Modified Renshen Wumei Decoction Alleviates Intestinal Barrier Destruction in Rats with Diarrhea

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Introduction

A single layer of epithelial cells lining the gut constitutes the intestinal barrier and mainly consists of enterocyte membranes and tight junctions (TJs) between differentiated enterocytes [1-3]. Intestinal barrier integrity is essential for digesting and absorbing nutrients, and maintaining the crucial physiological barrier against invasion by exogenous pathogenic microorganisms, both in humans and animals [4, 5]. However, numerous studies have indicated that persistent diarrhea with over 14 days of watery stool impairs the growth of the intestinal mucosa, thereby hampering the function of the mechanical barrier as well as ion transport, microbiota composition, and immune function [6, 7]. Malnutrition, adolescence, lack of breastfeeding, infection, and inappropriate use of antibiotics are risk factors for persistent diarrhea. In addition, antibiotic misuse during persistent diarrhea episodes has also been identified as a main risk factor [8]. At present, fluid infusion, dietotherapy, antibiotics, and intestinal mucosal protector use are common therapeutic methods for ameliorating persistent diarrhea in children [9, 10].

Traditional Chinese medicine (TCM) has offered complementary and alternative therapies for treating diarrhea; these therapies have been found to improve diarrheal symptoms, with good feedback being received from patients [11, 12]. Renshen, a popular TCM, is widely used to treat many diseases in Asian countries. It exerts beneficial effects through its primary active component, polysaccharides, which has been reported to help treat diarrhea [13]. Another famous Chinese herbal formula, Wumei Pill, is useful for treating diarrhea-predominant irritable bowel syndrome (IBS-D) by improving the ratio of Bifidobacterium/Enterobacteriaceae (B/E value) and reducing the hippocampal tissue glutamate (Glu), P α-amino butyric acid (P α-GABA), dopamine (DA) and 5-hydroxytryptamine (5-HT) contents [14]. Through more than two decades of clinical practice and research, we created a novel Chinese herb, called ‘modified Renshen Wumei decoction (MRWD),’ which could be an external...
therapeutic agent to help ameliorate symptoms of persistent diarrhea. However, to date, there is no specific information on the underlying mechanisms by which MRWD improves intestinal barrier function in a diarrhea model, and further studies are therefore needed on this intriguing topic.

Hence, our study aimed to elaborate the effects of MRWD administration on intestinal barrier function in rats with diarrhea, providing partial theoretical evidence about the efficacy of MRWD in preventing and curing diarrhea. Our findings will also have important practical implications for the use of MRWD as a therapeutic agent to ameliorate persistent diarrhea in children.

**Materials and Methods**

The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Sichuan Academy of Laboratory Animals (P202004091). All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health).

**Animal Care and Experimental Design**

Specific pathogen-free (SPF)-grade male Sprague–Dawley rats (weighing 70 ± 10 g) were supplied by Dossy Experimental Animals Co., Ltd. (China). The rats were kept in an animal room at a constant temperature (25 ± 2°C) and humidity (55 ± 10%) with 12 h of light per day and allowed food and water ad libitum before the experiment. Twelve out of 48 rats were randomly distributed to the blank group (CK group). To create diarrhea models, the other 36 rats were infused with 20 ml/kg BW senna fluid and were made to swim with a fuse wound around the tail root until they were exhausted. After successful modeling, the rats were randomly distributed to the model group (MC group), Western medicine group (Medilac-Vita) (MV group) and Chinese herb group (MRWD group). All the rats were fed a basal diet in a single cage. After 14 days following the creation of the diarrhea models, the rats in the CK and MC groups were infused with 0.9% NaCl, while those in the MV and MRWD groups were infused with 0.7 g/kg BW MV and 35 g/kg BW MRWD, respectively. The infusion was given once a day for 7 days.

**Preparation of Chinese Medicines**

Preparation of 30% senna fluid: In total, 300 g of senna leaves were immersed in 2,000 ml water at 90°C for 5 min, decoccted for 15 min and then filtered through four layers of gauze. After concentration using a rotary evaporator and dissolution to obtain 1,000 ml solution, the solution was stored at 4°C.

