



Therapeutic Efficacy of Acupuncture Targeting GV-1 Acupoint on Diarrhea Induced by Sennae Folium in Rabbits

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ABSTRACT

Diarrhea is among one of the significant digestive disorders having significant implications related to rabbit farming. This study aimed to investigate the effects of GV-1 (*Hou-hai*) acupuncture on behavior, intestinal structure and immune function in rabbits having diarrhea induced by Sennae Folium. The mechanism underlying GV-1 acupuncture's efficacy related to treatment of diarrhea was explored by measuring serum levels of various brain-gut peptides along with the expression of key protein molecules involved in 5-HT signaling pathway in colon tissue. After establishing diarrhea using Sennae Folium the rabbits were subjected to aqua-acupuncture or dry-needle acupuncture at GV-1 point for 7 days. The rabbits' behavior, body weight and diarrhea index were observed and recorded. The therapeutic effects of GV-1 acupuncture on diarrhea were evaluated using histopathological techniques, enzyme-linked immunosorbent assay and western blotting assay. Results revealed rabbits with GV-1 acupuncture significantly improved the production performance of rabbits and effectively relieved the intestinal tract damage caused by diarrhea. Inflammatory factors including IL-2, IL-1 β , TNF- α and IFN- γ were downregulated following dry-needle acupuncture and aqua-acupuncture at GV-1 points. Moreover, 5-HT, CCK, SS, SP, and VIP brain-gut peptides were downregulated while the levels of MTL, GAS, ENK and β -EP were upregulated. Results of western blotting show that compared to CG protein level of TPH-1 and 5HT₃R increased significantly in MG and BCG. However, treatment by acupuncture significantly overcomes the expression of TPH-1 and 5HT₃R in ATG and DTG ($P < 0.05$). SERT showed an opposite trend with the expression levels of TPH-1 and 5HT₃R. It was concluded that acupuncture at GV-1 point is a favorable therapeutic option for alleviating diarrhea. Both dry-needle acupuncture and aqua-acupuncture treatments demonstrate significant efficacy in reducing FSAE-induced diarrhea with aqua-acupuncture having superior therapeutic effects compared to dry-needle acupuncture at the GV-1 point. Acupuncture targeting GV-1 relieves diarrhea by regulating various inflammatory cytokines and brain-gut peptides as brain-intestine peptides potentially serving as a crucial mediator in its mechanism of action.

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Authors' Contribution

HD, XW, ML and KL designed and conceived the study, carried out the research, analysed the data and wrote the manuscript. KL, ARA, RAA, AM and DS arranged funding and resources, supervised and investigation the study, and critically reviewed and revised the manuscript.

Key words

Acupuncture, GV-1, Diarrhea, Rabbit, Brain-gut peptide, 5-HT signaling pathway

INTRODUCTION


Diarrhea is a serious and common health concern in rabbits which leads to damage to gut mucosa and

death resulting in significant losses to rabbit farming industry (Chen *et al.*, 2017; Li *et al.*, 2022). Diarrhea can be attributed to pathogenic infections or gastrointestinal distress induced by environmental pollutants however, antibiotics and probiotics have been widely used to cure diarrhea (Zubair *et al.*, 2022; Pasha *et al.*, 2022; Rehan *et al.*, 2023). The administration of antibiotics and probiotics is associated with high costs reducing profit gain at numerous farms. Due to this reason antibiotic replacement therapy has been studied extensively. Acupuncture is an ancient therapeutic modality of traditional Chinese medicine which has been used for many years (Zhuang *et al.*, 2012). Extensive research has demonstrated that the widespread utilization of acupuncture in managing diverse

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gastrointestinal (GI) disorders including diarrhea, gastric paralysis syndrome, dyspepsia and irritable bowel disease (Li *et al.*, 2015; Rehman *et al.*, 2022; Younas *et al.*, 2023). The efficacy of acupuncture has been extensively verified making it a well-established therapeutic intervention however, the details related to the mechanisms regarding acupuncture therapy are still insufficient and require further investigations.

Governing vessel (GV) is called Du Mai in traditional Chinese medicine (TCM). Du means to govern or dominate, Du Mai (GV) can dominate all Yang Channels, while GV-1 (*Hou-hai*) is the first acupoint of the Du Mai, and it is located in the caldera of dorsomeson between archos and veuro of the tail. Acupuncture is recognized for its local efficacy and GV-1 is commonly applied in veterinary clinics to cure different ailments such as diarrhea, constipation and perianal diseases (Li *et al.*, 2015; Long *et al.*, 2016). However, there is diminished literature on the therapeutic effects and mechanism of GV-1 in treating diarrhea it is possible that the location of GV-1 acupoints can alter its therapeutic effects. Given the easy accessibility of GV-1 for acupuncture in veterinary medicine and investigating its effects on diarrhea would offer valuable theoretical support for utilizing acupuncture as a suitable treatment option for diarrhea.

