors, closely associated with bidirectional communication issues in the brain-gut axis.[51] Prior research has affirmed that tuina therapy effectively modulates the brain-gut interaction.[52] Employing fMRI technology, our team has demonstrated that treating SDD in children by stimulating the spleen-meridian and large-intestine-meridian points activates several brain regions linked to visceral, sensory, and motor functions, such as the posterior cingulate cortex, precuneus, middle frontal gyrus, temporal lobe, and insula. The insula notably constitutes a neural pathway governing the brain-gut axis, including the thalamus, cerebellum, basal ganglia, and insula.[53] MCs, as key effectors in the brain-gut axis, release various active substances upon axis activation, impacting intestinal physiological function and permeability.[54–56] This study investigated how pediatric tuina affects MC expression in the brain region of mice with diarrhea. The results revealed a decrease in MCs and tryptase in the brain region following tuina therapy, indicating a potential role of MCs and their released substances in the brain-gut circuitry for treating diarrhea through specific acupoints.

This study underscores the significant role of the spleen-meridian point and the large-intestine-meridian point in pediatric diarrhea treatment, elucidating their effect on MCs. This study serves as a crucial reference for treating pediatric diarrhea by focusing on the spleen-meridian point and the large-intestine-meridian point as primary acupoints in clinical practice. It establishes a foundational framework for clinical applications centered around these points.

Nonetheless, this study has limitations and primarily explores the effects of tuina therapy on diarrhea in young mice. Further investigations are necessary to delve into the release of additional active substances from MCs and the signaling pathways involved. This will deepen our comprehension of the roles of MCs in the neuroendocrine and immune network of the skin-brain-gut axis and elucidate the mechanisms underlying the effects of pediatric tuina on diarrhea.

5. Conclusion

This study utilized a mouse model of spleen-deficiency diarrhea to elucidate the biological mechanism of pediatric tuina on specific acupoints concerning the skin-brain-gut axis and MC involvement. It introduces a novel avenue for fundamental research into pediatric tuina on specific acupoints and furnishes scientific support for using pediatric tuina in diarrhea treatment.

Author contributions

Conceptualization: YW, ML. Methodology: YW, SF, YL. Software: YL and FL. Validation: SF, FL, YG and YC. Formal analysis: FL, FR and RL. Investigation: YL, FL, YG and YC. Resources: YL, SF, FL. Data curation: YL and FL. Writing – Original Draft: YW, YL. Writing – Review & Editing: YW, YL. Visualization: YW, SF, ML. Supervision: YW, ML. Project administration: YW. Funding acquisition: YW.

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Ethical statement

The experimental was approved by the Ethics Committee of the Animal Experimental Center of Tianjin University of Traditional Chinese Medicine (TCM-LAEC2022162).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

