ORIGINAL RESEARCH

Impacts of Acupuncture Plus Acupoint Application on Intestinal Function and Expression of MAPK and ERK in Colon Tissue of Rats

Fei Hua, MD; Shanshan Rong, BS; Lan Zhang, BS; Yaping Weng, BS; Wei Shen, MD; Chunbo Liu, MD

ABSTRACT

Context • Slow transit constipation (STC) has a high incidence worldwide, which not only seriously affects patients' normal lives but also may cause malignant intestinal lesions. Among the limited treatment options for STC, traditional Chinese medicine (TCM) is considered to be the key to STC treatment in the future.

Objective • The study intended to examine the impacts of acupuncture plus acupoint application on MAPK and ERK in STC rats, with the aim of preliminarily exploring the relevant mechanisms for treating STC as well as providing new ideas and means for future clinical treatment.

Design • The research team designed a randomized, controlled animal study.

Setting • The study was carried out at department of Department of Rehabilitation, Affiliated Hospital of Medical School of Ningbo University, Ningbo, Zhejiang, China.

Animals • The animals were 30 six-to-eight-week-old, Sprague Dawley (SD) rats, half male and half female and weighing a mean of 200 ± 20 g.

Intervention • The rats were randomly assigned to one of three groups, 10 rats in each group: a negative control group that wasn't induced with STC and received no treatments; a positive control group, the model group (MG), that was induced with STC and received no treatments; and an intervention group that was induced with STC and received the investigated treatments. The intervention group was treated with acupuncture at Tianshu point (ST25) and received acupoint application from Chinese medicine.

Outcome Measures • The study measured the alterations in the rats' body weight and feces, as well as the rats' intestinal motility, using intragastric administration of activated carbon. The rats were killed to obtain their intestinal tissues, for measuring expression of mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) using Western blotting and polymerase chain reaction (PCR).

Results • Postintervention, at 28 days after induction of STC, the rats' weights weren't significantly different in the intervention and control groups ($P > .05$) but were significantly higher than that in the model group ($P < .05$). The rats' weights in the intervention and control groups gradually increased significantly, while those in the model group gradually decreased significantly ($P < .05$). The defecation volume and fecal water content (FWS) decreased in the significantly model group but increased significantly in the intervention group ($P < .05$). The intestinal motility test revealed no significant differences in the propulsion rate between the intervention and control groups ($P > .05$), but the rate was significantly lower in the model group than that of the intervention group ($P < .05$). The intestinal fecal residue in the model group was the highest among the three groups, followed in descending order by the intervention group and the control group, with the differences being statistically significant ($P < .05$). In addition, the MAPK and ERK in the model group significantly increased, and the values were significantly higher in the intervention group than those of the model group ($P < .05$).

Conclusions • Acupuncture plus acupoint application can validly improve the defecation and intestinal motility of STC rats, possibly through inhibiting MAPK and ERK. (*Altern Ther Health Med.* 2022;28(8):30-37).

Fei Hua, MD, Nurse; **Lan Zhang**, **BS**, Nurse; **Wei Shen, MD**, Physician; and **Chunbo Liu, MD,** Physician, Department of Rehabilitation, Affiliated Hospital of Medical School of Ningbo University, Ningbo, Zhejiang, China. **Shanshan Rong, BS**, Nurse, Geriatric Department, Affiliated Hospital of Medical School of Ningbo University, Ningbo, Zhejiang, China. **Yaping Weng, BS**, Physician, Department of Nephrology, Affiliated Hospital of Medical School of Ningbo University, Ningbo, Zhejiang, China.

Corresponding author: Chunbo Liu MD
E-mail: liuchunbo_ndfy@163.com

Constipation not only seriously affects patients' normal lives but also can cause malignant lesions of intestinal tissues and even induce tumors in severe cases, threatening patients' lives.[1] Slow transit constipation (STC) is the most common type of constipation, and dysfunction of the large intestine causes it.[2] Its most typical manifestations are prolonged defecation time and difficulty in defecation as well as frequent abdominal pain and complications.[2]

While seriously affecting patients' normal excretions, STC can also lead to severe changes in most patients' lives, diets, and psychology.[3] Long-term STC may not only give rise to secondary hemorrhoids, anal fissures, and cardiovascular diseases but also may cause malignant lesions of intestinal tissues and intestinal infection, induce intestinal tumors in severe cases, and threaten patients' lives.[4,5] Some epidemiological surveys have indicated that the global prevalence of constipation among adults is 2.5%-7.9%, and the proportion of those adults having STC exceeds 60%-80%.[6,7]

The pathological mechanism of STC is intricate and isn't yet thoroughly defined, but diet types, drugs, depression, genetic factors, other colorectal diseases, irregular eating patterns, and living habits are all predisposing factors for STC.[8] Black and Ford found that STC mainly results from gastrointestinal dysmotility and transport dysfunction, which is extremely difficult to cure completely and has a very high recurrence rate.[9]

Currently, STC treatment mainly uses conservative therapies, including over-the-counter laxatives, prescription drugs, fiber supplements, and dietary changes in addition to traditional dilative laxatives and secretory laxatives.[10] However, these drugs often come with side effects. Long-term oral laxatives can aggravate injuries to the intestinal wall's nerve and can damage intestinal mucosa, leading to a repeated vicious circle of constipation, and irritating laxatives may even induce tumors.[11-13]

Surgical resection is a last resort if other therapies have failed, including total colectomy and subtotal colectomy,[14] with confirmed curative effects. But due to many postoperative complications and great trauma, the surgical treatment of STC isn't widely used in clinics. Because of the ever-increasing incidence of STC, an urgent need exists to find more effective, safer, and more stable treatments for clinical practice.