Braingut peptides have crucial functions in along the digestive tract and central nervous system (Bonaz and Sabate, 2009). Gastrin and cholecystokinin are peptides which effectively regulate gastrointestinal motility. It has been reported that electroacupuncture at ST-36 promoted excretion of MTL and CCK which can reveal the mechanism of acupuncture enhancing gastrointestinal myoelectric activity (Niu *et al.*, 2007). Crucial functions of brain gut peptides (Gastrin, Serotonin), cytokines (Tumor necrosis factor- α , Interleukin-1 β) and signaling mediators establishing connections between the immune system and various cell types (Anderson and Delgado, 2008; Wei *et al.*, 2020). Neuropeptides and cytokines can modulate the inflammation response by affecting phagocytosis of phagocytes, apoptosis of epithelial cells and the release of inflammatory molecules (Anderson and Delgado, 2008).

A close association has been reported between the 5-HT signaling pathway, oncogenesis of IBS and intestinal inflammation (Koopman *et al.*, 2021). The 5-HT is synthesized by two types of cells in intestine that are enterochromaffin cells and enteric serotonergic neurons. Those cells produce tryptophan hydroxylase-1 or TpH2 to generate 5-HT. Serotonin reuptake transporter is the releasing regulator of 5-HT whereas, the functional effects of 5-HT are exerted through activation of specific 5-HT receptors (Gershon and Tack, 2007). The 5-HT₃ receptor

plays a crucial role in regulating inflammatory reactions (Najjar *et al.*, 2023).

Acupuncture generates a noxious stimulus that leads to inflammatory response enhancing the therapeutic potential by activating the nerve-endocrine-immune systems and is the important medium connecting brain-gut axis (Li *et al.*, 2019). To investigate the therapeutic effect of GV-1 acupuncture on diarrhea and to compare the efficacy of dry-needle acupuncture at GV-1 and aqua-acupuncture treatment at GV-1 diarrhea was induced in rabbits by sennae folium aqueous extract (FSAE). Moreover, bladder meridian -37 (BL-37) point was set as the acupoint control of GV-1 to verify the effectiveness and specificity of GV-1 acupoint. The 37th acupoint (BL-37, Han-gou) of the bladder meridian is located on the caudo-lateral aspect of the pelvic, 1/3 of the distance ischial tubercles to the middle of biceps femoris tendon, in the rut between the semitendinosus and musculus vastus intermedius. This acupoint is not often used, and no studies have shown that it has a therapeutic effect on diarrhea. This study was conducted to investigate the underlying mechanism of GV-1 acupuncture including rabbit behavior, pathological alterations in intestinal tissue, serum inflammatory factors, brain-gut peptides and expression levels of key proteins in the 5-HT signaling pathways. This study will highlight the results aimed at identifying brain-gut peptides and cytokines functions in diarrhea development through acupuncture treatment.

MATERIALS AND METHODS

Preparation of sennae folium

Sennae folium (8kg) was obtained from Tongrentang Co., Ltd., Beijing, China. Sennae folium (100g) was soaked with 1 L of water in beaker for 30 min and placed in water bath preset to 100°C for 25 min. The obtained filtrate was filtered with a gauze. This procedure was repeated 80 times to produce about 49 L of sennae folium aqueous extract. Subsequently, the aqueous extract was concentrated to 8L using multi-functional miniature extractor concentrator under a vacuum of 55°C that was equal to 1 g/mL of sennae folium aqueous extract (FSAE) which was then stored at 4°C for further use according to previous study.

Experimental animals

Thirty male New Zealand rabbits aged at 5 months (2.0~2.5 kg of bodyweight) were purchased from the Quanyou farm (Baoding, China). All rabbits were housed in the animal facility of Hebei Agricultural University. Each rabbit was kept in a single cage having 22±3°C temperature and 50% ~ 60% relative humidity with free

access to water. Each rabbit was provided with a daily ration of 160g divided equally into two meals of 80g in the morning and evening throughout the experiment.

Model evaluation criteria

The evaluation criteria for the diarrhea model were as follows: (1) loose feces with mucous or watery consistency; (2) reduced appetite and body weight; (3) peripheral vasoconstriction, dorsal curvature, and arching posture; (4) a lack luster and soiled coat or presence of fecal matter on the coat; (5) easy fatigue and lethargy. (1) to (3) were the major symptoms, and (4) and (5) were the concurrent symptoms. The successful establishment of a rabbit diarrhea model requires the presence of at least 2 major symptoms and 2 concurrent symptoms (Li *et al.*, 2023; Zhu *et al.*, 2018).

