Xiangsha Liujunzi Decoction Alleviates Symptoms in Rats With Functional Dyspepsia Through EGC-Derived NGF

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Research

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Abstract

Background: Xiangsha Liujunzi (XSLJZ) decoction is a traditional Chinese prescription that shows to be effective in functional dyspepsia (FD). However, the underlying mechanism remains to be elucidated. Nerve growth factor (NGF) secreted from enteric glial cells (EGCs) is associated with visceral hypersensitivity (VH), which has been wildly accepted as the main pathological mechanism of FD. But it is not clear whether and how EGC-derived NGF contributes to VH in FD. The present study aimed to evaluate the effects of XSLJZ decoction on FD and investigate whether XSLJZ decoction relieves VH by inhibiting EGC-derived NGF and TrkA-Akt-TRPV1 axis.

Methods: FD rat models were established using iodoacetamide and modified multiple platform method. The validation of FD model and effects of XSLJZD decoction on FD were evaluated in terms of weight, food intake, hematoxylin-eosin staining and Von Frey test. The expression levels of glial fibrillary acidic protein (GFAP), NGF and transient receptor potential vanilloid receptor 1 (TRPV1) were detected by immunohistochemistry and western blot analysis. The expression of TrkA and Akt was determined by western blot analysis. The co-localization of GFAP and NGF was observed by double-label immunofluorescence.

Results: XSLJZ decoction increased the weight, food intake and pain threshold of FD rats. The expression levels of GFAP and NGF, together with NGF-positive EGCs were increased in FD rats, but decreased following XSLJZ decoction treatment. Furthermore, XSLJZ decoction downregulated the over-expression of TrkA, Akt and TRPV1 in FD rats.

Conclusions: XSLJZ decoction can effectively improve the symptoms of FD and alleviate VH in FD, which may be mediated via EGC-derived NGF and TrkA-Akt-TRPV1 axis.

Background

Functional dyspepsia (FD) is one of the most prevalent functional gastrointestinal disorders and affects 5%~40% of the population worldwide\textsuperscript{[1]}. It is defined by Rome IV criteria as postprandial fullness, early satiety, epigastric pain, and epigastric burning in the absence of organic abnormalities\textsuperscript{[2]}. Several pathological mechanisms have been thought to underlie symptom occurrence, such as visceral hypersensitivity, disturbed gastrointestinal motility, duodenal low-grade inflammation, gut-brain axis dysregulation and intestinal microbiota imbalance\textsuperscript{[3]}. Despite extensive research, the definite pathophysiology of FD still remains unclear.

Visceral hypersensitivity (VH), which refers to increased sensation of both physiological and noxious stimuli\textsuperscript{[4]}, has been wildly accepted as the main underlying mechanism in FD \textsuperscript{[5]}. The nervous system, including central nervous system and enteric nervous system (ENS), plays active roles in the pathogenesis of VH \textsuperscript{[6]}. Enteric glial cells (EGCs) are the major component of ENS and are distributed across all layers of the gastrointestinal wall\textsuperscript{[7]}. Emerging evidence suggests that EGCs are pivotal...
regulators of gastrointestinal homeostatic functions by controlling mucosal sensation, motility, and immune responses\(^8\). EGCs can be activated upon stimuli. Reactive EGCs express the typical identification marker glial fibrillary acidic protein (GFAP) and secrete neurotrophic factors including nerve growth factor (NGF)\(^9\). NGF secreting EGCs has been proved to induce VH in rats with irritable bowel syndrome (IBS)\(^10\). However, it is not clear whether EGC-derived NGF contributes to VH in FD. Fortunately, there is evidence to suggest that the increased expression of GFAP and NGF is positively correlated with dyspeptic symptoms of epigastric pain, postprandial fullness and early satiation in FD patients\(^11\). NGF is the first discovered member of a family of neurotrophic factors. It binds primarily to the high affinity receptor tropomyosin receptor kinase A (TrkA). Binding of NGF to TrkA can further promote sensitization of transient receptor potential vanilloid receptor 1 (TRPV1) via the phosphatidylinositol 3 kinase (PI3K)/Akt pathway\(^12-14\). Research indicates that the expression of NGF and TRPV1 is increased in the gastric mucosa of FD patients, which is associated with VH\(^15\). Thus, we hypothesized that NGF is derived from EGCs and mediates VH in FD via its downstream TrkA-Akt-TRPV1 axis.