In recent years, traditional Chinese medicine's (TCM's) treatments for constipation have gained increasing attention in domestic and foreign research. Commercial formulations such as Guilun Runchang granules and Maren pills as well as other TCMs have been shown to be alternative therapies for STC. These medicines are safe and effective and can significantly alleviate the symptoms of STC patients.[15,16] In addition, the efficacy of TCM treatments in relieving STC symptoms has been documented, including acupoint catgut embedding—the implantation of absorbable catgut sutures at traditional Chinese meridian acupoints; acupuncture; and abdominal massage.[17,18]

Some clinicians believe that acupuncture plus acupoint application—the application of an herbal medication to an acupoint—may become the optimal treatment for constipation in the future.[19,20] Acupoint application combined with drugs has been found to help constipated patients recover gastrointestinal peristalsis and coordinate gastrointestinal function through acupoint-meridian viscera,[21] but its specific mechanism remains uncharacterized.

Acupuncture, as one of the most traditional methods of TCM, has been shown to exert stable therapeutic effects on infantile diarrhea, bronchial asthma, intestinal dysfunction and other diseases, with high levels of safety.[22,23] Acupuncture, through needling acupuncture points, has been found to activate the nervous system or inhibit sympathetic nerves, and it can promote the blood-gas movement of meridians, thus resolving diseases.[24,25]

Moreover, acupuncture has many advantages, such as simple operation, high levels of safety and lasting curative efficacy, allowing for a wide range of clinical uses.[26] At present, acupuncture therapy plus acupoint application has been extensively used in the treatment of intestinal inflammation and other diseases, and its efficacy for the treatment of constipation has also been confirmed.[27,28]

Some studies have pointed out that mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) in the colon tissue of STC rats differed significantly from those of normal rats.[29] The critical role of MAPK and ERK in intestinal function has also been repeatedly confirmed.[30-33] Other research has shown that MAPK and ERK can regulate the dysfunction of intestinal mucosa and the obstruction of normal function of intestinal cells caused by aquaporin (AQP) stimulation, which may be the mechanism for their participation in the onset and progression of STC.[34] One study found that MAPK and ERK can activate AQP expression, which causes the reabsorption of water by colonic mucosa and the decrease of intestinal water, leading to the occurrence of dry and hard stools and constipation.[35]

Therefore, the current study intended to examine the impacts of acupuncture plus acupoint application on MAPK and ERK in STC rats, with the aim of preliminarily exploring the relevant mechanisms for treating STC as well as providing new ideas and means for future clinical treatment.

METHODS

Animals

The research team designed a randomized, controlled animal study. The study was carried out at department of Department of Rehabilitation, Affiliated Hospital of Medical School of Ningbo University, Ningbo, Zhejiang, China. The animals were 30 six-to-eight-week-old, Sprague Dawley (SD) rats, half male and half female and weighing a mean of 200 ± 20 g. They were fed adaptively for one week in an environment with 12h light/dark alternately, at a temperature of 18-26°C and a 40-70% humidity, with food and water ad libitum. Then, 20 rats were randomly selected for inducement of STC, and the other 10 became the control group.

Procedures

Modeling methods. The research team conducted STC modeling with reference to Ling et al's research.[36] The 20 rats that the team had selected for modeling received 8mg/kg/d of diphenoxylate-containing feed continuously for 120 days, to induce STC and establish the STC model. The rats having dry fecal granules with a shortened granular shape, laborious defecation, and a lower daily defecation volume than that of the control group were regarded as being successfully modeled. The control group ate a normal diet with normal feeding.

Groups. Ten of the 20 rats induced with STC were randomly selected for acupuncture plus acupoint application and became the intervention group. The other 10 rats with induced STC became the model group and received no treatments. The control group also received no treatments.

Acupoint application. The TCM pharmacy at the research team's hospital provided all of the herbs used for the intervention group: rhubarb, mirabilite, magnolia officinalis, cassia seed, aloe and borneol. According to a ratio of 1:2:1:1:1:1:1, the herbs were ground into a powder, which was then sifted through a 200-mesh sieve, with an appropriate amount of ginger juice added to make it into a paste, and then stirred thoroughly and refrigerated for later use.

