Atractylodes chinensis volatile oil up-regulated IGF-1 to improve diabetic gastroparesis in rats

Hongzeng Li 1*, Yitong Wang 1*, Yuxin Tian 1, Feiyue Tian 1, Zhiyang Xing 1, Yunfei Wang 2, Meixing Yan 3*, Yanling Gong 1*

1 Department of Pharmacy, College of Chemical Engineering, Qingdao University of Science and Technology, Qingdao, China
2 Shandong Xinhua Pharmaceutical Company Limited, Zibo, China
3 Qingdao Women and Children’s Hospital, Qingdao, China

ORIGINAL ARTICLE

Article type: Original

Article history:
Received: Sep 6, 2021
Accepted: Apr 9, 2022

Keywords:
Atractylodes
Gastroparesis
Insulin-like growth factor I
Interstitial cells of cajal
Stem cell factor

ABSTRACT

Objective(s): Diabetic gastroparesis (DGP) is one of the main complications of diabetes, and more than half of diabetes cases are accompanied by gastroparesis. This study aims to explore the effect of Atractylodes chinensis volatile oil (ACVO) on DGP rats.

Materials and Methods: The rats were injected with STZ combined with a high-sugar and high-fat diet in an irregular manner to establish the DGP model. ACVO at different doses (9.11 mg/kg, 18.23 mg/kg, and 36.45 mg/kg) were given by intragastric administration. A mixture of cisapride and metformin was used as the positive control. After treatment with ACVO, body weight increased and blood glucose decreased when compared with rats in the DGP group. Gastric emptying and intestinal propulsion were accelerated, and gastric acid secretion increased. The serum insulin-like growth factor-1 (IGF-1) level was compared with rats in the DGP group. Gastric emptying and intestinal propulsion were accelerated, and gastric acid secretion increased. The serum insulin-like growth factor-1 (IGF-1) level was increased. Protein expressions and positive cells of IGF-1 receptor (IGF-1R), acetylcholine transferase (CHAT), and stem cell factors (SCF) in the stomach were significantly increased determined by western blot and immunofluorescence staining. The morphology and the number of interstitial cells of Cajal (ICCs) in the stomach were restored, determined by hematoxylin and eosin staining and immunohistochemical staining, respectively.

Conclusion: ACVO effectively alleviated DGP in rats, and its mechanism may be related to the up-regulation of IGF-1/IGF-1R signaling.

Introduction

Diabetic gastroparesis (DGP) is a complication of diabetes and about 50% of people with diabetes suffer from it. Some DGP patients have no obvious symptoms, and some patients will have accompanying indigestion and delayed gastric emptying (1), characterized by early satiety and persistent nausea. DGP not only brings physical pain to the patients but also reduces the quality of life and happiness of the patients. Drug therapy and dietary changes are helpful to increase gastric motility and improve the symptoms of gastroparesis. However, adverse reactions caused by drugs have greatly limited the development and use of gastric motility drugs (2-4). Therefore, it is particularly important to develop a new drug with low toxicity and side effects for the treatment of DGP.

The volatile oil of Atractylodes chinensis (ACVO) is extracted from the rhizome of A. chinensis. Studies have found that ACVO has high medicinal values such as antibacterial, anti-hypoxia, hypoglycemic, anti-ulcer, diuretic, anti-gastritis, etc (5-8). Sesquiterpenes, the main components of ACVO, have obvious anti-inflammatory and insecticidal activity (9, 10). Therefore, ACVO is widely used in the fields of medicine and agriculture. Some studies have shown that A. chinensis rhizome can be used to treat gastrointestinal diseases (11). Its volatile oil composition has a potential effect for the treatment of colitis (colitis syndrome) (12). However, the effects of volatile oil from A. chinensis on DGP and its mechanism have not been reported.