References

1. Local Burden of Disease Diarrhoea Collaborators. Mapping geographical inequalities in childhood diarrhoeal morbidity and mortality in low-income and middle-income countries, 2000-17: analysis for the Global Burden of Disease Study 2017. *Lancet*. 2020;395(10239):1779–1801.
2. Public Health Sciences Data Center. Other infectious diarrheal diseases[R/OL]. http://www.phsciencedata.cn/Share/index.html?766c8437-9d73-4360-aa40-47c420767105; 2023-4-27.
3. WHO. Diarrhea disease[R/OL] [2023-4-27] http://www.who.int/mediacentre/factsheets/fs330/en/; 2017-03-02.
4. King CK, Glass R, Bresee JS, Duggan C, Centers for Disease Control and Prevention. Managing acute gastroenteritis among children: oral rehydration, maintenance, and nutritional therapy. *MMWR Recomm Rep (Morb Mortal Wkly Rep)*. 2003;52(RR-16):1–16.
5. Ni X, Wang BX, Wang Q, et al. Code of practice for the treatment of acute infectious diarrheal diseases in children (2020 version). *China Medicine and Pharmacy*. 2020;10(21):249–256.
6. Chen SC, Yu BY, Suen LK, et al. Massage therapy for the treatment of attention deficit/hyperactivity disorder (ADHD) in children and adolescents: a systematic review and meta-analysis. *Compl Ther Med*. 2019 Feb;42:389–399.
7. Lee MS, Kim JI, Ernst E. Massage therapy for children with autism spectrum disorders: a systematic review. *J Clin Psychiatry*. 2011 Mar;72(3):406–411.
8. Fu S, Li Y, Li R, et al. Pediatric tuina for allergic rhinitis in children: a systematic review and meta-analysis of randomized controlled trials. *Front Pediatr*. 2022 Nov 14;10, 1043322.
9. Liang SB, Lai BY, Cao HJ, et al. Pediatric tuina for the treatment of anorexia in children under 14 years: a systematic review and meta-analysis of randomized controlled trials. *Compl Ther Med*. 2020 Jun;51, 102411.
10. Lai BY, Liang N, Cao HJ, et al. Pediatric Tui Na for acute diarrhea in children under 5 years old: a systematic review and meta-analysis of randomized clinical trials. *Compl Ther Med*. 2018;41:10–22.
11. Li HT. Study on the spectrum of modern infantile Tuina diseases. *Tianjin University of Traditional Chinese Medicine*. 2021.
12. Lai BY, Jia LY, Yang LH, Yan YQ, Wang Y, Liu JP. Tuina for rotavirus diarrhea in children:A systematic review and meta-analysis of randomized clinica trials. *World Chinese Medicine*. 2019;14(4):868–875.
13. Wang HL, Zhang SQ, Zhang PL, et al. Multi-central randomized controlled investigation on the massage for supplementing qi and removinoobstruction in the Governor Vessel for treatment of infantile diarrhea due to spleen deficiency. *Chin Acupunct Moxibustion*. 2008;(11):813–816.
14. Wang YG, Guo XQ. Study on rules of using specific acupoints in lnfantile diarrhea for the massage treatment of ancient literature. *Lishizhen Medicine and Materia Medica Research*. 2013;24(1):194–195.
15. Lai BY, Liang N, Cao HJ, et al. Pediatric Tui Na for acute diarrhea in children under 5 years old: a systematic review and meta-analysis of randomized clinical trials. *Compl Ther Med*. 2018;41:10–22.
16. Huang Y, Ma Q, He J, et al. Abdominal massage alleviates functional diarrhea in immature rats via modulation of intestinal microbiota and tight junction protein. *Front Pediatr*. 2022;10, 922799.
17. Bian L, Fu SF, Li RR, Ren FJ, Wang YG. Study on the law of massage acupoint selection for children diarrhea based on the Traditional ChineseMedicine Inheritance Support System. *Tianjin Journal of Traditional Chinese Medicine*. 2020;37(7):784–787.
18. Lu T, Yin L, Chen R, et al. Chinese pediatric Tuina on children with acute diarrhea: a randomized sham-controlled trial. *Health Qual Life Outcome*. 2021;19(1):4.
19. Peng Y, Leng L, Chen Z, et al. Acute infantile diarrhea treated with infantile Tuina: a multicentre randomized controlled trial. *Zhongguo Zhen Jiu*. 2011;31(12):1116–1120.
20. Wang LN, Wang XZ, Li YJ, et al. Activation of subcutaneous mast cells in acupuncture points triggers analgesia. *Cells*. 2022;11(5):809.
21. Chen LZ, Kan Y, Zhang ZY, et al. Neuropeptide initiated mast cell activation by transcutaneous electrical acupoint stimulation of acupoint LI4 in rats. *Sci Rep*. 2018;8(1), 13921.
22. Janssens AS, Heide R, den Hollander JC, Mulder PG, Tank B, Oranje AP. Mast cell distribution in normal adult skin. *J Clin Pathol*. 2005;58(3):285–289.