Preparation of MRWD: In total, 50 g of MRWD, consisting of 8 g of raw sun-dried Renshen, 8 g of Wumei, 5 g of hawthorn, 5 g of Chinese Yam, 5 g of pomegranate peel, 5 g of Pogostemon cablin, 5 g of tuckahoe, 5 g of lotus seed, 1 g of baked ginger and 3 g of prepared liquorice, was boiled twice in pure water. After filtration with a 200-mesh filter cloth, the solution was combined and concentrated to 1.74 g/mL. The aforementioned Chinese medicines were provided and identified by the Pharmacy Department of Chengdu University of Traditional Chinese Medicine.

**Sample Collection**

At the end of the experiment, the average body weight of rat in different groups was shown in Table 1. The rats were anesthetized by intraperitoneally injecting 3.5% chloral hydrate (350 mg/kg BW). Following this, 4 ml of abdominal aortic blood was collected and centrifuged in an EDTA anticoagulant tube at 3,500 rpm for 15 min to acquire serum samples; the samples were kept at −20°C until analysis. After blood sampling, colon samples were immediately collected from the upper 4 cm of the anus. Three pieces of a 2-cm colon sample were gently flushed with 0.9% NaCl and then fixed in 4% paraformaldehyde.

**Serum Electrolyte Detection**

The concentrations of Na⁺, K⁺, Ca²⁺, and Cl⁻ were determined using spectrophotometric kits in line with the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China).

**Enzyme-Linked Immunosorbent Assay (ELISA)**

The D-lactate, diamine oxidase (DAO), IL-1, IL-6, TNF-α, IFN-γ, calcitriol, taurine, leukotriene B4 (LTB4) and cortisone contents were determined using spectrophotometric kits in line with the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute).

**Table 1. The average body weight of rats in different treatment groups at the time of sacrifice.**

| Groups | Average body weight (g) |
|--------|------------------------|
| CK     | 197.68 ± 23.24         |
| MC     | 158.24 ± 13.33***      |
| MV     | 156.60 ± 13.47         |
| MRWD   | 174.55 ± 9.70          |

*Values are means with standard deviations (n = 8).
*Compared with the CK group, **p < 0.001. †Compared with the MC group, ‡p < 0.05.
Western Blot Analysis

Frozen colon samples (approximately 0.1 g) were homogenized in 1 ml ice-cold RIPA lysis buffer (1% Triton X100, 10% SDS, 0.15 M NaCl, 15.4 mM Tris–HCl, 0.5% deoxycholic acid, 1 mM Na orthovanadate and Roche Mini EDTA-Free Protease Inhibitor Cocktail; pH 8.0). Following this, ultrasonication was performed to break the cells. The cells were then centrifuged at 10,000 × g for 15 min at 4°C. The proteins in the supernatant containing 4× Laemmli sample buffer (Bio-Rad, USA) were denatured in a 98°C metal bath for 10 min. Equal amounts of samples were then subjected to SDS–PAGE, and the expression levels of toll-like receptor (TLR4), MyD88, p-NF-κB p65, NF-κB p65, occludin, claudin-1 and ZO-1 proteins were assessed by western blotting using the indicated antibodies. The expression level of β-actin was assessed to ensure equal protein sample loading.

Serum Metabolomics Analysis

In total, we mixed 100 μl serum samples and 400 μl prechilled methanol (Thermo Fisher, USA) by vortexing. The samples were incubated on ice for 5 min and then centrifuged at 15,000 × g for 5 min. Some supernatants were diluted to a final concentration containing 60% methanol by LC–MS-grade water (Thermo Fisher). The samples were subsequently transferred to a fresh Eppendorf tube using a 0.22-μm filter and then centrifuged at 15,000 × g at 4°C for 10 min. Finally, the filtrate was injected into the LC–MS/MS system for analysis. LC–MS/MS analysis was performed by the Vanquish UHPLC System (Thermo Fisher) coupled with an Orbitrap Q Exactive HF-X mass spectrometer (Thermo Fisher). The samples were injected onto a Hypersil Gold column (100 × 2.1 mm, 1.9 μm) using a 16 min linear gradient at a flow rate of 0.2 ml/min. The eluents for the positive polarity mode were as follows: eluent A (0.1% FA in water) and eluent B (methanol). The eluents for the negative polarity mode were as follows: eluent A (5 mM ammonium acetate 153, pH 9.0) and eluent B (methanol). The solvent gradient was set as follows: 2% B, 1.5 min; 2–100% B, 12 min; 100% B, 14 min; 100–2% B, 14.1 min and 2% B, 16 min. The Q Exactive HF-X mass spectrometer was operated with a spray voltage of 3.2 kV, capillary temperature of 320°C, sheath gas flow rate of 35 arb and aux gas flow rate of 10 arb.