Acupuncture. The acupuncture needles were purchased from Gushi Gongyuan Medical Device Company (Henan, China). The acupoint used was the ST25, which is as an important acupoint for modulating intestinal function, and it can regulate intestinal qi, restore the large intestine's conduction function, promote secretion of intestinal mucus, and strengthen intestinal peristalsis and rectal contraction.[37] Also, Liang et al found that the ST25 can improve intestines and stomach and relieve constipation symptoms.[38]

Outcome measures. The study measured the alterations in the rats' body weight and feces, as well as the rats' intestinal motility, using intragastric administration of activated carbon. The activated carbon suspension was purchased for measurement of the intestinal propulsion rate from Changpu Chemical (Taizhou, China).

The rats were killed to obtain their intestinal tissues for measuring expression of mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) using Western blotting and polymerase chain reaction (PCR).

For Western blotting, the research team purchased: (1) a radioimmunoprecipitation assay (RIPA) lysate from Fisher Scientific (Waltham, Massachusetts, USA); (2) bicinchoninic acid (BCA) from Sigma-Aldrich (Shanghai, China); (3) a kit for the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) from Thermo Fisher Scientific (Waltham, Massachusetts, USA); (4) the polyvinylidene difluoride (PVDF) membrane from EMD Millipore, previously Merck Millipore, (Darmstadt, Midwest, Germany); and (5) the enhanced chemiluminescence (ECL) from Sigma-Aldrich (Shanghai, China). All antibodies were provided by Cell Signaling Technology (CST, Boston, Massachusetts, USA).

For PCR detection, the research team purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA): (1) the TRIzol reagent for isolation of RNA, (2) a reverse transcription kit; and (3) an amplification kit.

Intervention

Acupuncture. The acupuncture used the Tianshu point (ST25) was located at 5 mm away from the middle umbilicus of the rats, one on the left and one on the right, following Xu et al's procedures.[23] The research team removed the hair around the ST25 acupoint, and after conventional disinfection, the acupuncture needle was lifted with tweezers, and with the needle tip pointing to ST25, the practitioner penetrated the skin about 20 mm vertically, with the needle handle being attached to the skin's surface.

After acupuncture, the rat's stomach was gently pressed for 2 min every 30 min for 3min. During withdrawal of the needles, the rat was fixed with one hand, and the needle was quickly pulled out by holding the needle handle with tweezers in the other hand.

The intervention group received the acupuncture once a day, with an interval of one day after continuous acupuncture for three days, for four weeks.

Acupoint application. Upon the completion of that acupuncture treatment, 0.1 cm × 0.1 cm of the herbal paste were placed on a 3 cm x 3 cm sterile patch and applied to both sides of the ST25 of the rats for 6 h once a day for 4 weeks. A circle of breathable and hypoallergenic tape was used to fix the patch to the skin.

Outcome Measures

Stool-specimen collection and treatment. Dietary activities, and fecal characteristics of the rats were observed and recorded, and their weights were measured on days 7, 14, 21, and 28. Filter paper was placed in the feed cage to collect the rats' feces, and 10 uniform particles of moderate size that weren't soaked in the rats' urine or drinking water were weighed, and the measurements were recorded immediately. Then the fecal particles were dried in a constant-temperature air dryer at 105°C and weighed again to calculate the fecal water content (FWC).

Intestinal-specimen collection and processing. The propulsion speed in the small intestine of the rats was measured using intragastric administration of activated carbon. On an empty stomach, the rats were gavaged with 2 mL of 100 g/L activated carbon suspension. After 30 min, the rats were killed by cervical dislocation under anesthesia, and the whole intestinal tissue from the pylorus to the end of rectum was immediately removed.

In a relaxed state, the intestinal length and the advancing distance of activated carbon in the intestine were measured to calculate the intestinal propulsion rate. In addition, the amount of feces remaining in each rat's colon was calculated and recorded. Later, 2 cm × 2 cm of colon tissue was washed with precooled normal saline and soaked in 4% paraformaldehyde for subsequent tests.

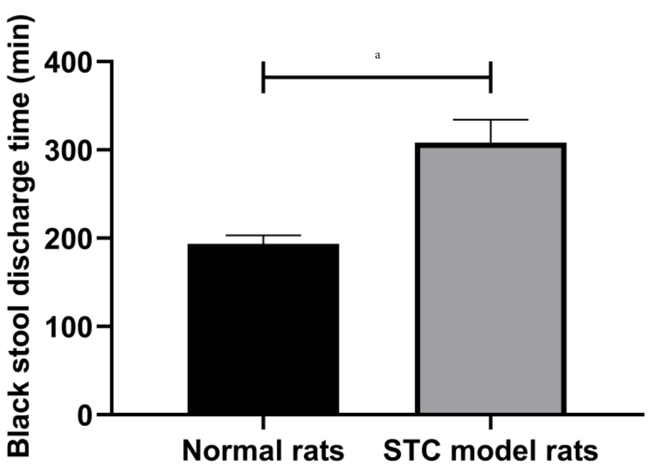
Western blotting. The collected colon tissue was transferred to Eppendorf (EP) tubes and subjected to lysis

Table 1. Primer Sequence

	F (5'-3')	R (5'-3')
MAPK	TGTTGACCGGAAGAACGTTGT	CAAAGTAGGCATGCGCAAGAG
ERK	CGCCAATGTACGTGCACTGA	CGACTGCAACGTCAC
GAPDH	CAACGGGAAACCCATCACCA	ACGCCAGTAGACTCCACGACAT

Abbreviations: ERK, extracellular signal-regulated kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MAPK, mitogen-activated protein kinase.