Experimental design

After one week of acclimatization period the rabbits were randomly divided into five groups comprising of a control group and four model groups. The control group was administered with saline at the dosage of 8 mL/kg daily for two weeks. The rabbits in model group were fed with sennae folium aqueous extracts by gavage (8 mL/kg) daily for two weeks. The gavage was administered daily at 10 am and the gavage procedures were all completed within a one-hour time frame. To ensure the efficacy of FSAE on rabbits no medication was given to rabbits. Moreover, acute toxicity of senna in mice demonstrate that senna is a well-tolerated laxative with no significant pain (Boeira *et al.*, 2010). The methodology was derived from preliminary experiments revealing the successful establishment of a rabbit diarrhea model by FSAE (8ml/kg) for 5 days. On the 7th day after confirming that all four model groups had successfully established diarrhea, which were randomly divided into GV-1 dry-needle acupuncture treatment group (DTG), GV-1 aqua-acupuncture treatment group (ATG), BL-37 acupoint control group (BCG) and diarrhea model group (MG). Subsequently, acupuncture protocol was initiated and applied for a duration of 7 consecutive days.

Interventions

The acupuncture treatment was performed by licensed acupuncturists. Sterile 0.25 mm disposable acupuncture needles used in this study was obtained from JingTang Herbal Co.Ltd (Baoding, China). Acupoints GV-1 and BL-37 were chosen for DTG and BCG animals. The acupoint site was disinfected and the dry-needle was inserted vertically to a depth of 1 *cun*. A dull needling consciousness was applied through raising, thrusting and entwisting maneuver for 20 min/day. In ATG treatment

was administered daily at GV-1 acupoint using 1 mL syringe to inject a mixed solution of vitamin B12 and normal saline in a ratio of 1:3 with a dosage of 0.2 mL. Rabbits in both the control group (CG) and the model group (MG) continued to receive gavage without any other interventions. Rabbits that experienced infections or premature death were excluded from the study.

Behavioral monitoring

Starting from the 7th day of the experiment the residual food in each cage was measured every morning before feeding. The daily feed intake of each rabbit was calculated and the total feed intake of each rabbit during the 7-13 days of experiment was recorded. Moreover, the mental well-being, physical activities, appetite and body coat condition of rabbits were observed and recorded during the acupuncture treatment procedure.

Body weight and diarrhea index detection

Body weight of rabbits was measured and documented on the 1st, 4th, 7th, 10th, and 13th day. Faecal status was observed and recorded 6 h after intragastric administration after which the rate of diarrhea and diarrhea index were calculated. The degree of diarrhea was divided into 5 grades as per previous studies reported (Blake *et al.*, 2016). Dry, hard, and dark feces were classified as grade 1 (constipation); round and uniform feces were classified as grade 2 (normal); soft and large feces that were attached to each other or with mucus are classified as grade 3 (mild diarrhea); small or unformed feces with mucus were classified as grade 4 (diarrhea); watery feces with mucus or blood were classified as grade 5 (severe diarrhea). The presence of diarrhea is defined as a stool grade of 3 or higher.

Sample collection

Experimental rabbits were euthanized by intravenous injection of pentobarbital sodium (100mg/kg) into the auricular vein. Blood samples from all rabbits were gathered from the jugular vein of animals, and the serum was obtained by centrifugation (1200 g, 4°C, 10 min) for ELISA. The colon tissues were excised, immediately immersed in liquid nitrogen and transferred to a freezer at -80 °C for cryopreservation. Simultaneously, duodenum, jejunum and colon samples were kept in paraformaldehyde (4%). The no of rabbits in each group was (n=6). All the obtained samples were labelled and the personnel who conducted the analyses were blinded to the grouping.

Pathomorphological examination and ELISA

The contents of serum interferon gamma, interlenkin 1 β , IL-2, tumor necrosis factor α , gastrin (GAS), motilin

(MLT), somatostatin, Substance P, vasoactive intestinal peptide, cholecystokinin (CCK), 5-hydroxytryptamine (5-HT), enkephalin (ENK), β -endorphins (β -EP) and acetylcholinesterase (AChE) were analyzed using ELISA kits purchased from Enzyme-linked Biotechnology (Shanghai, China). The duodenum, jejunum and colon tissues were collected, fixed and paraffin embedded. Then a 4 μ m section from each sample was cut and subjected to histopathological processing followed by staining with hematoxylin and eosin. Histopathological slide analysis was observed for pathological alterations under microscope (Olympus Corporation, Tokyo, Japan).

Western blot analysis

Protein extraction was performed through bicinchoninic acid protein assay kit (BioChain, USA). Then protein products were quantified using the BCA protein assay kit (Thermo, USA). Antibodies of β -actin, 5-HT₃R, TPH-1 and SERT were purchased from CST (USA). Image Quant LAS 4000 mini (USA) was used for densitometric values analysis.