The raw data files generated by UHPLC–MS/MS were processed using Compound Discoverer 3.0 (CD 3.0, Thermo Fisher) for peak alignment, peak picking and quantification of each metabolite. The metabolites were annotated using the KEGG database (http://www.genome.jp/kegg/), HMDB database (http://www.hmdb.ca/) and LIPID MAPS database (http://www.lipidmaps.org/). Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were performed using metaX (a flexible and comprehensive software program for processing metabolomics data). Univariate analysis (t-test) was performed to determine the statistical significance (p-value). Metabolites with a Variable Importance in Projection (VIP) score > 1, p-value < 0.05 and fold change (FC) ≥ 2 or FC ≤ 0.5 were considered differential metabolites. Volcano plots were used to filter metabolites of interest based on their log(FC) and −log10(p-value).

For clustering heat maps, the data were normalized using z-scores of the intensity areas of differential metabolites and plotted using the heatmap package in R language. The correlation between differential metabolites was assessed using the cor() function in R language (method = Pearson’s correlation). Statistical significance of the correlation between differential metabolites was calculated using cor.mtest() in R language. The functions of these metabolites and metabolic pathways were assessed using the KEGG database. Metabolic pathway enrichment of differential metabolites was performed. If x/n > y/N, the metabolic pathway was regarded as significantly enriched.

Statistical Analysis

SPSS 24.0 statistical software (IBM, USA) was used to analyze all the data. If the data conformed to a normal distribution and the variance was homogeneous, the LSD method was used after one-way ANOVA for multiple comparisons. Otherwise, Tamhane’s T2 multiple comparison was adopted. The data are displayed as the means ± SE. p-values < 0.05 were considered statistically significant.

Results

Physical and Physiological Effects in the MC and MRWD Groups

The diarrhea assessment results revealed that the prevalence of loose stools and diarrhea index were significantly higher (p < 0.01) in the MC group than that in the MV and MRWD groups (Table 2). Compared with the CK group, HE staining revealed obvious pathological changes in the colon in the MC group; however, these pathological changes were lesser in the MV and MRWD groups (Fig. 1A).

![Table 2. Assessment of diarrhea in rats.](image)

| Groups   | CK        | MC        | MV        | MRWD      |
|----------|-----------|-----------|-----------|-----------|
| Prevalence of loose stools | 89.46 ± 7.85 | 36.37 ± 4.61 * | 32.16 ± 4.37 ** |
| Diarrhea index | 2.32 ± 0.48 | 0.90 ± 0.16 * | 0.72 ± 0.17 ** |

*Values are means with standard deviations (n = 8).
*Compared with the MC group, p < 0.05 and **p < 0.01.

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however, the contents were lesser in the MV and MRWD groups (Figs. 1B and C). These results indicate that MRWD infusion alleviates the destruction of the intestinal morphology induced by diarrhea.

Optimization and Validation of UHPLC–MS/MS Methods

The differences in metabolites among the CK, MC, MV and MRWD groups were assessed by PCA. As shown in Fig. 2A, the PCA score plot provided the clustering images of each group; these images were well distinguished, indicating that MV and MRWD infusion could partly regulate the metabolic disorders caused by diarrhea. To further analyze the metabolomics model for each group and to identify the potential differential metabolites with

![Fig. 1. Physical and physiological effects of MRWD on the colon of rats. (A) Haematoxylin–eosin (HE) staining of colon tissue. (B) The concentration of D-lactate was detected by ELISA. (C) The concentration of DAO was detected by ELISA. *Compared with the CK group, \( p < 0.05 \) and ** \( p < 0.01 \). *Compared with the MC group, \( p < 0.05 \) and *** \( p < 0.01 \).](image)

![Fig. 2. Multivariate statistical analysis plot of CK, MC MV and MRWD groups. The PCA (A) and PLS-DA (B) score plots demonstrate complete separation of the serum samples among the groups.](image)
significant changes, PLS-DA was performed. Compared with PCA, PLS-DA emphasizes the differences between
groups and weakens the random differences unrelated to the purpose of the study. This enables a better
understanding of the overall characteristics and variation rules of multidimensional metabolomics data, which is
conducive to the discovery of intergroup differences and metabolic markers causing differences. The score plot
provided in Fig. 2B shows a clear separation among the four groups of serum samples.