Xiangsha Liujunzi (XSLJZ) decoction is a classical herbal formula that has been widely used for the treatment of FD. It is composed of the following eight herbs: *codonopsis pilosula, rhizoma atractylodis macrocephalae, poria cocos, liquorice, pinellia ternate, tangerine peel, costustoot, fructus amomi*. Previous studies show that XSLJZ decoction significantly improves the clinical symptoms of FD compared with prokinetic drugs or placebo and no adverse effects are observed\(^16,17\). Moreover, it has been demonstrated that XSLJZ decoction can alleviate VH of rats with FD\(^18\). However, the mechanism by which XSLJZ decoction relieves VH in FD remains elusive.

In this study, we established a rat model with FD and investigated whether XSLJZ decoction relieves VH via regulation of EGC-derived NGF and TrkA-Akt-TRPV1 axis.

**Methods**

**Drugs**

XSLJZ decoction was prepared by the Pharmaceutical Department of Xiyuan Hospital in accordance with good manufacturing practices, and included eight Chinese medicinal herbs (Table 1) at a mass ratio of 6:12:12:4:6:5:4:5. All herbs were purchased from qualified suppliers in China and identified as eligible medicinal material. The pure extracts of the components were prepared. The components were dissolved in sterile water. High-dose (11.2g/kg), middle-dose (5.6g/kg) and low-dose (2.8g/kg) of XSLJZ decoction were administered to the rats. Mosapride (1.6mg/kg Lunambett Pharmaceuticals Co. Ltd., China) was administered to rats in the Mosapride group. Meanwhile, the rats in the model and control group received 1mL/100g sterile water daily by oral gavage for 14 consecutive days.

**Table 1** Components of the XSLJZ decoction
| English name               | Chinese name | Latin name                                      | Weight (g) | Part used |
|---------------------------|--------------|-------------------------------------------------|------------|-----------|
| Codonopsis pilosula       | Dang Shen    | Codonopsis pilosula (Franch.) Nannf.            | 6          | Roots     |
| Rhizoma atractyloides     | Bai zhu      | Atractylodes macrocephala Koidz.               | 12         | Rhizome   |
| Poria cocos               | Fu Ling      | Poria cocos (Schw. ) Wolf.                     | 12         | Sclerotia |
| Liquorice                 | Gan Cao      | Glycyrrhiza uralensis Fisch.                   | 4          | Roots     |
| Pinellia ternata          | Ban xia      | Pinellia ternata (Thunb.) Breit.              | 6          | Tuber     |
| Tangerine Peel            | Chen Pi      | Citri Reticulatae Pericarpium                 | 5          | Pericarp  |
| Costustoot                | Mu Xiang     | Radix Aucklandiae                              | 4          | Roots     |
| Fructus Amomi             | Sha Ren      | Amomum villosum                                | 5          | Fruit     |

**Animals**

Healthy seven-day-old male Sprague-Dawley rats (12-15g) were purchased from SPF Biotechnology Co., Ltd., Beijing, China. The rats were housed in cages maintained on a 12-h light/dark cycle with the room temperature of 22-24°C and a humidity of 60%-70%. Animal procedures were approved by the Committee on Animal Care and Use of the Institute of Xiyuan Hospital, China Academy of Chinese Medical Sciences, and conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publications No. 85-23, revised 1996).

**Generation of functional dyspepsia model**

The FD model was established by neonatal gastric irritation combined with modified multiple platform method (MMPM). Previous studies revealed that the model rats exhibited delayed gastric emptying and VH, which are considered to be the major pathophysiological features of FD\textsuperscript{[19,20]}. As shown in Fig. 1, ten-day old rat pups were randomly divided into 2 groups, including the IA-treated group (n = 40) and control group (n=8). The IA-treated group received 0.2mL of 0.1% iodoacetamide (IA) in 2% sucrose by oral gavage daily for 6 days. The control group received 0.2 ml of 2% sucrose only\textsuperscript{[21]}. All the rats were fed normally until 42 days old when IA-treated rats were randomly divided into five groups (n = 8, each group): model group, mosapride group, low-dose XSLJZ group, middle-dose XSLJZ group, and high-dose XSLJZ group. Then, the 42-day-old IA-treated rats were exposed to MMPM. In brief, the rats were placed on top of the platforms (6.5cm in diameter) inside a water tank. The tanks were filled with water at room temperature until 1 cm of the platform. Exposure to the MMPM began at 17:00, with a duration of 14 hours per day for 14 days. After modeling, rats received 1ml/100g of each drug or sterile water daily...
by oral gavage for 14 days. At the end of treatment, rats were anesthetized with 1% pentobarbital sodium, and the gastric antrum was excised for the following experiments.