Studies have confirmed that the absence of interstitial cells of Cajal (ICCs) eventually leads to DGP (13). ICCs are a pacemaker in the gastrointestinal tract that triggers gastric motility. Meanwhile, ICCs are an intermediate that conduct neurotransmission from the enteric nervous system to the gastric smooth muscle. Stem cell factor (SCF) is responsible for phenotypic maintenance, proliferation, and differentiation of ICCs (14). Insulin-like growth factor-1 (IGF-1) is a homologous molecule that promotes cell growth and differentiation and is also a nutrient for neurons (15). Excitatory neuron acetylcholine transferase (CHAT) and inhibitory neuron nitric oxide synthase (nNOS) work together to balance gastric motility. In previous studies, it was found that nitric oxide synthase is abnormal in DGP. However, there is less research on CHAT. It has been reported

* Corresponding authors: Meixing Yan. Qingdao Women and Children’s Hospital, Qingdao 266011, China. Email: meixing@163.com; Yanling Gong. Department of Pharmacy, College of Chemical Engineering, Qingdao University of Science and Technology, 53 Zhongzhou Road, Qingdao 266042, China. Email: hanyu_ma@126.com
# These authors contributed equally to this work.
that IGF-1 has a significant improvement on peripheral neuropathy caused by diabetes (16, 17). The damage to its receptor (IGF-1R) promotes the development of DGP (18).

In this study, rats were injected with streptozocin and fed with high sugar and fat to establish a DGP model, and the effect of ACVO on DGP involved in IGF-1 signaling was explored. The results might lay a foundation for the development of new drugs to treat DGP.

Materials and Methods

Materials and reagents

ACVO was extracted from the dried rhizome of *A. chinensis*, which was purchased from Qingdao Guofeng Pharmacy (Qingdao, Shandong, China). Cisapride and metformin were purchased from Shandong Qikang Pharmaceutical Co., Ltd. (Dezhou, Shandong, China). Streptozotocin (STZ), enzyme-linked immunosorbent assay (ELISA) kits for serum IGF-1, and Bicinchoninic acid (BCA) kits were obtained from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Phenolic red solutions were purchased from the Tianjin Institute of Light and Fine Chemical Engineering (Tianjin, China). IGF-1R, CHAT, and SCF antibodies were purchased from Abcam (Cambridge, UK). Sodium dodecyl sulfate polyacrylamide gel, polyvinylidene difluoride (PVDF) membranes, and ECL substrate kit were purchased from Beijing Bios Biotechnology Co., Ltd. (Beijing, China). All other chemicals used were of analytical grade.

Experimental animals

One hundred and eighty-five adult male Wistar rats (180–200 g) were used in this study which were purchased from Qingdao Daren Fortune Animal Science And Technology Co., Ltd. (Qingdao, Shandong, China). The animals were placed in cages with a temperature of 22 ± 2 °C and humidity of 50–60% and fed a standard diet for 7 days of adaption. All animal experiments were approved by the Animal Care and Use Committee at the Qingdao University of Science and Technology (approval code: 20201015). The research involving animals was conducted according to the Guide for the Care and Use of Laboratory Animals.

Animal processing

At the end of adaptive feeding, the rats were randomly divided into the control group (NCG) (n=35) and the diabetic gastroparesis group (DGP) (n=155). Rats with DGP were intraperitoneally injected with STZ at a dose of 50 mg/kg, and rats with NCG were given the same volume of normal saline. Fasting blood glucose was determined 72 hr later. Fasting blood glucose of rats higher than 16.7 mmol/l for two consecutive weeks indicated that the rat developed diabetes. Otherwise, the rats were anesthetized to death with pentobarbital sodium (65 mg/kg). The stomach was removed and cut open along the greater curvature. The contents were poured into a beaker and 20 ml NaOH solution (0.5 mol/l) and 0.5 ml 20% trichloroacetic acid were added. Then the supernatant was obtained by centrifugation and its absorbance was determined at 560 nm with a Microplate reader (Infinite m plex, Tecan, Switzerland). The same method was used to determine the absorbance of the standard phenolic red solution. The gastric emptying rate was calculated according to the following equation:(1)(20).

\[
\text{gastric emptying rate} (\%) = \left(1 - \frac{A_s}{A_i}\right) \times 100
\]  

(1)

\(A_s\) represents the absorbance of the phenolic red solution in the stomach of each group, and \(A_i\) represents the absorbance of standard phenolic red solution.

\[
\text{intestinal propulsion rate} (\%) = \left(\frac{\text{length of the purple part of intestine}}{\text{length of the whole intestine}}\right) \times 100
\]  

(2)