Statistical analysis

Data is presented as mean \pm SD. One-way analysis of variance (ANOVA) was performed using SPSS (16.0, USA). Results were considered significant at $P > 0.05$.

RESULTS

Behavioral performance

Rabbits in the CG exhibited vigorous activity levels,

exhibited a healthy appetite, possessed well-maintained shiny fur and had regular grainy fecal pellets. While rabbits in the remaining four MG gradually exhibited signs of fatigue following intragastric administration, including activity, sluggish response, diminished appetite and loose stools. The rabbit diarrhea model was successfully established on the 7th day. Under the intervention of acupuncture negative behavior of rabbits in both the ATG and DTG groups exhibited a significant improvement. During the 7-13 days of the experiment as depicted in Figure 1A, a significant decrease in total feed intake per rabbit was observed in the MG compared to the CG. In contrast to MG both ATG and DTG groups demonstrated a substantial increase in total feed intake per rabbit.

Body weight and diarrhea index

As shown in Figure 1 body weight of CG increased significantly during the whole experiment whereas, it decreased significantly in other groups (Fig. 1B). Compared with the CG all rabbits in experimental group were significantly lower in body weight due to diarrhea at 7 days. After 7 days of acupuncture intervention GV-1 acupoint showed significant increase in the body weight of diarrheic animals ($P < 0.05$) (Fig. 1C). In contrast with the trend of body weight change animals of CG did not show any diarrhea symptom while the diarrhea rate and its index in experimental group increased (Fig. 1D, E). Compared to CG diarrhea was induced successfully in all experimental groups and the diarrhea index increased significantly ($P < 0.05$) after 7 days the diarrhea index in ATG and DTG decreased significantly ($P < 0.05$ or $P < 0.01$) (Fig. 1F).

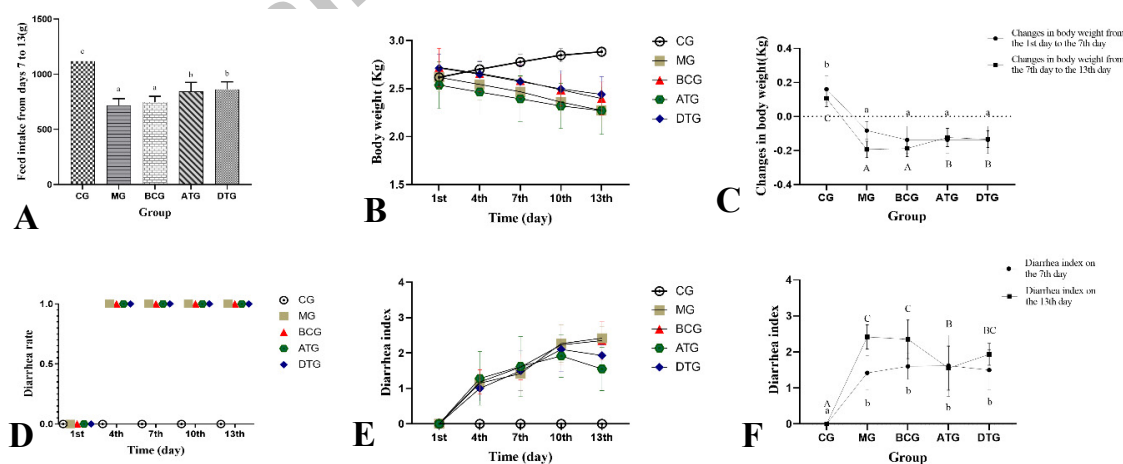


Fig. 1. Effect of acupuncture on feed intake, body weight and diarrhea index in experimental animals. A: The average feed intake of rabbits from days 7 to 13. B: The change trend of body weight in different treatment cycles. C: The difference analysis of body weight in each group after 7 days model construction and 7 days treatment. D: The change trend of diarrhea rate in different treatment cycles. E: The change trend of diarrhea index in different treatment cycles. F: The difference analysis of diarrhea index in each group after 7 days model construction and 7 days treatment.

Intestinal histopathology

The histomorphology changes of duodenal structures were observed by H and E staining on pathological sections from different experimental groups is shown in Figure 2. In CG the duodenal morphology and structures were normal. The surface of the duodenal mucosa appeared as dense with increased number of intestinal villi. The villous surface epithelial cells were normal and the goblet cells were evenly distributed among the epithelial cells (oval mark), the Lieberkühn crypts was clearly visible in the basal parts of intestinal villi (triangle mark) (Fig. 2A). The duodenal mucosa showed evidence of edema which was characterized by separation of epithelium and lamina propria to form substantial gaps. In MG (rectangular mark) accompanied by marked infiltration of inflammatory cells in certain sections (arrow mark) (Fig. 2B). The degree of injury in BCG was similar to that of MG (Fig. 2C) aqua-acupuncture and dry needle treatment significantly alleviated the inflammatory injury induced by sennae folium. After 7 days treatment the duodenal villi length and morphology in both the ATG and DTG did not fully recovered to normal state. However, there was a significant reduction in duodenal mucosa edema and inflammatory cell infiltration (Fig. 2D, E).