**Body weight and food intake**

The body weight and food intake of rats were detected before and after the drug treatment. After 12 h of fasting, the rats were housed individually. The food was provided for 24 h, and the food consumption was calculated.

**Von Frey Test**

The pain threshold in response to Von Frey filament stimulation was measured, as described previously\[22, 23\]. After acclimation for 30 minutes prior to testing, each rat was placed in a chamber on a platform with 10mm grids of iron wires. A series of Von Frey filaments were applied to the central part of the plantar surface of right hind-paw with the force in ascending gram (1 g, 1.4 g, 2 g, 4 g, 6 g, 8 g, 10 g, 15 g, and 26 g). Each stimulus was repeated 3 times following a 5-min interval. The mechanical pain threshold (MPT) was defined as the lowest force in grams required until the rat withdrew its hind-paw. The mean was calculated for each rat's pain threshold. The lower the value, the higher the sensitivity of rats to mechanical stimulation.

**Hematoxylin-eosin (HE) staining**

Gastric antrum tissues were embedded in paraffin after fixation in 10% formalin and cut into 4μm-thick sections. Then, the sections were stained with HE and photographed by a microscope.

**Immunohistochemistry (IHC)**

Embedded gastric antrum samples were deparaffinized, and antigen retrieved was performed. The sections were treated with 3% H₂O₂ for 30 min, followed by 5% bull serum albumin (BSA) for 20 min. Then, the sections were incubated overnight at 4°C with the following antibodies: polyclonal rabbit anti-NGF (1:300, Abcam), polyclonal rabbit anti-GFAP(1:200, Proteintech), polyclonal rabbit anti-VR1 (1:100, Abcam). After washing, the sections were incubated with Goat Anti-Rabbit horseradish peroxidase (HRP)-conjugated secondary antibody for 50 min. Diaminobenzidine (DAB) was employed to detect the immuno-complex, and hematoxylin was used for nuclear counterstaining. After dehydration through an ascending alcohol gradient and clarification with xylene, the sections were visualised under a microscope, and images were acquired using a LEICA camera. At least 5 fields per rat and 5-6 rats per group were analysed. Mean integrated optical density (MOD) was calculated using Image Pro Plus version 6.0 analysis software.

**Immunofluorescence (IF)**

Embedded gastric antrum samples were deparaffinized, antigen retrieved was performed, and the sections were blocked. The sections were incubated overnight at 4°C with polyclonal rabbit anti-NGF
(1:300, Abcam) and polyclonal rabbit anti-GFAP (1:200, Proteintech). Following three 5-min washing, the sections were then incubated with a FITC-labeled goat anti-rabbit antibody and a Cyanine3-labeled goat anti-rabbit secondary antibody (1:300, Abcam) at room temperature for 60 min. The nuclei were stained with DAPI (Genepool). Images were captured at 400× under a fluorescent microscope (Olympus, Japan).

**Western blot analysis**

Gastric antrum tissues were homogenized in ice-cold RIPA buffer containing 1 mM PMSF (Servicebio, China), and protein concentration was quantified using a BCA protein assay kit (Servicebio, China). Equal amount of total proteins (50 μg) was loaded on 10% SDS-PAGE gels, and then transferred to PVDF membranes (PALL, USA). The PVDF membranes were blocked with 1×Tris-buffered saline Tween (TBST) containing 5% non-fat milk at room temperature for 1h. Membranes were incubated overnight at 4°C with the following primary antibodies: anti-GFAP (1:1900, Proteintech), anti-NGF (1:1800, Abcam), anti-TrkA (1:1200, Abcam), anti-Akt (1:2000, Proteintech), anti-VR1 (1:1700, Abcam) and anti-β-actin (1:1500, Abcam). After washing three times with TBST, the membranes were incubated with the following secondary antibodies: Goat anti-rabbit IgG (1:2800, Abcam) or goat anti-mouse IgG (1:3200, Abcam) at room temperature for 2h. The protein bands were visualized using enhanced chemiluminescence substrate (Pierce, USA) and the protein levels were quantified with Image J software.

**Statistical analysis**

SPSS 19.0 software was used for all statistical analyses. All values were presented as mean ± SD. One way analysis of variance (ANOVA) was used to test the statistical significance among the groups. p < 0.05 was considered statistically significant.