Identification of Potential Metabolites in the Serum Samples of Diarrhea Model Rats Following MRWD
Infusion

Of the numerous compound signals detected in the CK, MC, MV and MRWD groups, variables that
significantly contributed to clustering and discrimination were identified according to the following threshold:
VIP score ≥ 1.0. This threshold was obtained after PLS-DA processing of the variables (Fig. 2B). Based on the VIP
score, variables were selected from the four groups for univariate analysis (t-test). When the p-value was < 0.05, the
compounds were screened as potential differential metabolites for identification. The differential metabolites
obtained from the four groups were analyzed by one-way ANOVA, and the results without significant differences
were removed. The screening results of the differential metabolites are presented as a volcano plot in Fig. 3A.

Table 3 summarizes the 20 potentially differentiated metabolites obtained from the four groups and their
retention times, mass charge ratios and molecular formulas. For clustering heatmaps, the data were assessed and
plotted in R language. The results showed that the metabolic pattern of the differential metabolites obviously
differed among the four groups (Fig. 3B).

Analysis of Signal Pathways Using the Serum Samples of Diarrhea Model Rats Following MRWD
Infusion

The KEGG database was used to assess the relevance of the pathways to determine the locations of the
differential metabolites among the groups. In fact, the bubble map of the metabolic pathway analysis indicated that
dramatically disturbed metabolic pathways included mineral absorption, carbohydrate digestion and
absorption, vitamin digestion and absorption, metabolic pathways, steroid biosynthesis, endocrine- and other
factor-regulated calcium reabsorption, protein digestion and absorption and oxidative phosphorylation (Fig. 4).

Validation of the Representative Metabolites

Based on metabolomics data, metabolites, including calcitriol, taurine, LTB4 and cortisone, were screened.
Following this, the effects of MRWD on the representative metabolites were assessed by ELISA. As shown in
Fig. 5A, the induction of diarrhea significantly reduced the calcitriol, taurine and cortisone contents (p < 0.01).
Compared with the MC group, the calcitriol, taurine and LTB4 contents were markedly increased in the MV and
MRWD groups (p < 0.01).

Analysis of Serum Electrolytes

Spectrophotometric kits were used to detect the concentrations of serum electrolytes, including Na⁺, K⁺, Ca²⁺,
and Cl⁻. As shown in Fig. 5B, the induction of diarrhea significantly decreased the electrolyte concentrations (p <
0.05). However, the electrolyte concentrations were increased by the MRWD infusion (p < 0.05).
Fig. 3. Analysis of differential metabolites in the serum samples obtained from CK, MC, MV and MRWD groups. (A) A volcano plot representing the significant variables for discriminating among the four groups. Significantly increased variables are presented in the red circle, while significantly decreased variables are presented in the green circle. (B) A heatmap of hierarchical clustering analysis (HCA) for differential metabolites. HCA is based on the Euclidean distance; colors from blue to red indicate elevated contents of metabolites.

Fig. 4. Pathway analysis of differential metabolites in the serum samples. A bubble plot for the identification of the most relevant metabolic pathways. The color of the circles indicates the significance of changes in the metabolites in the corresponding pathway, while the size corresponds to the pathway impact score. The pathway impact score represents the cumulative percentage from the matched metabolite to the total pathway importance.
Analysis of Intestinal Integrity

The differences in intestinal barrier-related variables among the four groups indicated that the expression levels of occludin, claudin-1 and ZO-1 proteins were lower in the MC group \( (p < 0.01) \) than that in the CK group. However, MV and MRWD infusion increased \( (p < 0.05) \) the expression levels of claudin-1 and ZO-1 proteins (Fig. 6).