23. Weber A, Knop J, Maurer M. Pattern analysis of human cutaneous mast cell populations by total body surface mapping. *Br J Dermatol*. 2003;148(2):224–228.
24. Li YM. The search of mobile target for acupuncture: why is mast cell? *Chin Acupunct Moxibustion*. 2021;41(9):965–970.
25. Shen D, Shen X, Schwarz W, Grygorczyk R, Wang L. $P2Y_{13}$ and $P2X_7$ receptors modulate mechanically induced adenosine triphosphate release from mast cells. *Exp Dermatol*. 2020;29(5):499–508.
26. Hu KK, Bruce MA, Butte MJ. Spatiotemporally and mechanically controlled triggering of mast cells using atomic force microscopy. *Immunol Res*. 2014;58(2-3):211–217.
27. —Espinosa E, Valitutti S. New roles and controls of mast cells. *Curr Opin Immunol*. 2018;50:39–47.
28. Liang Y, Huang S, Qiao L, et al. Characterization of protein, long noncoding RNA and microRNA signatures in extracellular vesicles derived from resting and degranulated mast cells. *J Extracell Vesicles*. 2019;9(1), 1697583.
29. Zhang Z, Ernst PB, Kiyono H, Kurashima Y. Utilizing mast cells in a positive manner to overcome inflammatory and allergic diseases. *Front Immunol*. 2022;13, 937120.
30. Li H, Zhang W, Ma F, Zhang X, Wang Y, Wang J. Abdominal massage improves the symptoms of irritable bowel syndrome by regulating mast cells via the trypase-PAR2-pkcε pathway in rats. *Pain Res Manag*. 2022;2022, 8331439.
31. Lin LL. *Effect of Chiropractic Therapy on Skin-Endogenous Cannabinoid System Based on Stress Effect of Mast Cells*. Fuzhou: Fujian University of Traditional Chinese Medicine; 2019.
32. Wang DY. *Effects of Massage on Peripheral Sensitization and Inflammatory Factors in Fibromyalgia Model Rats*. Fuzhou: Fujian University of Traditional Chinese Medicine; 2022.
33. Zou SJ, Zhou YS, Fan YL, et al. Primary study on standardization of animal model in the lnsufficiency of the spleen-qi Syndrome.Partition in macroscopic syndrome and symptoms. *Chinese Journal of Integrated Traditional and Western Medicine on Digestion*. 2001;5:264–267.
34. Xu J. Study on the effect-mechanism of Atractyloside A on spleen deficiencydiarrhea mice based on intestinal flora and TLR4/NF-KB pathway. *Hubei University of Chinese Medicine*. 2022.
35. State Pharmacopoeia Committee. *Pharmacopoeia of the People's Republic of China*. Beijing, China: Medical Science and Technology Press; 2015:22–23.
36. Zhang L, Ravipati AS, Koyyalamudi SR, et al. Antioxidant and anti-inflammatory activities of selected medicinal plants containing phenolic and flavonoid compounds. *J Agric Food Chem*. 2011 Dec 14;59(23):12361–12367.
37. Ren G, Li L, Hu H, Li Y, Liu C, Wei S. Influence of the environmental factors on the accumulation of the bioactive ingredients in Chinese rhubarb products. *PLoS One*. 2016 May 3;11(5), e0154649.
38. Li X, Zhu J, Wu Y, Tan Z. Correlation between kidney function and intestinal biological characteristics of adenine and folium SennaeInduced diarrhea model in mice. *Turk J Gastroenterol*. 2023;34(1):4–12.
39. Ge L, Cao X, Zhang Q, et al. Tuina for children with acute diarrhea: an evidence-based clinical guideline. *Chin J Evidence-Based Med*. 2021;21(7):745–753.
40. Wu XN, He SY, Li XL. Effect of addition and reduction of Shenling Baizhu Powder combined with pediatric massage therapy on pediatric autumn diarrhea. *Contemporary Medicine*. 2014;20(16):157–158.
41. Shang Y. Clinical observation on 45 cases of acute non-bacterial infections diarrhea in children treated with the combination of modified Weiling Decoction and Tuina. *Journal of Pediatrics of Traditional Chinese Medicine*. 2018;14(2):58–60.
42. Lv Y, Jiang YY. Efficacy of Yan Hu Ning plus pediatric massage on pediatric rotavirus enteritis. *Clinical Journal of Chinese Medicine*. 2020;12(7):26–28.
43. Liu WR, Sun YX, Wang W, Xing XL, Wang MX, Du MR. Clinical efficacy of infantile massage combined with acupoint herbal patching for rotavirus enteritis in children and its influence on gastrointestinal hormone and inflammatory index. *Hebei Journal of Traditional Chinese Medicine*. 2021;43(10):1690–1693.
44. Shi Y, Qi L, Wang J, et al. Moxibustion activates mast cell degranulation at the ST25 in rats with colitis. *World J Gastroenterol*. 2011;17(32):3733–3738.
45. Ding N, Jiang J, Qin P, Wang Q, Hu J, Li Z. Mast cells are important regulator of acupoint sensitization via the secretion of tryptase, 5-hydroxytryptamine, and histamine. *PLoS One*. 2018;13(3), e0194022.