Figure 1. Comparison of Discharge Time for Black Stools



[a]$P < .05$, indicating that the control group's discharge time was significantly shorter than that of the 20 STC rats in the intervention and model groups

Abbreviations: STC, slow transit constipation

using RIPA lysate to extract the total protein, and then subsequently protein quantification was performed using BCA. After the SDS-PAGE occurred, the protein was moved for one hour to a PVDF membrane for sealing and subsequently rinsed three times with tris-buffered saline (TBST).

Thereafter, it was immersed in I antibodies for MAPK (1:1,000), ERK (1:1,000) and internal reference β-actin (1:1,000) and incubated overnight at 4°C. The next day, the membrane was placed into a second antibody (1:5,000) after TBST washing and incubated for one hour. After development with ECL, the gray value was analyzed using the Quantity One software (Hercules, California, USA).

PCR detection. Total RNA from colon tissue, isolated with TRIzol, was reverse transcribed into complementary DNA (cDNA) using a reverse transcription kit following the manufacturer's instructions, and then amplified using an amplification kit.

The reaction conditions were 37°C for 15 min and 85°C for 5 s. The MAPK and ERK mRNA levels relative to β-actin were calculated using 2-ΔΔCt. Genscript Biotech (Hercules, California, USA) was responsible for the design and construction of the primer sequence, as shown in Table 1.

Statistical Analysis

Data were statistically analyzed using SPSS20.0 (IBM, Armonk City, New York, USA). All the experimental results were described as means ± standard deviations (SDs), and $P < .05$ was considered to be statistically significant. The independent sample *t* test was employed for intergroup comparisons, and a one-way analysis of variance (ANOVA) and a least significant difference (LSD) post-hoc test were used for multigroup comparisons.

RESULTS

Modeling Results

No rats died during the modeling. As Figure 1 shows, the 20 STC rats in the intervention and model groups took significantly longer to discharge black stool than did the control group ($P < .05$).

Weight Changes in Rats

No significant difference existed in body weight between the intervention and model groups after STC inducement on day 7, with $P > .05$, but the control group's was significantly higher than that of the model group on days 7, 14, 21, and 28 and significantly higher than that of the intervention group on days 7, 14, and 21, with $P < .05$ (Table 2). On days 14, 21, and 28, the rats' mean weight was significantly higher in the intervention group that of the model group, with $P < .05$. On day 28, the rats' weight in the intervention and control groups weren't significantly different, with $P > .05$, but both were significantly higher than that in the model group, with $P < .05$. The rats' weights gradually and significantly increased in intervention and control groups, while the weights decreased gradually in the model group, with $P < .05$.

Comparison of Rat Feces

As Figure 2 shows, the defecation volume and FWC were significantly lower in the model group than those of the intervention and control groups ($P < .05$), and the intervention group's defecation volume and FWC were significantly lower than those of the control group ($P < .05$).

Comparison of Intestinal Motility

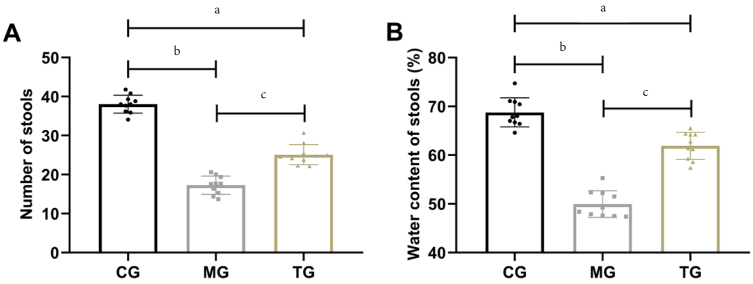
Figure 3 shows the results. The rats' intestinal lengths among the three groups weren't significantly different ($P > .05$). The intervention group had a propulsion rate that wasn't significantly different from that of the control group ($P > .05$), but propulsion rate was significantly lower in the model group than in the intervention group ($P < .05$). The

Table 2. Weight Changes in Rats

	Day 7	Day 14	Day 21	Day 28	F	*P* value
Control Group	220.45 ± 16.18	229.86 ± 15.82[a]	237.24 ± 16.23[a,b]	248.45 ± 16.18[a,b,c]	5.399	.004
Model Group	204.00 ± 13.72[d]	195.53 ± 14.39[a,d]	187.26 ± 15.64[a,b,d]	171.82 ± 13.67[a,b,c,d]	9.097	<.001
Intervention Group	206.15 ± 13.59[d]	214.36 ± 15.46[a,d,e]	225.15 ± 13.68[a,b,d,e]	245.70 ± 14.26[a,b,c,e]	14.380	<.001
F	3.779	12.730	29.340	86.950		
P value	0.036	<0.001	<0.001	<0.001		