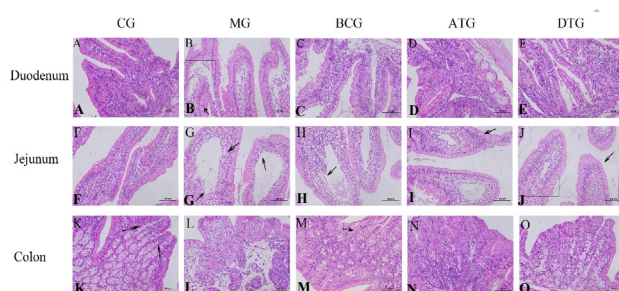


Fig. 2. Effect of acupuncture on histological structure of duodenum, jejunum, colonic tissue of sennae folium induced diarrhoeal rabbits. Stain H&E, Magnification: 200 \times .

The effect of acupuncture on jejunum histomorphology were shown in Figure 2. The villi of the jejunum of healthy animals were intact and there were no significantly visible signs of edema and prominent inflammatory reaction (Fig. 2F). In MG and BCG severe edema in mucosa and submucosa could be found in some local jejunum tissue (arrow mark), whereas, the villi length of jejunum significantly decreased due to diarrhea (Fig. 2G, H); The mucosal edema (arrow mark) and the decreased connective tensile tissue strength (rectangular mark) were partially enhanced in both ATG and DTG however, complete recovery was not achieved (Fig. 2I, J).

There were no significant pathological changes in

CG (Fig. 2). The colon was structurally intact with clear morphology and goblet cells (arrow mark). In MG the mucous membrane was damaged, the basal layer edema was observed (rectangular contour mark), and the goblet cell apoptosis (Fig. 2L). The colon morphology of the BCG was basically consistent with that of MG whereas, the presence of hydropic degeneration in the epithelial cells within the mucosal layer was evident (Fig. 2M). The colonic injury in the ATG and DTG were significantly improved (Fig. 2N, O).

ELISA profile of inflammatory mediators

The impact of acupuncture treatment on serum inflammatory cytokines was assessed using the ELISA assay. As depicted in Figure 3 there was a significant increase in the release of TNF- α , INF- γ , IL-1 β and IL-2 in MG and BCG ($P < 0.01$). However, these effects were significantly decreased in ATG and DTG after 7 days of intervention ($P < 0.05$) (Fig. 3).

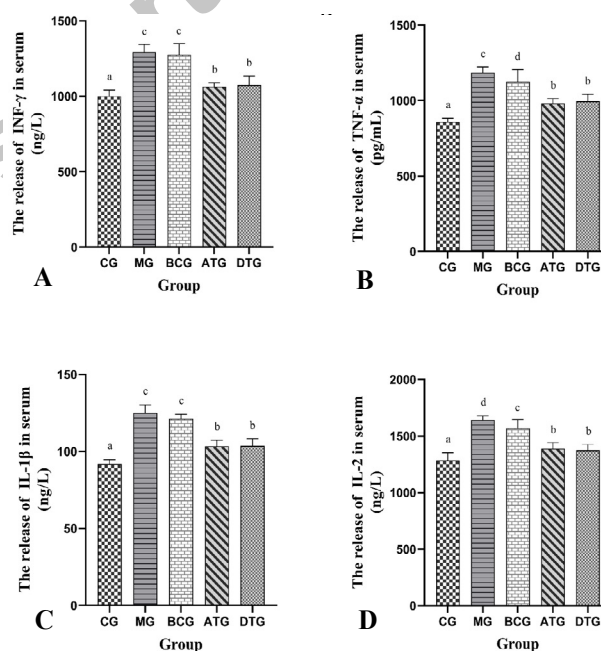


Fig. 3. The effect of acupuncture treatment on the release of cytokines in serum of rabbits suffering from sennae folium induced diarrhea. A, The release of IFN- γ ; B, The release of TNF- α ; C, The release of IL-1 β ; D, The release of IL-2.