Fasting blood glucose, serum IGF-1, gastric juice volume, and gastric acid

The remaining rats were used for determination of fasting blood glucose, serum IGF-1, and gastric acid. After measuring the fasting blood glucose, the rats were anesthetized with pentobarbital sodium (65 mg/kg), and then the blood was collected from the heart to separate serum by centrifugation. Serum IGF-1 concentration was determined by the ELISA method. Thereafter, the entire stomach was cut open and the blood was wiped off. Cut along with one side of the great bend of the stomach to collect the gastric juice and centrifuged at 3500 rpm/min. The supernatant was the gastric juice volume. 2 ml supernatant gastric juice was taken out and added with two drops of phenolphthalein reagent, and then titrated with 0.02 mol/l NaOH in a burette until the solution turned red with no more color change. Then the volume of the consumed NaOH solution was recorded. The gastric acid secretion amount of the rats was calculated according to the following equation:(3)(21).

\[
\text{gastric acid secretion} (\mu\text{Eq/L}) = \frac{V_{\text{NaOH}}}{0}
\]  

(3)
Western blotting

The same part of fresh stomach tissues of rats in each group was taken and protein lysates were added. After 30 min, the homogenate was centrifuged at 12000 rpm at 4 °C for 10 min. The BCA kit was used to determine the protein concentration. The protein samples were separated by sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) and transferred to the PVDF membrane. Then, the protein samples were sealed with 5% skimmed milk powder for 2 hr and washed with TBST. Subsequently, the primary antibody against IGF-1R (rabbit polyclonal, 1:1000 dilution), CHAT (rabbit polyclonal, 1:1000 dilution), SCF (rabbit polyclonal, 1:1000 dilution) was added and incubated for 2 hr. Then the membranes were washed with TBST 5 times and 5 min each time. HRP-conjugated secondary antibody (1:1000 dilution) was added and incubated for 1 hr. Finally, ECL was applied evenly to the membranes for development. The membranes were imaged using an automated imaging system (Bio-Rad Gel Doc EZ, Bole Life Medicine Products Co. Ltd, Shanghai, China). Image J was used to analyze the gray value of the bands with GAPDH as the internal reference.

Immunohistochemical staining

The same part of the stomach was soaked in 4% paraformaldehyde, embedded in paraffin, sectioned, and then antigen repaired after dewaxing. The treated slices were incubated in 3% H2O2 for 20 min and washed with PBS three times, 5 min each time. The tissues were then sealed with goat serum for 10 min, rabbit anti-mouse C-Kit antibody (1:100) was added, and then incubated at 4 °C overnight. After washing with PBS, goat anti-rabbit secondary antibody (1:1000) labeled with biotin was added to the tissue and incubated at room temperature for 20 min. Then 3, 3-diaminobenzidine (DAB) was added and incubated for 8 min, and the slices were dyed with hematoxylin. After being sealed with neutral gum, the slices were observed under a microscope (BX53, Olympus, Tokyo, Japan).

Hematoxylin and eosin staining

Slices of rat stomach tissue were taken and stained with hematoxylin and eosin (H&E) according to standard operating procedure (22).

Immunofluorescence staining

Gastric tissue was removed after perfusion fixation and soaked in 4% paraformaldehyde for 6–8 hr, and then transferred to 30% sucrose solution. The same part of gastric tissue was frozen and then sectioned. The prepared slices were sealed with goat serum for 2 hr, then primary antibody against IGF-1R (rabbit polyclonal, 1:200 dilution), CHAT (rabbit polyclonal, 1:200 dilution), SCF (rabbit polyclonal, 1:200 dilution) was added and incubated at 4 °C overnight. The sections were cleaned with PBS 3 times, 5 min each time, and then incubated with secondary antibody (cy3-conjugated or FITC-conjugated, 1:100) for 2 hr. Finally, the sections were sealed and observed under a microscope (BX53, Olympus, Tokyo, Japan).

Statistical analysis

The results were presented in the form of mean ± standard deviation, and then one-way ANOVA was performed using SPSS. LDS and S-N-K were used for pairwise comparisons. When P≤0.05, it was considered statistically significant.