[a] *P*<.05, indicating a significant decrease in weight for the model group and increase in weight for the control and intervention groups between day 7 and days 14, 21, and 28
[b] *P*<.05, indicating a significant decrease in weight for the model group and increase in weight for the control and intervention groups between day 14 and days 21 and 28
[c] *P*<.05, indicating a significant decrease in weight for the model group and increase in weight for the control and intervention groups between day 21 and day 28
[d] *P*<.05, indicating a significant difference in weight between the control group and the model group on days 7, 14, 21, and 28 and between the control group and the intervention group on days 7, 14, and 21
[e] *P*<.05, indicating a significant difference in weight between the model group and the intervention group on days 14, 21, and 28

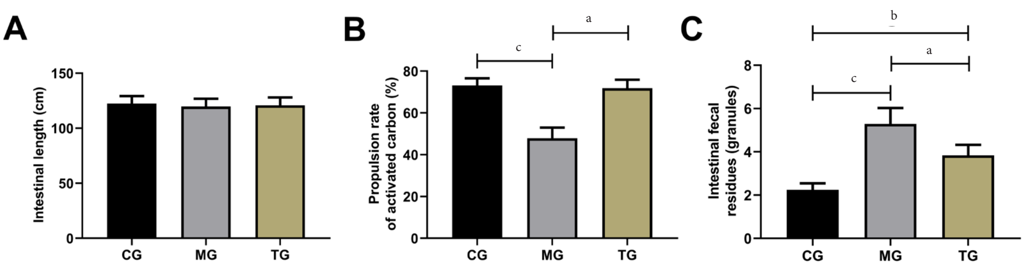
Figure 2. Comparison of the Rats' Feces for the Three Groups. Figure 2A shows the comparison of defecation volume, and Figure 2B shows the comparison of the FWC.



[a] *P*<.05, indicating that the intervention group's defecation volumes and FWC were significantly lower than those of the control group
[b] *P*<.05, indicating that the model group's defecation volumes and FWC were significantly lower than those of the control group
[c] *P*<.05, indicating that the model group's defecation volumes and FWC were significantly lower than those of the intervention group

Abbreviations: CG, control group; FWC, fecal water content; MG, model group; TG, intervention group.

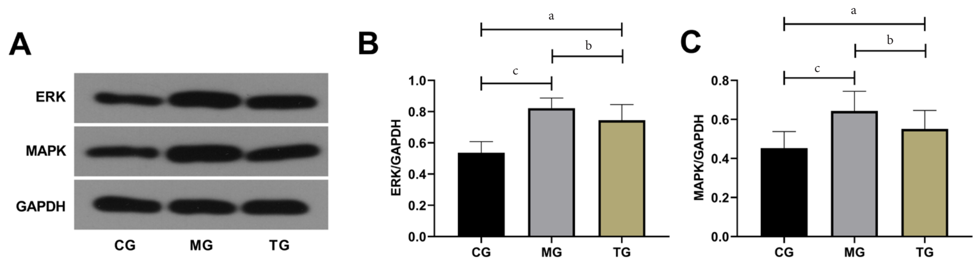
Figure 3. Comparison of Rats' Intestinal Motility. Figure 3A shows the comparison of intestinal length; Figure 3B shows the comparison of propulsion rate of activated carbon; and Figure 3C shows the comparison of intestinal fecal residues.



c*P*<.05, indicating a significantly lower propulsion rate and higher intestinal fecal residues in the model group than those in the control group
a*P*<.05, indicating a significantly lower propulsion rate and higher intestinal fecal residues in the model group than those in the intervention group
b*P*<.05, indicating significantly lower intestinal fecal residues in the control group than those in the intervention group

Abbreviations: CG, control group; ERK, extracellular signal-regulated kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MAPK, mitogen-activated protein kinase; MG, model group; TG, treatment (intervention) group.

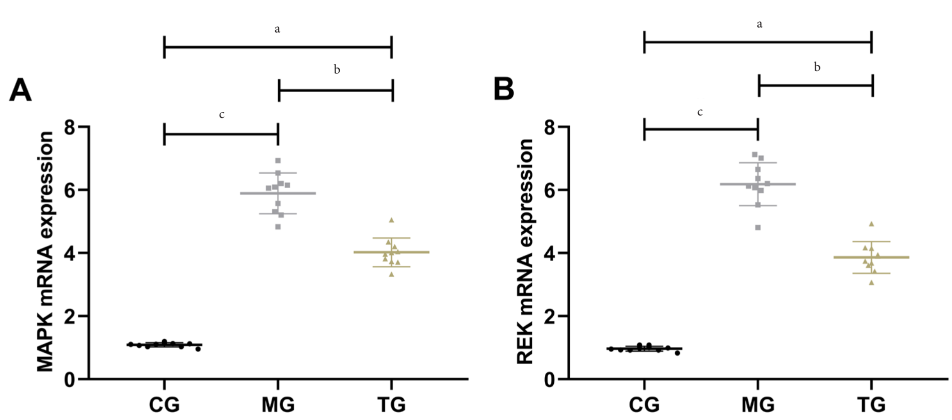
Figure 4. MAPK and ERK Protein Expression in Rats' Intestinal Tissue. Figure 4A shows the Western blotting diagram; Figure 4B shows the MAPK protein expression in the intestinal tissue; and Figure 4C shows the ERK protein expression in the intestinal tissue.