**Results**

**HE staining of the gastric antrum**

HE staining was performed on the gastric antrum tissues of rats for histological evaluation. As shown in Fig. 2, the mucosa of gastric antrum in each group was smooth and intact without any sign of inflammation or deep damage. No obvious changes were observed in the gastric histology among the groups.

**XSLJZ decoction ameliorates the symptoms of the rats with FD**

To investigate the effects of XSLJZ decoction on FD, we detected the body weight (Fig. 3a), food intake (Fig. 3b) and VH of rats in all groups. Before treatment, the rats in the model group exhibited a significant decrease in body weight and food intake compared with the control group (p<0.01). After treatment for 14 days, the rats in the XSLJZ groups and mosapride group gained more weight than those...
in the model group (p < 0.05, p< 0.01). The food consumption of rats in the XSLJZ groups and mosapride group were increased as well (p<0.01), implying that the appetite of FD rats was promoted.

Previous studies found out that a threshold to painful somatic stimulus in FD patients was lower than in normal subjects, and this phenomenon was attributed to central sensitization mechanisms\cite{14, 24}. Thus in the present study, Von Frey test was performed to evaluate VH by exploring somatic sensation. As shown in Fig. 3c, the MPT from Von Frey filaments in the model group was significantly lower than that in the control group (p < 0.01). While MPT was increased in the XSLJZ groups and mosapride group by varying degrees compared with the model group. These findings indicated that the rat model with FD was established successfully and that XSLJZ decoction could regulate the disorder state.

**XSLJZ decoction inhibits EGCs activation in FD rats**

GFAP is considered as a specific marker of EGCs activation\cite{25}. We thus examined the expression of GFAP in gastric antrum to assess the influence of XSLJZ decoction on EGCs regulation. IHC revealed that GFAP was positively stained as brownish yellow and mainly expressed in the lamina propria of gastric mucosa (Fig. 4a). The expression of GFAP in the model group was higher than that in the control group (p < 0.01), while XSLJZ decoction and mosapride significantly decreased GFAP expression of rats with FD (Fig. 4b). We also detected the expression levels of GFAP using western blot (Fig. 4c, Fig. 4d), and the expression trend was similar to the IHC results.

**XSLJZ decoction reduces the expression of EGC-derived NGF in FD rats**

Given that activated EGCs acts as a major source of NGF, and NGF plays a significant role in modulating gastrichypersensitivity\cite{9, 12}. We hypothesized that EGCs contribute to visceral sensitivity in FD rats by secreting NGF. We addressed this question by examining the protein expression of NGF using IHC (Fig. 5a, Fig. 5b) and western blot (Fig. 5c, Fig. 5d),as well as the colocalization of NGF and GFAP using double-label IF (Fig. 5e).

IHC indicated that NGF was expressed in the cytoplasm of lamina propria. The expression of NGF was higher in the gastric mucosa of rats with FD compared with those of the control rats (p < 0.05). different-dose XSLJZ decoction and mosapride reduced NGF expression in FD rat. Western blot analysis showed that the expression of NGF was increased significantly in the FD group than those in the control group. By contrast, the middle-dose and high-dose XSLJZ groups exhibited significantly decreased expression of NGF compared with the model group (p < 0.05). There was no significant difference among the model group, low-dose XSLJZ group and mosapride group.

We next examined whether NGF colocalized with EGCs. double-label IF showed that NGF and GFAP co-expressed in the lamina propria of gastric mucosa, and NGF was observed in GFAP-positive cells. The fluorescence intensities of NGF and GFAP in the FD group were higher than those in the control group, which made the co-expression of NGF and GFAP more distinct.
XSLJZ decoction inhibits TrkA-Akt-TRPV1 axis in FD rats

TRPV1 as a hallmark of VH, is activated by NGF\cite{26, 27}, and the fact that NGF exerts the function through its receptor TrkA and downstream PI3K/Akt pathway has been recently revealed\cite{13}. Thus the potential effects of XSLJZ decoction on TrkA-Akt-TRPV1 axis in FD rats were assessed.

Western blot analysis showed that the expressions of TrkA, Akt and TRPV1 were upregulated significantly in the model group compared with those in the control group. The levels of these proteins were downregulated after the treatment of FD rats with different-dose XSLJZ decoction. However, these protein levels showed no significant differences between the model group and mosapride group (Fig. 6a, Fig. 6b). We also examined the expression of TRPV1 in gastric mucosa by IHC (Fig. 6c). The TRPV1 level in the gastric mucosa of FD rats was consistent with the western blot result (p < 0.05). While, TRPV1 expression was decreased in the middle-dose XSLJZ group compared with the rats with FD (p < 0.05). No significant difference was observed among the model group, low-dose XSLJZD group, high-dose XSLJZD group and mosapride group.