Results

Effects of ACVO on body weight and blood glucose in DGP rats

At the end of the experiment, there was a significant decrease in body weight and an increase in blood glucose in the DGP group compared with the NCG group (Figure 1a and b, P<0.01), indicating successful establishment of diabetes. During the whole experiment, NCG rats fed a normal diet showed a steady weight gain (Figures 1a and b, compared with before treatment, P>0.01) and no significant changes in blood glucose (Figures 1a and b, compared with before treatment, P>0.05). However, the weight of DGP rats decreased significantly and blood glucose increased significantly (Figures 1a and b, compared with before treatment, P<0.01). After the ACVO treatment, the weight of rats in ACVO.L, ACVO.M, and ACVO.H groups increased significantly while the blood glucose decreased significantly (Figures 1a and b, compared with before treatment in the same group, P<0.05 or P<0.01). Furthermore, with the increase in dose, the effect of ACVO increased (Figure 1a and b, compared with the DGP group after treatment, P<0.05 or P<0.01) and the effect of a high dose of ACVO was similar to that of CMG. The results showed that ACVO exhibited a certain hypoglycemic effect.

Effects of ACVO on gastric emptying, intestinal propulsion, gastric acid, and gastric juice volume in DGP rats

Compared with the NCG group, DGP rats had significantly slower gastric emptying, intestinal propulsion, less gastric acid secretion, and gastric juice volume, (Figures 1a and b, compared with before treatment, P<0.01). After the ACVO treatment, the gastric emptying and intestinal propulsion in rats treated with medium and high doses of ACVO were significantly faster than those in the DGP group (Figures 1a and b, compared with before treatment in the same group, P<0.05 or P<0.01). Furthermore, with the increase in dose, the effect of ACVO increased (Figure 1a and b, compared with the DGP group after treatment, P<0.05 or P<0.01) and the effect of a high dose of ACVO was similar to that of CMG. The results showed that ACVO exhibited a certain hypoglycemic effect.
of IGF-1R, CHAT, and SCF in the gastric tissues of DGP rats were significantly decreased (Figures 4a and 4b, $P<0.01$). After treatment with ACVO, IGF-1R, CHAT, and SCF protein expressions in the gastric tissue increased with the increase of the dose of ACVO, showing statistical significance in the medium and high dose group (Figures 4a and 4b, compared with the DGP group, $P<0.01$).

**Effect of ACVO on the immune activity of IGF-1R, CHAT, and SCF in DGP rats**

Immunofluorescence was used to analyze the expression of IGF-1R, CHAT, and SCF in the gastric tissues of rats in each group. The results revealed that the immunopositive reaction of IGF-1R, CHAT, and SCF in the gastric tissues of DGP rats was significantly reduced compared with the NCG group (Figures 5a, b, and c). However, the immunopositive reactions of IGF-1R, CHAT, and SCF were both increased with the increase in ACVO dose (Figures 5a, b, and c).

**Effect of ACVO on gastric histomorphology of DGP rats**

The results of HE staining were shown in Figure 6. The gastric tissues of the NCG rats were intact without any significant damage. The cells were orderly arranged, and there was no inflammatory infiltration, showing a normal morphology (Figure 6a). In contrast, the gastric tissue cells of DGP rats were disordered and some of the cells were deformed (Figure 6b). Compared with the DGP, the morphology of rat gastric tissue cells in ACVO treatment groups was improved to varying degrees (Figures 6c, 6d, and 6e). With the increase of the dose, the morphology of gastric tissue was gradually restored to normal. There was no significant difference between ACVO, H and NCG groups (Figures 6a and 6e).

**Effect of ACVO on c-kit expression of ICCs in the gastric tissues of DGP rats**

The expression of c-kit in the gastric tissues of the rats in each group was shown in Figure 7. Compared with the
NCG group, the expression of ICCs positive cells in the gastric tissues of DGP rats was significantly decreased (Figures 7a and 7b). After the treatment of ACVO, the expression of ICCs positive cells in the gastric tissues of DGP rats was up-regulated to varying degrees with the increase of the dose (Figures 7c, 7d, and 7e). The results suggested that ACVO could restore ICCs positive cells in the gastric tissues of DGP rats.