[a]$P < .05$, indicating significantly lower ERK/GAPDH and MAPK/GAPDH in the control group than those in the intervention group
[b]$P < .05$, indicating significantly higher ERK/GAPDH and MAPK/GAPDH in the model group than those in the intervention group
[c]$P < .05$, indicating significantly lower ERK/GAPDH and MAPK/GAPDH in the control group than those in the model group

Abbreviations: CG, control group; ERK, extracellular signal-regulated kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MAPK, mitogen-activated protein kinase; MG, model group; TG, treatment (intervention) group.

Figure 5. MAPK mRNA and ERK mRNA Levels in Rats' Intestinal Tissue. Figure 5A shows the MAPK mRNA level in the intestinal tissue, and Figure 5B shows the ERK mRNA level in the intestinal tissue.



[a]$P < .05$, indicating significantly lower MAPK mRNA and ERK mRNA levels in the control group than those in the intervention group
[b]$P < .05$, indicating significantly lower MAPK mRNA and ERK mRNA levels in the intervention group than those in the model group
[c]$P < .05$, indicating significantly lower MAPK mRNA and ERK mRNA levels in the control group than those in the model group

Abbreviations: CG, control group; ERK, extracellular signal-regulated kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MAPK, mitogen-activated protein kinase; MG, model group; TG, treatment (intervention) group.

intestinal fecal residue wad significantly higher in the model group than that in the control and intervention groups (both $P < .05$), and it was significantly lower in the control group compared to that of the intervention group .

MAPK and ERK Protein Expression

As Figure 4 shows, the MAPK and ERK protein levels in the rats' intestinal tissues were significantly lower in the control group than in the intervention and model groups (both $P < .05$), while the levels were significantly higher in the model group than those in the intervention group ($P < .05$).

MAPK and ERK mRNA Levels

Figure 5 shows the results. The MAPK and ERK mRNA levels in the rats' intestinal tissues were significantly lower in the control group than in the intervention and model groups ($P < .05$) and were also significantly lower in the intervention group as compared to those in the model group ($P < .05$).

DISCUSSION

In the current study, the research team first found that the discharge time for the black stool of STC rats in the model and intervention groups increased significantly. The model group's weights decreased significantly over time, but the intervention group's weights gradually increased, with no evident difference between the intervention group and the control group at day 28, suggesting that acupuncture therapy plus acupoint application can effectively overcome the pathological state of STC, which agrees with the results of Lee et al's study.[39]

Then, the current research team compared the defecation status of the rats and found that the defecation volume and FWC in the model group decreased remarkably, while the intervention group's increased, indicating that acupuncture plus acupoint application can improve the defecation status of STC.

The intestinal function test indicated that the intervention group had a, intestinal propulsion rate not significantly different from that of the control group and significantly higher than that of the model group, with notably fewer intestinal fecal residues than the model group, which confirms the efficacy of acupuncture plus acupoint application in STC.

The current research team speculates that that finding occurred because of the use of the ST25 acupoint, which other studies have shown to be an important acupoint for modulating intestinal function.[37,38] The results of this experiment also verify the significance of ST25 for intestinal function.

Confirming the critical role of MAPK and ERK in the intestinal tract as found in other studies,[34,40,41] the current study found that MAPK and ERK increased in the model group. After the acupuncture plus acupoint application, the MAPK and ERK decreased significantly in the intervention group, which suggests that MAPK and ERK might be the targets for STC treatment. However, more studies are warranted for confirmation.

CONCLUSIONS

Acupuncture plus acupoint application can effectively improve the defecation of STC rats and their intestinal motility, and its mechanism may be through inhibiting MAPK and ERK.

AUTHORS' DISCLOSURE STATEMENT

The Ningbo Medical Science and Technology Project (No.: 2020Y11) supported the study. The research team declares that it has no conflicts of interest.