Analysis of Inflammation-Related Parameters

As shown in Fig. 7A, the induction of diarrhea increased the expression levels of TLR4, MyD88 and p-NF-κB p65 proteins \( (p < 0.05) \). The expression levels of TLR4, MyD88 and p-NF-κB p65 proteins were markedly lower in
the MV and MRWD groups than that in the MC group ($p < 0.01$). In addition, the IL-1 and TNF-α contents were higher in the MC group than that in the MC and MRWD groups ($p < 0.05$). Nevertheless, IL-6 and IFN-γ were not prominently influenced ($p > 0.05$) by the induction of diarrhea (Fig. 7B).

**Discussion**

Maintenance of the integrity of intestinal epithelial cells (IECs) and control of the permeability between adjacent IECs are the minimum requirements to ensure the intestinal barrier functioning properly [15]. However, the intestinal function is often compromised in patients with persistent diarrhea. Consequently, maintaining normal intestinal architecture and functioning is essential for alleviating diarrhea. In our study, we noticed that MRWD obviously decreased the diarrhea index and restored the impaired intestinal architecture in rats with diarrhea. Serum D-lactic acid and DAO are two well-established markers for monitoring changes in intestinal permeability [16]. The increase in the serum D-lactate and DAO contents has been reported to correlate with the extent of intestinal barrier dysfunction [17, 18]. Consistent with this finding, we noted a decrease in the serum D-lactate and DAO contents following MRWD infusion. Moreover, TJs connect neighboring IECs and play a vital role in paracellular solute permeability [19]. The reconfiguration of TJs makes the epithelial barrier more conducive to the paracellular permeation of fluid and cations, predominantly Na+, which can decrease transepithelial electric resistance (TEER) [20]. Besides, it was reported that a single material of MRWD possesses the protective function of TJ proteins to alleviate the destruction in different disease models. Pomegranate peel polyphenols (PPPs) alleviated HFD-induced depressed colonic tight junction protein expression level in rats. In addition, it has been confirmed that PPPs increased the LPS-induced decreased tight junction protein expression level in Caco-2 cells [21]. Chinese yam phenanthrene 4 (CYP4) pretreatments substantially improved tight junction protein occludin in dextran sulfate sodium (DSS)-treated mice [22]. Hence, TJs and serum ion distribution were also assessed. The expression levels of occludin, claudin-1 and ZO-1 proteins and the electrolyte concentrations were markedly abrogated by the induction of diarrhea; however, this finding was not noted in the MV and MRWD groups. These results support the notion that MRWD exerts beneficial effects on the intestinal barrier integrity in rats with diarrhea.

In addition to damaging the intestinal integrity and disrupting electrolyte homeostasis in the colon of rats, the induction of diarrhea triggers intestinal inflammation marked by upregulated expression levels of pro-inflammatory cytokines in the intestine [23, 24]. Previous studies revealed that pro-inflammatory cytokines such as IL-1 and TNF-α could increase the intestinal TJ permeability, which has been postulated to be an important factor in the exacerbation of intestinal inflammation [25-27]. In our study, MV and MRWD infusion was found to decrease the IL-1 and TNF-α contents induced by diarrhea; this finding is consistent with the improved intestinal barrier integrity. The existing scientific evidence states that inflammatory responses can be regulated by various signaling pathways. Hence, we also assessed the molecular mechanisms by which MRWD affects intestinal inflammatory responses in rats with diarrhea.

TLR is a type of protein with a type I transmembrane receptor, which is rich in extracellular leucine repeat domains and intracellular transcription/IL-1 receptor domains [28]. TLR4 is the best-characterized member of the TLR family; it is activated by bacterial lipopolysaccharide (LPS), thus initiating an innate immune response and inflammation in higher animals [29, 30]. In our study, we found that the colonic expression levels of TLR4 protein and its downstream target, MyD88, were decreased in the MV and MRWD groups. A previous study
demonstrated that the genetic knockout of TLR4 (Tlr4−/−) protected against the development of barrier disorder and mitigated the duration and severity of diarrhea [31]. Therefore, the downregulation of the TLR4 signaling pathway and its downstream cytokine production may help improve the intestinal integrity following MRWD infusion. At the same time, single materials of MRWD, such as pugosotenomon cablin, Chinese yam, ginger, and pomegranate peel, protect the host via suppressing TLR4/ NF-κB signaling and its downstream inflammatory cytokines [32-34]. Subsequently, we assessed the expression of p-NF-κB p65 protein. As expected, MV and MRWD infusion suppressed the expression level of p-NF-κB p65 protein. Therefore, we reasoned that the inhibition of intestinal pro-inflammatory cytokines was linked to the blocking of NF-κB expression via the suppression of the TLR4 signaling pathways in the MRWD group.