**Discussion**

The link between gastroparesis and diabetes has been noted for the last century, and DGP is a serious complication of diabetes. However, DGP is less likely to be detected before the onset of the disease and is difficult to treat (23). According to recent reports, some Chinese herbal ingredients can effectively treat DGP (24). In this study, we explored the therapeutic effect of ACVO on DGP rats and its potential mechanism. The results showed that ACVO significantly reduced blood glucose and promoted gastrointestinal motility in DGP rats. The mechanism of its effect may be related to elevation of serum IGF-1, up-regulation of IGF-1R, CHAT, SCF expression, and the restoration of ICCs number positive cells in the gastric tissues.

The DGP model induced by intraperitoneal injection of...
STZ and irregular high-sugar and high-fat diet is a widely used modeling method (25). DGP is characterized by weight loss, increased blood glucose, delayed gastric emptying, and reduced excretion of gastric acid. Gastric acid is the main substance that activates pepsinogen in the gastric juice converting it to active peptic, which digests food in the acidic environment provided by gastric acid, thereby promoting gastric emptying. In DGP rats, a sharp drop in body weight and a significant increase in blood sugar were intuitive indicators of DGP. Gastric emptying was delayed accompanied by a significant reduction in intestinal propulsion. After the treatment of ACVO, the body weight and blood glucose were recovered, and the status of delayed gastric emptying and reduced intestinal propulsion rate were also significantly alleviated. To our knowledge, the therapeutic effect of ACVO on DGP is revealed for the first time.

ICCs are a kind of mesenchymal cells which are scattered in the gastrointestinal smooth muscle and autonomic nerve endings. It is recognized as a pacemaker to transduce electrical activity to the gastric smooth muscle and trigger muscle contraction. C-kit protein is the main protein expressed in ICs (26). A decrease in C-Kit expression indicates ICs abnormalities, resulting in gastric emptying delay (27).

IGF-1 acts as a nutritional factor that promotes the growth of cells in the gastrointestinal tract. IGF-1R is activated by IGF-1. Activation of the IGF-1R stimulates multiple pathways which finally results in multiple biological effects in a variety of tissues and cells. IGF-1/IGF-1R signaling was implicated in the development of the nervous system by promoting neuronal growth, survival, proliferation, and differentiation. Previous studies have shown that there is no direct relationship between ICs and IGF-1. However, IGF-1 is expressed in gastric smooth muscle cells and induces gastric smooth muscle cells to produce SCF (27-29). SCF is a ligand of C-Kit and plays an important role in the phenotypic differentiation and maintenance of ICs. The SCF/C-Kit pathway is the main way to regulate ICs (30). The decrease in IGF-1 signal transduction leads to a decrease in SCF expression, which leads to depletion of ICs (31). In our present study, the influence of ACVO on IGF-1 and ICs has been explored. The results showed that the expressions of IGF-1R, SCF, and C-Kit in the gastric tissues of the DGP rats were greatly reduced, and the number of ICs also declined significantly. After the treatment with ACVO, the expressions of IGF-1, SCF and ICs were significantly increased. In addition, the level of serum IGF-1 also increased with the increase of the dose, suggesting that ACVO could up-regulate the expressions of IGF-1/IGF-1R signaling and SCF/C-Kit to restore ICs and further promote gastric movement.

CHAT, an excitatory neuron (32, 33), maintains gastric movement together with inhibitory neurons, and IGF-1 plays an important role in the development of neurons. In this study, the expression of IGF-1/IGF-1R in DGP rats was significantly reduced, and the normal survival of CHAT could not be maintained, which was a factor in the delay of gastric emptying in rats. After the treatment of ACVO, CHAT expression was restored and increased with the increase of the dose of ACVO. The up-regulation of CHAT might be related to IGF-1/IGF-1R signaling which needs to be further explored.

Conclusion
In general, ACVO increased body weight and decreased blood glucose in the DGP rats. ACVO effectively promoted gastric emptying and intestinal propulsion in the DGP rats and increased gastric acid secretion. Furthermore, after the treatment of ACVO, the serum IGF-1 level in DGP rats was increased, and the protein expressions and positive cell expressions of IGF-1, CHAT, and SCF in the gastric tissue were significantly increased. The morphology and arrangement of the cells and the number of ICs in the gastric tissue were restored. ACVO plays a positive role in the treatment of DGP, and its mechanism may be related to increment of serum IGF-1 and up-regulation of IGF-1R in the gastric tissues, thereby up-regulating the expression of SCF, further restoring the number of ICs, and promoting gastric movement. Meanwhile, the gastric expression of CHAT was restored and gastric movement was increased. The above research provides further insight into the potential role of ACVO in the development of DGP.