46. Li L, ed. *Mast Cell Fundamentals and Clinical Applications*. vol. 6. BeiJing: China Science Publishing & Media Ltd; 2020.
47. Albert-Bayo M, Paracuellos I, González-Castro AM, et al. Intestinal mucosal mast cells: key modulators of barrier function and homeostasis. *Cells*. 2019;8(2):135.
48. Katinios G, Casado-Bedmar M, Walter SA, et al. Increased colonic epithelial permeability and mucosal eosinophilia in ulcerative colitis in remission compared with irritable bowel syndrome and Health. *Inflamm Bowel Dis*. 2020;26(7):974–984.
49. Chen HY, Liu J, Weng DZ, et al. Ameliorative effect and mechanism of Si-Ni-San on chronic stress-induced diarrhea-irritable bowel syndrome in rats. *Front Pharmacol*. 2022;13, 940463.
50. Li HN, Wang YY, Zhang XF, Ma F, Dong H, Wang JG. Mechanism of abdominal massage in preventing visceral hypersensitivity in IBS-D rats andits effect on intestinal mast cells and TRPV1 sensitization. *Liaoning Journal of Traditional Chinese Medicine*. 2023;10:1–12.
51. Cangemi DJ, Lacy BE. Management of irritable bowel syndrome with diarrhea: a review of nonpharmacological and pharmacological interventions. *Therap Adv Gastroenterol*. 2019;12, 1756284819878950.
52. Zhang Y, Cong D, Liu P, et al. Study on the mechanism of regulating the hypothalamic cortical hormone releasing hormone/corticotropin releasing hormone type I receptor pathway by vibro-annular abdominal massage under the brain-intestine interaction in the treatment of insomnia. *Medicine (Baltim)*. 2021;100(19), e25854.
53. Bian L. Study on brain functional magnetic resonance of childrenwith spleen deficiency diarrhea by using tuina PituAcupoint for different duration. *Tianjin University of Traditional Chinese Medicine*. 2020.
54. Farhadi A, Fields JZ, Keshavarzian A. Mucosal mast cells are pivotal elements in inflammatory bowel disease that connect the dots: stress, intestinal hyperpermeability and inflammation. *World J Gastroenterol*. 2007;13(22):3027–3030.
55. Bhuiyan P, Chen Y, Karim M, Dong H, Qian Y. Bidirectional communication between mast cells and the gut-brain axis in neurodegenerative diseases: avenues for therapeutic intervention. *Brain Res Bull*. 2021;172:61–78.
56. Traina G. The role of mast cells in the gut and brain. *J Integr Neurosci*. 2021;20(1):185–196.