Serum protein profile of brain-gut peptides

The serum samples of each group were collected and the contents of 9 different types of brain-gut peptides including 5-HT, CCK, SP, VIP and SS were significantly increased in MG and BCG (Fig. 4). Compared to MG

the expression of the above 5 types of brain-gut peptides were significantly down-regulated by aqua-acupuncture treated via dry needle ($P < 0.05$ or $P < 0.01$). However, CCK, SP and SS in ATG and DTG animals did not exhibit significant differences compared to those in BCG which proved that these above 3 peptides were not only affected by acupuncture at GV-1 (Fig. 4A, E). Contrary to the results of 5-HT and other peptides diarrhea significantly down-regulated the level of GAS, MTL, ENK and β -EP ($P < 0.01$). The expression levels of the above 4 types of peptides were significantly up-regulated in ATG and DTG ($P < 0.05$ or $P < 0.01$) compared to GM. However, ENK and EP did not show any significant differences among BCG, ATG and DTG groups which showed that the above 2 peptides were not regulated by GV-1 point (Fig. 4F, I). Compared to CG, SP, ENK and β -EP in ATG did not exhibit significant differences. However, significant differences were observed between DTG and CG. These findings suggest that aqua-acupuncture at GV-1 point exhibited superior therapeutic efficacy compared to dry-needle acupuncture in rabbits with diarrhea.

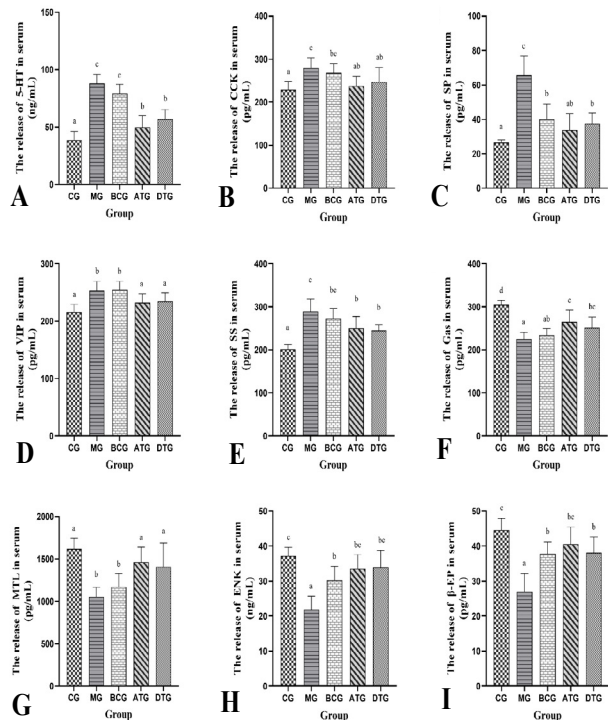


Fig. 4. The effect of acupuncture treatment on the release of brain-gut peptides in serum of rabbits suffering from sennae folium induced diarrhea. A, The release of 5-HT; B, The release of CCK; C, The release of SP; D, The release of VIP; E, The release of SS; F, The release of GAS; G, The release of MTL; H, The release of ENK; I, The release of β -EP.

Regulation effects of acupuncture treatment on 5-HT signaling pathway

Compared to CG, the protein levels of TPH-1 and 5HT₃R significantly increased in MG ($P < 0.05$) and BCG ($P < 0.05$). However, acupuncture treatment suppressed the expression of TPH-1 and 5HT₃R in ATG and DTG ($P < 0.05$) (Fig. 5A, C). The SERT was opposite with the trend that of TPH-1 and 5HT₃R (Fig. 5A, D).

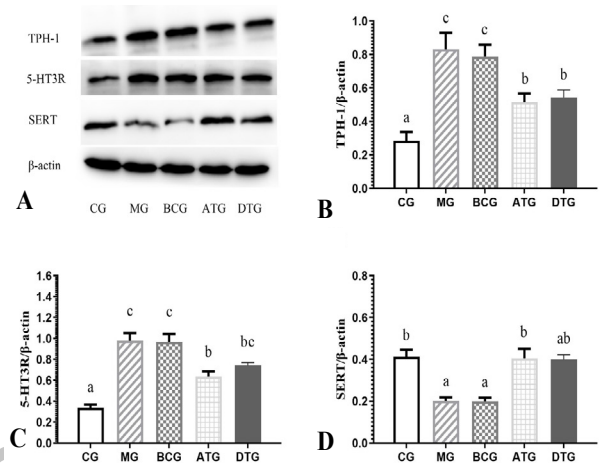


Fig. 5. The effect of acupuncture treatment on the expression levels of 5-HT signaling pathway associated proteins in rabbits suffering from sennae folium induced diarrhea. A, Western blot analysis: The protein level of TPH-1, 5-HT₃R, SERT. B, protein level of TPH-1. C, protein level of 5-HT₃R. D, Protein level of SERT. β -actin was used as control.