Acknowledgment
This work was supported by the Postgraduate funding of Qingdao University of Science and Technology, Qingdao, China (project No. 3280-120202190565).

The results presented in this paper were part of a student thesis.

Authors’ Contributions
HL, YW, FT, and ZX Conceived the study and design; YW Analyzed the data and prepared the draft manuscript; YG and MY Critically revised the paper; YG Supervised the research; HL and YW Approved the final version to be published.

Conflicts of Interest
None of the authors has personal or financial conflicts of interest.

References
1. Bharucha AE, Kudva YC, Prichard DO. Diabetic gastroparesis. Endocr Rev 2019; 40:1318-1352.
2. Kumar M, Chapman A, Javed S, Alam U, Malik RA, Azmi S. The investigation and treatment of diabetic gastroparesis. Clin Ther 2018; 40:850-861.
3. Avalos DJ, Sarosiek I, Loganathan P, McCallum RW. Diabetic gastroparesis: Current challenges and future prospects. Clin Exp Gastroenterol 2018; 11:347-363.
4. Sullivan A, Temperley L, Ruban A. Pathophysiology, aetiology and treatment of gastroparesis. Dig Dis Sci 2020; 65:1615-1631.
5. Zhang H, Han T, Sun LN, Huang BK, Chen YF, Zheng HC, et al. Regulative effects of essential oil from Atractylodes lancea on delayed gastric emptying in stress-induced rats. Phytomedicine 2008; 15:602-611.
6. Luo XQ, Gu YH, Wu ZY. [Comparison of the effect of eight kinds of volatile oil of Chinese material medica on percutaneous absorption of ibuprofen in vitro]. Zhong Yao Cai 2007; 30:571-573.
7. Ishii T, Okuyama T, Noguchi N, Nishidono Y, Okumura T, Kaibori M, et al. Antiinflammatory constituents of Atractylodes chinensis thizome improve glomerular lesions in immunoglobulin A nephropathy model mice. J Nat Med 2020; 74:51-64.
8. Hossen MJ, Chou JY, Li SM, Fu XQ, Yin C, Guo H, et al. An ethanol extract of the rhizome of Atractylodes chinensis exerts anti-gastritis activities and inhibits Akt/NF-kappaB signaling. J Ethnopharmacol 2019; 228:18-25.
9. Shimato Y, Ota M, Asai K, Atsumi T, Tabuchi Y, Makino T. Comparison of byakujutsu (Atractylodes rhizome) and sojutsu (Atractylodes lancea rhizome) on anti-inflammatory and immunostimulative effects in vitro. J Nat Med 2018; 72:192-201.
10. Chu SS, Jiang GH, Liu ZL. Insecticidal compounds from the essential oil of Chinese medicinal herb Atractylodes chinensis. Pest Manag Sci 2011; 67:1253-1257.
11. Kim JH, Doh EJ, Lee G. Chemical differentiation of genetically identified atractylodes japonica, A. macrocephala, and A. chinensis rhizomes using high-performance liquid chromatography with chemometric analysis. Evid Based Complement Alternat Med 2018; 2018:4860371.
12. Feng W, Ao H, Yue S, Peng C. Systems pharmacology reveals the unique mechanism features of Shenzhu Capsule for treatment of ulcerative colitis in comparison with synthetic drugs. Sci Rep 2018; 8:16160.
13. Yang S, Wu B, Sun H, Sun T, Han K, Li D, et al. Impaired insulin/IGF-1 is responsible for diabetic gastroparesis by damaging myenteric cholinergic neurones and interstitial cells of Cajal. Biosci Rep 2017; 37.
14. Ren K, Yong C, Yuan H, Cao B, Zhao K, Wang J. TNF-alpha inhibits SCF, ghrelin, and substance P expressions through the NF-kappaB pathway activation in interstitial cells of Cajal. Braz J Med Biol Res 2018; 51:e7065.