REFERENCES

1. Schiano di Visconte M, Pasquali A, Cipolat Mis T, Brusciano L, Docimo L, Bellio G. Sacral nerve stimulation in slow-transit constipation: effectiveness at 5-year follow-up. *Int J Colorectal Dis*. 2019;34(9):1529-1540. doi:10.1007/s00384-019-03351-w
2. Sailer M. Slow transit constipation. *Zentralbl Chir*. 2019;144(2):179-189.
3. Sharma A, Rao S. Constipation: pathophysiology and current therapeutic approaches. *Handb Exp Pharmacol*. 2017;239:59-74. doi:10.1007/164_2016_111
4. Bharucha AE, Wald A. Chronic Constipation. *Mayo Clin Proc*. 2019;94(11):2340-2357. doi:10.1016/j.mayocp.2019.01.031
5. Vriesman MH, Koppen IJN, Camilleri M, Di Lorenzo C, Benninga MA. Management of functional constipation in children and adults. *Nat Rev Gastroenterol Hepatol*. 2020;17(1):21-39. doi:10.1038/s41575-019-0222-y
6. Camilleri M, Ford AC, Mawe GM, et al. Chronic constipation. *Nat Rev Dis Primers*. 2017;3(1):17095. doi:10.1038/nrdp.2017.95
7. Ge X, Zhao W, Ding C, et al. Potential role of fecal microbiota from patients with slow transit constipation in the regulation of gastrointestinal motility. *Sci Rep*. 2017;7(1):441. doi:10.1038/s41598-017-00612-y
8. Wei D. [Progress in the treatment of surgical procedures for slow transit constipation]. *Zhonghua Wei Chang Wai Ke Za Zhi*. 2018;21(3):357-360.
9. Black CJ, Ford AC. Chronic idiopathic constipation in adults: epidemiology, pathophysiology, diagnosis and clinical management. *Med J Aust*. 2018;209(2):86-91. doi:10.5694/mja18.00241
10. Rao SS, Rattanakovit K, Patcharatrakul T. Diagnosis and management of chronic constipation in adults. *Nat Rev Gastroenterol Hepatol*. 2016;13(5):295-305. doi:10.1038/nrgastro.2016.53
11. Zhao JS, Tong WD. [Pathophysiology of slow transit constipation]. *Zhonghua Wei Chang Wai Ke Za Zhi*. 2012;15(7):758-760.
12. Bassotti G, Villanacci V. Slow transit constipation: a functional disorder becomes an enteric neuropathy. *World J Gastroenterol*. 2006;12(29):4609-4613. doi:10.3748/wjg.v12.i29.4609
13. Wang R, Su Q, Yan Z. Treatment of slow transit constipation-induced ileus during pregnancy by colectomy with ileorectal anastomosis: A case report. *Medicine (Baltimore)*. 2020;99(18):e19944. doi:10.1097/MD.0000000000019944
14. Altomare DF, Picciariello A, Di Ciaula A, Rinaldi M, De Fazio M, Portincasa P. Effects of temporary sacral nerve stimulation on gastrointestinal motility and function in patients with chronic refractory slow-transit constipation. *Tech Coloproctol*. 2021;25(3):291-297. doi:10.1007/s10151-020-02367-7
15. Wang B, Chen Y, Chen S. [Clinical observation of slow transit constipation treated with acupuncture and modified *weitong xiaopi* formula]. *Zhongguo Zhenjiu*. 2017;37(2):130-134.
16. Sun Y, Yan C, Jin S, et al. Curative Effect and mechanism of Guiren Runchang Granules on morphine-induced slow transit constipation in mice. *Evid Based Complement Alternat Med*. 2020;2020:5493192. doi:10.1155/2020/5493192
17. Xie Y, Fan Y, Fan W, Yang X, Xiang Y, Zhao T. Application effect of catgut-embedding therapy combined with minimally invasive surgery for slow transit constipation: A protocol for systematic review and meta-analysis. *Medicine (Baltimore)*. 2020;99(35):e21875. doi:10.1097/MD.0000000000021875
18. Deng Z, Fu Z, Yan W, et al. The different effects of Chinese Herb Solid Drink and lactulose on gut microbiota in rats with slow transit constipation induced by compound diphenoxylate. *Food Res Int*. 2021;143:110273. doi:10.1016/j.foodres.2021.110273
19. Li S, He Y, Zhang H, et al. Formulation of traditional Chinese medicine and its application on intestinal flora of constipated rats. *Microb Cell Fact*. 2020;19(1):212. doi:10.1186/s12934-020-01473-3
20. Alsalimy N, Madi L, Awaisu A. Efficacy and safety of laxatives for chronic constipation in long-term care settings: A systematic review. *J Clin Pharm Ther*. 2018;43(5):595-605. doi:10.1111/jcpt.12721
21. Zheng SZ, Guo Q, Zhang GM, Pan LH, Zeng KW. [Therapeutic effect and mechanism of Shouhui Tongbian Capsules on slow transit constipation model mice]. *Zhongguo Zhongyao Zazhi*. 2021;46(3):520-525.
22. Yao CJ, Li YL, Pu MJ, Luo LH, Feng PM. Traditional Chinese medicine for irritable bowel syndrome: A protocol for meta-analysis. *Medicine (Baltimore)*. 2020;99(48):e23394. doi:10.1097/MD.0000000000023394
23. Xin P, Kuang HX, Li XL, et al. [Proteomics and its application to determine mechanism of action of traditional Chinese medicine]. *Zhongguo Zhongyao Zazhi*. 2018;43(5):904-912.
24. Zhuang Y, Xing JJ, Li J, Zeng BY, Liang FR. History of acupuncture research. *Int Rev Neurobiol*. 2013;111:1-23. doi:10.1016/B978-0-12-411545-3.00001-8
25. Acar HV. Acupuncture and related techniques during perioperative period: A literature review. *Complement Ther Med*. 2016;29:48-55. doi:10.1016/j.ctim.2016.09.013
26. Zhao J. [Interpretation of acupuncture theory from acupuncture application]. *Zhongguo Zhenjiu*. 2017;37(10):1115-1118.
27. Liu Z, Yan S, Wu J, et al. Acupuncture for chronic severe functional constipation: A randomized trial. *Ann Intern Med*. 2016;165(11):761-769. doi:10.7326/M15-3118
28. Pei L, Geng H, Guo J, et al. Effect of acupuncture in patients with irritable bowel syndrome: A randomized controlled trial. *Mayo Clin Proc*. 2020;95(8):1671-1683. doi:10.1016/j.mayocp.2020.01.042
29. Fan YH, Zhang GS, Li YL, Zhang L, Lü B. [The effect of exogenous glial cell line-derived neurotrophic factor on expression of gastric and colonic Akt, MAPK in slow transit constipation rats]. *Zhonghua Nei Ke Za Zhi*. 2011;50(4):328-332.
30. Wei G, Gao N, Chen J, et al. Erk and MAPK signaling is essential for intestinal development through Wnt pathway modulation. *Development*. 2020;147(17):dev185678. doi:10.1242/dev.185678
31. Gao W, Wang C, Yu L, et al. Chlorogenic Acid attenuates dextran sodium sulfate-induced ulcerative colitis in mice through MAPK/ERK/JNK pathway. *BioMed Res Int*. 2019;2019:6769789. doi:10.1155/2019/6769789
32. Qu M, Li D, Qiu Y, Wang D. Neuronal ERK MAPK signaling in response to low-dose nanopolystyrene exposure by suppressing insulin peptide expression in Caenorhabditis elegans. *Sci Total Environ*. 2020;724:138378. doi:10.1016/j.scitotenv.2020.138378
33. Mao J, Qi S, Cui Y, et al. Lactobacillus rhamnosus GG attenuates lipopolysaccharide-induced inflammation and barrier dysfunction by regulating MAPK/NF-kappaB signaling and modulating metabolome in the piglet intestine. *J Nutr*. 2020;150(5):1313-1323. doi:10.1093/jn/nxaa009
34. Cheng B, Rong A, Zhou Q, Li W. CLDN8 promotes colorectal cancer cell proliferation, migration, and invasion by activating MAPK/ERK signaling. *Cancer Manag Res*. 2019;11:3741-3751. doi:10.2147/CMAR.S189558
35. Zhang Z, Liu Q, Zang H, Shao Q, Sun T. Oxymatrine protects against l-arginine-induced acute pancreatitis and intestine injury involving Th1/Th17 cytokines and MAPK/NF-κB signalling. *Pharm Biol*. 2019;57(1):595-603. doi:10.1080/13880209.2019.1657906