In the present study, serum samples were analyzed by a UHPLC–MS/MS-based metabolomics approach to detect the effect of MRWD infusion on the endogenous metabolites; significant differences in the levels of the endogenous metabolites were observed among the four groups. Studies have shown that phosphoric acid can promote intestinal maturation and the growth rate of longfin yellowtail fish by stimulating digestive enzymes [35]. KEGG enrichment results indicated that phosphoric acid plays an important role in mineral absorption as well as oxidative phosphorylation. The induction of diarrhea significantly decreased the content of phosphoric acid, while MRWD infusion alleviated this decrease, with the content in the MRWD group being higher than that in the CK group. In the present study, the calciotritol content in the MC group was found to be lower than that in the CK group. An MRWD/MC ratio greater than 1 indicated that MRWD infusion increased the calciotritol content. The altered calciotritol content indicated that intestinal calcium absorption was abrogated by the induction of diarrhea but alleviated by MRWD infusion [36]. Similarly, the taurine content was lower in the MC group than in the CK group; however, MRWD infusion increased the taurine content in rats with diarrhea. KEGG enrichment results indicated that taurine is vital to the transport of minerals and organic ions. These changes indicated that intestinal absorption was markedly hampered by the induction of diarrhea but was alleviated by MRWD infusion. Moreover, the content of LTB4, which is related to the PPAR pathway, was significantly increased by MRWD infusion. Given the damage to intestinal barrier function by inflammatory cytokines, e.g., TNF-α, IL-1 and IL-6, studies have revealed that PPARs plays an anti-inflammatory role by subverting NF-kB activation; this finding is consistent with the results of western blotting and ELISA [37, 38]. In brief, the serum metabolites showed an abnormal change, which might be closely related to abnormal intestinal absorption and anti-inflammatory function. MRWD infusion could alleviate diarrhea by improving intestinal absorption and immune function.

In summary, the present findings indicate that MRWD administration can improve intestinal barrier function by promoting electrolyte transport, intestinal integrity and inhibiting the TLR4/NF-κB signaling pathways. These changes were found to be accompanied by the alleviation of diarrhea in rats. Our findings provide a scientific basis for the use of MRWD for treating persistent diarrhea.

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Conflicts of Interest
The authors have no financial conflicts of interest to declare.