DISCUSSION

Acupuncture is a widely applied method in traditional medicine with high efficacy. Studies have revealed that acupuncture therapy is effective for more than one hundred diseases and symptoms including diarrhea (Wang *et al.*, 2022; Zhang *et al.*, 2021). Though acupuncture-related techniques were relatively commonly used in treating diarrhea. Limited knowledge is available related to the mechanisms and therapeutic effects (Li *et al.*, 2015). In this study, we employed sennae folium to induce diarrhea in rabbits for exploring the treatment effects of aqua-acupuncture with dry needle and the potential mechanisms of acupuncture method.

Rabbit diarrhea induced by FSAW is a recognized model used to study the pathology and mechanism of diarrhea (Zhang *et al.*, 2020). The efficacy of GV-1 as an acupuncture point in the treatment of diarrhea has been demonstrated in various clinical studies (Yang *et al.*, 2022). Therefore, this stimulatory was selected using both

aqua-acupuncture and dry-needle acupuncture in a FSAE-induced diarrhea rabbit to detect the treatment effects and explore its mechanisms. It was found that rabbits treated by aqua-acupuncture treatment and dry-needle acupuncture could ameliorate body weight loss and diarrhea index after 7 days and inhibit the severe inflammation leading to necrosis in duodenal, jejunal and colonic mucosa which was different in BCG and MG. Our results are in corroboration with previous studies (Dahlgren *et al.*, 2022; Li *et al.*, 2019) and provide reference for suitable treatment modalities of diarrhea.

Diarrhea is considered as a severe inflammatory disorder and inflammatory cytokines have important relationship with development of diarrhea (Dong *et al.*, 2017). Acupuncture therapy affect immune and endocrine function in host, it was hypothesized that the treatment effect of acupuncture in diarrhea is related to its regulation of immune cells and endocrine function. The pivotal involvement of IFN- γ and α , IL-1 β and 2 in immune and inflammatory responses has been widely acknowledged (Liu *et al.*, 2021). It was found that the expression levels of inflammatory cytokines in both aqua-acupuncture and dry needle treatment significantly improves the release of four inflammatory cytokines induced by diarrhea. Our results are consistent with a previous study (Dong *et al.*, 2017; Yang *et al.*, 2021).

Clinical and experimental results indicated that during acupuncture treatment brain-gut peptides are related to the modulation of diarrhea (Augustyniak *et al.*, 2021). It has been reported that 9 types of brain-gut peptides associated with diarrhea, which were screened and detected in our research. The result shows that acupuncture at GV-1 point regulated the levels of serum 5-HT, VIP, GAS and MTL in rabbits with diarrhea. However, no significant regulatory effect on these brain-gut peptides was observed with acupuncture at BL-37. Moreover, both acupuncture at GV-1 and BL-37 acupoints exhibited significant down-regulation of serum SP levels and up-regulation of serum ENK and β -EP levels in rabbits with diarrhea.

The 5-HT is mainly derived from enteroendocrine cells playing crucial roles in the gastrointestinal inflammatory response. Excessive release of 5-HT leads to diarrhea whereas, the administration of 5-HT receptor antagonists induce constipation (Yang *et al.*, 2021). The VIP is a commonly found mammalian neuropeptide innervating gastrointestinal tract, which regulates physiological processes like gut secretion and motility (Iwasaki *et al.*, 2019). Excessive secretion of VIP has been demonstrated to induce severe watery diarrhea in humans (Ito *et al.*, 2012). However, the function of VIP in acute diarrhea remains imperfectly established. The VIP is recognized as a biomarker of inflammatory bowel disease as higher VIP

concentrations are reported in these inflammatory diseases (Abad *et al.*, 2012). Our findings are in agreement with the increase of VIP plasma concentrations which indicated a drastic intestinal inflammatory response induced by sennae folium (Xie *et al.*, 2021). Compared to MG rabbits the animals in ATG and DTG exhibited significant decrease ($P < 0.05$) of serum concentrations of 5-HT and VIP after acupuncture at GV-1 indicating that this treatment method might have effectively cured diarrhea and suppressed intestinal inflammation by regulating levels of 5-HT and VIP. Furthermore, the impact of acupuncture treatment duration on serum concentrations of 5-HT and VIP remains unclear as further research is required to explain the findings of this study.

Gastrin stimulates acid secretion and fundic mucosal growth (Heilmann *et al.*, 2017). The content of gastrin can be affected by factors of diarrhea, hypercalcemia and use of antacids. The mechanisms of acupuncture treatment on gastrin expression and release in rabbits with diarrhea yet remains unexplored. It was observed that serum gastrin concentrations in rabbits treated with aqua-acupuncture and dry-needle acupuncture were significantly lower when compared with that in healthy rabbits, but significantly higher compared to the MG. In contrast to our findings, in dogs with chronic enteritis the serum gastrin concentrations can reach up to as high as double in concentration as compared to dogs without gastrointestinal disease (Garcia-Sancho *et al.*, 2005). The difference of gastrin contents between chronic enteritis and acute diarrhea is due to chronic inflammation leading to gastric lesions and decreased acidity in gastric antrum. The variation trend of the gastrin seen in this study is associated with the inflammation as inflammatory cytokines affect gastrin concentrations (Hopman *et al.*, 2003; Suzuki *et al.*, 2001).