Discussion

FD is a common gastrointestinal disorder associated with multiple factors. VH has been believed to have an important role in the pathogenesis of FD\cite{28}. The present study confirmed the effects of XSLJZ decoction on relieving symptoms and demonstrated that it occurred by regulating EGC-derived NGF and its downstream TrkA-Akt-TRPV1 axis in FD rats.

We established a FD rat model through neonatal gastric irritation and MMPM, which has been verified in our previous study\cite{19}. All model rats displayed characteristic features of decreased body weight, poor appetite, and no detectable gastric pathological changes, which was similar to that experienced by humans with FD. XSLJZ decoction could increase the body weight and food intake of FD rats.

Previous researches measured the gastric sensitivity by inserting a balloon or pressure transducer into the rat stomach via surgical intervention\cite{18, 29}. However, it is likely that surgical procedures affect the gastric physiological function, and that animal studies do not necessarily reflect human physiology. Based on this, we tried to adopt a method without the need for surgical intervention. It has been reported that patients with FGIDs exhibit lower pain tolerance to somatic stimulus in addition to VH\cite{14, 30}, which suggests that somatic and gastrointestinal afferents converge on common spinal cord neurons\cite{31}. This explanation is further supported by animal studies in which the rats treated with TNBS that displayed somatic hypersensitivity to mechanical stimuli (Von Frey) were exactly the same rats that exhibited VH to colonic distension\cite{23, 24}. Moreover, retrograde tracing studies have indicated that splanchnic nerve afferent fibers from the stomach of male SD rats enter the spinal cord T_6^-L_1, which overlap with those from the hind paw\cite{32, 33}. Thus in this study, we performed Von Frey test applied to the hind paw for evaluating somatic sensation to reflect the gastric sensitivity state. Our finding showed that
hypersensitivity from mechanical stimulation, which was evident in FD rats, could be reduced after treatment with XSLJZ decoction. It suggests that XSLJZ decoction has the potential of relieving VH.

To further affirm that XSLJZ decoction relieves VH, we examined the expression of TRPV1, which is essential for development of VH. A growing body of evidence suggests that TRPV1 antagonists attenuate VH\[^{34}\], whereas TRPV1 knock-out animals have decreased perception of noxious stimuli \[^{35}\]. Uprogulation of TRPV1 promotes calcium ion influx into sensory neurons causing their depolarization, and ultimately induces VH. Despite classically described as an inflammatory mediator, NGF is also a key factor in inducing non-inflammatory VH by upregulating TRPV1\[^{26}\]. The present study revealed that the expression levels of NGF and TRPV1 were significantly increased in the gastric mucosa of FD rats without any sign of inflammation. This finding is consistent with the previous report in FD patients\[^{15}\]. Moreover, the most important finding was that middle-dose XSLJZ decoction significantly reduced these protein levels. The results provide further evidence that XSLJZ decoction relieves VH, and that this effect of XSLJZ decoction is involved in modulating NGF-induced upregulation of TRPV1.

It is widely acknowledged that NGF induces upregulation of TRPV1 via its high affinity receptor TrkA. The mechanisms that link TrkA with TRPV1 involve multiple signaling pathways, and PI3K/Akt is a major one of them\[^{36}\]. It has been proved that the PI3K/Akt pathway plays a critical role in regulating colonic VH through mediating NGF-induced TRPV1 protein synthesis\[^{13}\]. However, It is not known whether PI3K/Akt pathway participates in VH of FD. In this study, the expression levels of TrkA and Akt were significantly upregulated in FD rats but significantly downregulated after XSLJZ decoction treatment. Therefore, it is plausible that XSLJZ decoction modulates NGF-induced upregulation of TRPV1 by inhibiting TrkA-Akt-TRPV1 axis.

Multiple cell types in the gastrointestinal tract contribute to NGF secretion\[^{37}\]. EGCs appear to be an important source of NGF and become activated by many factors, such as pro-inflammatory cytokines, bacteria, and neurotrophins\[^{38}\]. All these factors are capable of stimulating EGCs to increase NGF secretion and express its receptor TrkA\[^{9,39}\]. In the present study, a significant increase in the expression level of GFAP was observed, supporting EGCs activation in FD rats. Furthermore, NGF-positive EGCs were more evident in FD rats. This points to the possibility that the increased expression of NGF is derived from EGCs. Of note, it was also demonstrated for the first time that XSLJZ decoction suppressed the activation of EGCs and inhibited its secretion of NGF.