References
1. Groschwitz KR, Hogan SP. 2010. Intestinal barrier function: molecular regulation and disease pathogenesis. J. Allergy Clin. Immunol. 124: 3-20.
2. Chelakkot C, Ghim J, Sung HR. 2018. Mechanisms regulating intestinal barrier integrity and its pathological implications. Exp. Mol. Med. 50: 103.
3. Maake, V, Severine V. 2017. The intestinal barrier: a fundamental role in health and disease. Expert Rev. Gastroenterol. Hepatol. 11: 821-834.
4. Lin XW, Liu X, Xu JJ, Cheng KK, Cao JN, Liu T, et al. 2018. Alginate oligosaccharide enhances intestinal integrity of weaned pigs through altering intestinal inflammatory responses and antioxidant status. RSC Adv. 8: 13482-13492.
5. König J, Wells J, García-Ródenas C, MacDonald T, Mercenier A, et al. 2016. Human intestinal barrier function in health and disease. Clin. Transl. Gastroen. 7:e196.
6. Sarmin M, Hossain MI, Islam SB, Alam NH, Sarker SA, Islam MM, et al. 2020. Efficacy of a green banana-mixed diet in the management of persistent diarrhea: protocol for an open-labeled, randomized controlled trial. JMIR Res. Protoc. 9:e15759.
7. Shafiqul S, Tahmeeed A, Harald B. 2017. Persistent diarrhea: a persistent infection with enteropathogens or a gut commensal dysbiosis? Environ. Microbiol. 19: 3789-3801.
8. Mahalanabis D, Alam AN, Rahman N, Hsannat A. 1991. Prognostic indicators and risk factors for increased duration of acute diarrhea and for persistent diarrhea in children. Int. J. Epidemiol. 20: 1064-1072.
9. Jeannette M, Sommer C, Olesen TM, Iverson P, Gustav A, Seth J, et al. 2018. Persistent symptoms in patients with Crohn’s disease in remission: an exploratory study on the role of diet. Scand. J. Gastroenterol. 53: 573-578.
10. Bandi G, Squit, K, Bhutta Z. 2020. Persistent diarrhoea: current knowledge and novel concepts. Paediatr. Int. Child Health 39: 41-47.
11. Liu YL, Liu KL, Yang M, Han Y, Zhang Q, Conde J, et al. 2019. Gastric parietal cell and intestinal goblet cell secretion: a novel cell-mediated in vivo metal nanoparticle metabolic pathway enhanced with diarrhea via Chinese herbs. Nanoscale Res. Lett. 14: 79.
12. Lin XY, Lin X, Xu JJ, Cheng KK, Cao JN, Liu T, et al. 2019. Metabolomics analysis of herb-partitioned moxibustion treatment on rats with diarrhea-predominant irritable bowel syndrome. Chin. Med. 14: 18.
13. Li SS, Qi YL, Chen LX, Qu D, Li ZM, Gao K, et al. 2019. Effects of Panax ginseng polysaccharides on the gut microbiota in mice with antibiotic-associated diarrhea. Int. J. Biol. Macromol. 124: 931-937.
14. Ding XJ, Sun XL, Yu XF, Zhao ST, Yang Y, Xu WJ, et al. 2019. The effects of wuzei pill on intestinal flora and neurotransmitters in rats with diarrhea-predominant irritable bowel syndrome (IBS-D). AIP Conf. Proc. 2079: 020028.
15. Laura R, Long J, Thorsten C. 2020. Barrier integrity and chronic inflammation mediated by HIF-1 impact on intestinal tumorigenesis. *Cancer Lett.* 490:186-192.

16. Assadiana A, Assadizadeh O, Senekowitsch C, Rotter R, Bahrami S, Furst W, et al. 2006. Plasma D-lactate as a potential early marker for colon ischaemia after open aortic reconstruction. *Euro. J. Vasc. Endovasc.* 31:470-474.

17. Hui S, Wu BY, Wan J, Liu WH, Su BB. 2015. The role of serum intestinal fatty acid binding protein levels and D-lactate levels in the diagnosis of acute intestinal ischemia. *Clin. Res. Hepatol. Gas.* 39:373-378.

18. Fukudaz T, Tsukanos K, Nakatsuji H, Suzuki K. 2019. Plasma dianzine oxidae activity decline with diarrhea severity in calves indicating systemic dysfunction related to intestinal mucosal damage. *Res. Vet. Sci.* 126:127-130.

19. Cathleen MC, Emily JO, Keely GM, Allie ES, Anne MS, Kristen MS, et al. 2012. Small bowel resection increases paracellular gut barrier permeability via alterations of tight junction complexes mediated by intestinal TLR4. *J. Sur. Res.* 258:73-81.

20. Anica SB, Moorthy K, Fan S, Jimenz J, Hernande, Gibson K, et al. 2019. The JAK-inhibitor tofacitinib rescues human intestinal epithelial cells and colonoids from cytokine-induced barrier dysfunction. *Inflamm. Bowel Dis.* 26:407-422.

21. Zhao R, Long X, Yang J, Du L, Zhang X, Li J, et al. 2019. Pomegranate peel polyphenols reduce chronic low-grade inflammatory responses by modulating gut microbiota and decreasing colonic tissue damage in rats fed a high-fat diet. *Food Funct.* 10:8273-8285.

22. Li Q, Li K, Hu T, Liu F, Liao S, Zou Y, et al. 2021. 6,7-Dihydroxy-2,4-Dimethoxyphenanthrene from Chinese yam peels alleviates DSS-induced intestinal mucosal injury in mice via modulation of the NF-κB/COX-2 signaling pathway. *J. Agric. Food Chem.* 69:4720-4731.