36. Ling J, Zhang W, Xu B, Qiu W, Wang Y. [Experimental study of Bisacodyl in the treatment of slow transit constipation model rats]. *Zhonghua Wei Chang Wai Ke Za Zhi*. 2016;19(12):1365-1369.
37. Li P, Luo Y, Wang Q, et al. Efficacy and safety of acupuncture at Tianshu (ST25) for functional constipation: evidence from 10 randomized controlled trials. *Evid Based Complement Alternat Med*. 2020;2020:2171587. doi:10.1155/2020/2171587
38. Liang C, Wang KY, Gong MR, Li Q, Yu Z, Xu B. Electro-acupuncture at ST37 and ST25 induce different effects on colonic motility via the enteric nervous system by affecting excitatory and inhibitory neurons. *Neurogastroenterol Motil*. 2018;30(7):e13318. doi:10.1111/nmo.13318
39. Xu G, Xiao Q, Zhou J, et al. Acupuncture and moxibustion for primary osteoporosis: an overview of systematic review. *Medicine (Baltimore)*. 2020;99(9):e19334. doi:10.1097/MD.0000000000019334
40. Wang PM, Hsu CW, Liu CT, Lai TY, Tzeng FL, Huang CF. Effect of acupressure on constipation in patients with advanced cancer. *Support Care Cancer*. 2019;27(9):3473-3478. doi:10.1007/s00520-019-4655-1
41. Lee HY, Kwon OJ, Kim JE, et al. Efficacy and safety of acupuncture for functional constipation: a randomised, sham-controlled pilot trial. *BMC Complement Altern Med*. 2018;18(1):186. doi:10.1186/s12906-018-2243-4