**Conclusions**

Our results demonstrate that reactive EGCs promotes NGF secretion, which further triggers activation of its downstream TrkA-Akt-TRPV1 axis, and ultimately contributes to VH in FD model rats. Moreover, This is the first report indicating that XSLJZ decoction improves the symptoms of FD and alleviates VH in FD by inhibiting EGC-derived NGF and TrkA-Akt-TRPV1 axis. These findings provide an insight into the pathogenic mechanism of FD and a potential therapeutic target of XSLJZ decoction that will benefit the treatment of FD.
Abbreviations

XSLJZ: Xiangsha Liujunzi; FD: Functional dyspepsia; NGF: Nerve growth factor; EGCs: Enteric glial cells; VH: Visceral hypersensitivity; MMPM: Modified multiple platform method; GFAP: Glial fibrillary acidic protein; TRPV1: Transient receptor potential vanilloid receptor 1; ENS: Enteric nervous system; TrkA: Tropomyosin receptor kinase A; PI3K: Phosphatidylinositol 3 kinase; IA: Iodoacetamide; MPT: Mechanical pain threshold; HE: Hematoxylin-eosin; IHC: Immunohistochemistry; MOD: Mean integrated optical density; IF: Immunofluorescence.

Declarations

Ethics approval and consent to participate

All procedures were performed according to the ethical guidelines and approved by the Ethics Committee for Animal Experimentation of Xiyuan Hospital, China Academy of Chinese Medical Sciences.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

FW and XT designed the study; JL coordinated the experiments and wrote the manuscript; LX participated in the design and data analysis of the experiment; LL provided manuscript preparation; EZ and ZZ carried out the experiments; LL, FW and XT revised the manuscript. All authors read and approved the final manuscript.

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Figures
Figure 1

Flow chart of FD model induction and treatment options.

- Oral gavage of IA
- Day 10 → Day 16
- sterile water mosapride XSLJZ
- Day 42 → MMMP → Administration
- Day 56 → Day 70
- FD model finished
- Sacrifice

| Control | Model | Mosapride |
|---------|-------|-----------|
| ![Control Image](image1) | ![Model Image](image2) | ![Mosapride Image](image3) |
| Low-dose XSLJZ | Middle-dose XSLJZ | High-dose XSLJZ |
Figure 2

Gastric morphology of rats in each group was detected by HE staining (Scale bars, 100μm).

![Figure 2]

Figure 3

Effects of XSLJZ decoction on dyspeptic symptoms in FD rats. a Body weight. b Food intake. c MPT from the Von Frey test. Data are presented as means ± SD, n=8 rats in all groups. **p < 0.01 compared with the control group; #p < 0.05, ##p < 0.01 compared with the model group.
Figure 4

The expression level of GFAP in the gastric antrum. a The expression of GFAP in gastric mucosa of each group was detected by IHC staining (Scale bars, 50μm). b Immunohistochemical expression of GFAP was showed as MOD value (n=5/6). c Western blot analysis of GFAP in the gastric antrum. d Relative grey value of GFAP protein levels in gastric tissues (n=3). **p<0.01 compared with the control group; #p < 0.05, ##p < 0.01 compared with the model group.
Figure 5

The expression level of EGC-derived NGF in the gastric antrum. a The expression of NGF in gastric mucosa of each group was detected by IHC staining (Scale bars, 50µm). b Immunohistochemical expression of NGF was showed as MOD value (n=5/6). c Western blot analysis of NGF in the gastric antrum. d Relative grey value of NGF protein levels in gastric tissues (n=3). e Co-expression of cell
nucleus (blue), GFAP (green), NGF (red) in gastric antrum (Scale bars, 25μm). *p < 0.05 compared with the control group; #p < 0.05, ##p < 0.01 compared with the model group.

Figure 6

The expression levels of TrkA, Akt, and TRPV1 in the gastric antrum. a Western blot analysis of TrkA, Akt, and TRPV1 in the gastric antrum. b Relative grey value of TrkA, Akt, and TRPV1 protein levels in gastric tissues (n=3). c The expression of TRPV1 in gastric mucosa of each group was detected by IHC staining (n=5/6, Scale bars, 50μm). *p < 0.05, **p<0.01 compared with the control group; #p < 0.05, ##p < 0.01 compared with the model group.