23. Beheshtipour J, Raeeszadeh M. 2020. Evaluation of interleukin-10 and pro-inflammatory cytokine profile in calves naturally infected with neonatal calf diarrhea syndrome. *Arch. Razi Inst.* 75:213-218.

24. Xu X, Gao LM, Liu YL, Xie CY. 2020. Maternal dietary uridine supplementation reduces diarrhea incidence in piglets by regulating the intestinal mucosal barrier and cytokine profiles. *J. Sci. Food Agr.* 100:3709-3718.

25. Sadi RA, Te DM, Said HM, Ma TY. 2010. IL-1β-induced increase in intestinal epithelial tight junction permeability is mediated by MEKK-1 activation of canonical NF-κB pathway. *Ann. J. Pathol.* 177:2310-2322.

26. Capalco CT, Nusrat A. 2009. Cytokine regulation of tight junctions. *Biochim. Biophys. Acta* 1788:864-871.

27. Sadi RA, Boivin M, Ma T. 2013. Mechanism of cytokine modulation of epithelial tight junction barrier. *Front. Biosci.* 14:2765-2778.

28. Kim HK. 2018. Do Toll-like receptors play a new role as a biomarker of irritable bowel syndrome?. *J. Neurogastroenterol.* 24:510-511.

29. Nikolay K, Konstantin S, Vladimir C, Porozov Y, Savateeva-Lyubimova, T. 2015. TLR4 signaling pathway modulators as potential therapeutics in inflammation and sepsis. *Vaccines* 5:34.

30. Peri F, Graus F, Weiss J. 2015. Endotexin, TLR4 signaling and beyond. *Mol. Immunol.* 63:125-126.

31. Wardill HR, Bowen J, Van Sebille YZ, Kate RS, Janet KC, Imogen AB, et al. 2016. TLR4-dependent claudin-1 internalization and secretagoue-mediated chloride secretion regulate irinotecan-induced diarrhea. *Mediators Inflamm.* 2016:1-10.

32. Meng X, Hu W, Wu S, Zhu Z, Lu R, Yang G, et al. 2019. Chinese yam peel enhances the immunity of the common carp (Cyprinus carpio L.) by improving the gut defence barrier and modulating the intestinal microbiota. *Fish Shellfish Immunol.* 95:528-537.

33. Du L, Li J, Zhang X, Wang L, Zhang W, Yang M, et al. 2019. Pomegranate peel polyphenols inhibits inflammation in LPS-induced RAW264.7 macrophages via the suppression of TLR4/NEK-xB pathway activation. *Food Nutr. Res.* 63:doi:10.29219/fnr.v63.3392.

34. Kim N, Ryu S, Hwang B, Park S, Kim Y, Hong J, et al. 2012. 1-Dehydro-[10]-gingerdione from ginger inhibits IKKβ activity for NF-κB activation and suppresses NF-κB-regulated expression of inflammatory genes. *Br. J. Pharmacol.* 167:128-140.

35. Suástegui JM, Leiva JS, Teles A, Dariel TR. 2020. Evaluation of homeopathic phosphoric acid, silica and pathogenic vibrio on digestive enzyme activity of longfin yellowtail fish (*Seriola rivoliana*). *Homeopathy* 109:3-13.

36. Kirby BJ, Ryan BA, Berit SK, St-Arnaud R, Kovacs CS. 2019. Intestinal calcium absorption upregulates during lactation without the vitamin D receptor (VDR) but is downregulated without calcitriol: evidence of an alternate receptor?. *Calcif. Tissue Int.* 104:S101.

37. Monsalve FA, Pyarasani RD, Lopez FD, Rodrigo MC. 2013. Peroxinsome proliferator-activated receptor targets for the treatment of metabolic diseases. *Mediators Inflamm.* 2013:539627.

38. Su CG, Wen X, Bailey ST, Jiang W, Rangwala SM, Keilbaugh,SA, et al. 1999. A novel therapy for colitis utilizing PPAR-gamma ligands to inhibit the epithelial inflammatory response. *J. Clin. Invest.* 104:383-389.