15. Gligorijevic N, Robajac D, Nedic O. Enhanced platelet sensitivity to IGF-1 in patients with type 2 diabetes mellitus. Biochemistry (Mosc) 2019; 84:1213-1219.
16. Brussee V, Cunningham FA, Zochodne DW. Direct insulin signaling of neurons reverses diabetic neuropathy. Diabetes 2004; 53:1824-1830.
17. Zhuang HX, Snyder CK, Pu SF, Ishii DN. Insulin-like growth factors reverse or arrest diabetic neuropathy: effects on hyperalgesia and impaired nerve regeneration in rats. Exp Neurol 1996; 140:198-205.
18. Byrn J, Targher G. NAFLD: A multisystem disease. J Hepatol 2015; 62:S47-64.
19. Mule F, Amato A, Serio R. Gastric emptying, small intestinal transit and fecal output in dystrophic (mdx) mice. J Physiol Sci 2010; 60:75-79.
20. Improta G, Broccardo M. Effect of selective mu 1, mu 2 and delta 2 opioid receptor agonists on gastric functions in the rat. Neuropharmacology 1994; 33:977-981.
21. Brage R, Cortijo J, Esplugues J, Esplugues JV, Marti-Bonmati E, Rodriguez C. Effects of calcium channel blockers on gastric emptying and acid secretion of the rat in vivo. Br J Pharmacol 1986; 89:627-633.
22. Zhang GQ, Yang S, Li XS, Zhou DS. Expression and possible role of IGF-IR in the mouse gastric myenteric plexus and smooth muscles. Acta Histochem 2014; 116:788-794.
23. Krishnasamy S, Abell TL. Diabetic gastroparesis: Principles and current trends in management. Diabetes Ther 2018; 9:1-42.
24. Wang B, Zeng KW, Hong ZF, Ti GX, Wang LY, Lu P, et al. Banxia xieinv decoction treats diabetic gastroparesis through PLC-IP3-Ca(2+)/NO-cGMP-PKG signal pathway. Chin J Integr Med 2020; 26:833-838.
25. Zhang MH, Fang XS, Guo YJ, Jin Z. Effects of AMPK on apoptosis and energy metabolism of gastric smooth muscle cells in rats with diabetic gastroparesis. Cell Biochem Biophys 2019; 77:165-177.
26. Sanders KM, Ward SM, Koh SD. Interstitial cells: Regulators of smooth muscle function. Physiol Rev 2014; 94:859-907.
27. Chen Y, Wang H, Li H, Liu S. Long-pulse gastric electrical stimulation repairs interstitial cells of cajal and smooth muscle cells in the gastric antrum of diabetic rats. Gastroenterol Res Pract 2018; 2018:6309157.
28. Wang Y, Xu XY, Tang YR, Yang WW, Yuan YF, Ning YJ, et al. Effect of endogenous insulin-like growth factor and stem cell factor on diabetic colonic dysmotility. World J Gastroenterol 2013; 19:3324-3331.
29. Torihashi S, Yoshida H, Nishikawa S, Kunisada T, Sanders KM. Enteric neurons express Steel factor-lacZ transgene in the murine gastrointestinal tract. Brain Res 1996; 738:323-328.
30. Rich A, Miller SM, Gibbons SJ, Malyss J, Szurszewski JH, Farrugia G. Local presentation of Steel factor increases expression of c-kit immunoreactive interstitial cells of Cajal in culture. Am J Physiol Gastrointest Liver Physiol 2003; 284:G313-320.
31. Horvath VJ, Vittal H, Lorincz A, Chen H, Almeida-Porada G, Redelman D, et al. Reduced stem cell factor links smooth myopathy and loss of interstitial cells of cajal in murine diabetic gastroparesis. Gastroenterology 2006; 130:759-770.
32. Anetsberger D, Kurten S, Jabari S, Brehmer A. Morphological and immunohistochemical characterization of human intrinsic gastric neurons. Cells Tissues Organs 2018; 206:183-195.
33. Bu J, Qiao X, He Y, Liu J. Colonic electrical stimulation improves colonic transit in rotenone-induced Parkinson's disease model through affecting enteric neurons. Life Sci 2019; 231:116581.