The primary physiological effects of MTL include increased pepsin secretion, migrating motor complex (MMC) and promoting gastrointestinal motility (Kitazawa and Kaiya, 2021). The MTL and CCK controls hunger and is related to gut-brain signaling (Tack *et al.*, 2021). The CCK has been shown to enhance satiety and reduce food consumption. Consistent with these effects, we observed a significant increase in serum CCK levels and a notable drop in MTL levels among rabbits within the diarrhea model group leading to increased abdominal satiety and decreased feed intake. The SP is a pro-inflammatory medium which releases upon nociceptive stimulation (Mazzoni *et al.*, 2024). Furthermore, SP is a reliable indicator of peripheral nociceptive afference in several studies (Ma *et al.*, 2023). Compared to the diarrhea model group, both the aqua-acupuncture and the dry-needle acupuncture at GV-1 exhibited significantly decreased plasma concentrations of CCK and SP. This suggests that GV-1 effectively mitigates

gastrointestinal tissue injury and inflammation induced by FSAE in rabbits with diarrhea which is consistent with the histopathological results of this study. However, the concentrations of SP were significantly reduced by acupuncture at BL-37 acupoints whereas, no significant difference was observed in CCK levels compared with GM. These findings suggest that acupuncture at BL-37 has the potential to modulate brain-gut peptide levels while its application is not favorable for animals with diarrhea.

The ENK and β -EP belonging to endogenous opioid polypeptides that contribute to ease pain, self-governing regulation of immune responses and better intestine functioning (Bodnar, 2023). This endogenous opioid system could be broken by pain and exogenous opioids (Roedel *et al.*, 2016). In this study, the concentrations of ENK and β -EP in the serum of rabbits with FSAE-induced diarrhea were significantly decreased compared to GM indicating a potential correlation between pain and diarrhea. However, the levels of ENK and β -EP at both GV-1 and BL37 points exhibited significant upregulation following acupuncture treatment, thereby suggesting that acupuncture exerts a universal analgesic effect. However, further investigation is required to establish the correlation between the analgesic efficacy of acupuncture at BL-37 points and its potential for alleviating diarrhea.

The 5-HT signaling pathway is involved in sustaining gut homeostasis, and the regulation of 5-HT in intestinal tissue is mediated by TPH-1 and SERT, which are key enzymes involved in the biosynthesis and reuptake of serotonin (Koopman *et al.*, 2021). The SERT is related to the termination of 5-HT in the mucosa and low levels of SERT is associated with intestinal inflammation (Gershon, 2004). The abnormal expression of 5-HT and 5-HT₃R has been extensively documented to induce inflammation in intestinal tissues. Recently, several 5-HT₃ receptor antagonists have demonstrated their utility in various intestinal inflammatory diseases. These antagonists effectively inhibit the releasing of inflammatory cytokines, like IL-1 β and TNF- α (Kato, 2013). Following the administration of FSAE to rabbits, a significant spike in peripheral free 5-HT levels was observed. The potential inactivation defect caused by reduced SERT expression is due to the underlying cause. Moreover, elevated 5-HT concentrations prolong its interaction with the 5-HT₃R thereby augmenting the effects of 5-HT₃R and subsequently leading to gastrointestinal disorders. After acupuncture at GV-1 point, the expression of SERT in colon tissue was increased whereas, the expression of 5HT₃R was decreased which is consistent with the observed changes in serum 5-HT and inflammatory cytokines levels. These findings suggest that acupuncture may alleviate FSAE-induced diarrhea and inflammation by modulating the

5-HT signaling pathway in rabbits.

CONCLUSION

Nexus to the above it is concluded that acupuncture at GV-1 point is a favorable therapeutic option for alleviating diarrhea. Both dry-needle acupuncture and aqua-acupuncture treatments demonstrate significant efficacy in reducing FSAE-induced diarrhea, with aqua-acupuncture exhibiting superior therapeutic effects compared to dry-needle acupuncture. Acupuncture exerts regulatory effects on the 5-HT signaling pathway thereby regulating brain-gut peptides production. The antidiarrheal effect of acupuncture at G-V1 is attributed to the regulation of various inflammatory cytokines and brain-gut peptides.

DECLARATIONS

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Ethical approval

The experimental procedures were reviewed and approved by the Laboratory Animal Ethics Committee of Hebei Agricultural University (Approval No. 2021066).

Statement of conflict of interest

The authors have declared no conflict of interest.

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