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Effects of Jiawei Huangqi Guizhi Decoction on the expression of GAS, MTL, GC, and TGF-β3 signaling pathways in CAG rats

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Abstract

To study the effect of Jiawei Huangqi Guizhi Decoction on gastrointestinal hormones and TGF-β3 signal pathways in rats with chronic atrophic gastritis (CAG). The model was created by methylnitrosoguanidine (MNNG) comprehensive method. After successful modeling, it was divided into three groups: model group (MC group), frolic acid group (AC group), modified Huangqi Guizhi Decoction high dose group (HH group), modified Huangqi Guizhi Decoction middle dose group (HM group), and modified Huangqi Guizhi Decoction low dose group (HL group). Gastric pathology was observed by HE staining. The levels of gastrin (GAS) and motilin (MTL) in rat serum were detected by ELISA assay. After HE staining, the histopathological sections were analyzed. Inflammatory factors infiltration and disorder of mucosal epithelial cells were found in the model group, which were improved to some extent after administration. According to the pathological chart analysis and score of rats in each group, compared with the model group, the gastric tissue damage of rats in each administration group has been improved in different degrees, among which the pathological scores of HH and HM groups were lower, and the corresponding damage degree was smaller. According to the test results of ELISA, Jiawei Huangqi Guizhi Decoction could reduce the contents of GAS, TGF-β3, and increase MTL abundance in the serum of rats, thus accelerating the apoptosis of tumor cells and inhibiting cancer. Jiawei Huangqi Guizhi Decoction played a positive role in preventing the occurrence of gastric cancer through reducing the concentrations of GAS and TGF-β3 in serum, and increasing the concentration of MLT in serum. This results provided a new treatment idea for CAG.

Keywords: Jiawei Huangqi Guizhi Decoction; GAS; MTL; GC; TGF-β3 signaling pathways; CAG rats.

Practical Application: Through rat model experiments, it can be seen that Jiawei Huangqi Guizhi Decoction can accelerate the apoptosis of tumor cells, inhibit the growth of tumor cells, and play a positive role in preventing the occurrence of gastric cancer.

1 Introduction

According to the statistics of the World Health Organization Cancer Agency in 2021, about 23.7% of the new cases of gastric cancer in the world come from China, ranking first in the world. In the annual cancer statistics in China, the new cases and death rate of gastric cancer rank among the top three of all cancers (World Health Organization, 2021). Gastric cancer, as a malignant tumor disease which is difficult to diagnose in the early stage and treat in the late stage, is disturbing the life of modern people, so it is very important to prevent and treat gastric cancer in China's social medical work. In developed countries like the United States, the medical system is relatively perfect and advanced, so that people can screen cancer earlier, and novel treatment methods can be used more quickly. According to the statistics of the American Cancer Society, the incidence of gastric cancer is decreasing year by year, and it is no longer one of the most serious cancers in its country (Islami et al., 2021).

Some studies have indicated that chronic atrophic gastritis (CAG) is the main precancerous lesion of intestinal type gastric cancer (Tang et al., 2022). Patients with CAG have an increased risk of gastric cancer, with an estimated annual risk of 0.1% per year (Zhang et al., 2020). Therefore, early detection, accurate prevention and early treatment of CAG are important ways to block the occurrence of canceration. The research group uses the theory of Chinese medicine to prevent diseases and adjust the internal environment of the body, which has a good clinical effect in the process of clinical treatment of CAG. In order to further explore the synergistic effect of Chinese medicine on the molecular network of inflammatory cancer transformation, this project studies CAG rats, and utilizes Jiawei Huangqi Guizhi Decoction for treatment. To observe the therapeutic effect, pathological changes, gastrointestinal hormone changes, immune system, and TGF-β3 signal pathway of Huangqi Guizhi Decoction on CAG, so as to evaluate the therapeutic value of Jiawei Huangqi Guizhi Decoction on CAG and provide scientific experimental basis for TCM syndrome differentiation and treatment of CAG.

The purpose of this study is to explore the preventive and ameliorative effects of Zhongjing Jingfang-Jiawei Huangqi Guizhi Decoction on CAG model rats by modeling gastric precancerous lesion rats, and to explore the preventive and therapeutic effects of Jiawei Huangqi Guizhi Decoction on gastric cancer model rats by observing the effects of Jiawei Huangqi Guizhi Decoction on TGF-β3 mRNA and protein expression during the transformation of gastric precancerous lesions in rats. The research results are as following.

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2 Materials and methods

2.1 Experimental materials

Animals: SD rats, weighing about 200-300 g, male, from the Experimental Animal Center of Guangzhou University of Traditional Chinese Medicine.

Reagents: methylnitrosoguanidine (MNNG, Guangzhou Kangming Biotechnology Co., Ltd., batch number: 20210601), ranitidine (Guangdong Hengjian Pharmaceutical Co., Ltd., H44021173), folic acid tablets (Fuzhou Haiwang Fuyao Pharmaceutical Co., Ltd., Lot number: 21042810). The primary antibodies were Rabbit polyclonal to GAPDH (ab8245; 1:1000; Abcam, Cambridge, MA, USA), anti-TGF-β (ab215715; 1:1000; Abcam).

Preparation of medicine: Astragalus and Guizhi Decoction (Astragalus, Guizhi, Codonopsis, Poria, Baishao, Atractylodes, Licorice, Ginger, Black Medicine, Chenpi, Cimicifuga, etc.) Decoction-free granules of traditional Chinese medicine (Sichuan New Green Pharmaceutical. Technology Development Co., Ltd.) were prepared with warm distilled water into a concentrate equivalent to 2 g/mL of the original medicinal materials, and put into a medicine bottle at high temperature and high pressure (gauge pressure: 0.7 kg/cm^2, temperature: 115°C, time 30 min), cooled and put into a sterilized bottle, and placed in a 4°C refrigerator for later use.

Materials and equipment: TGF-β3 kit, hematoxylin, eosin staining solution, semi-automatic rotary paraffin microtome (HM340E), automatic staining machine (Gemini AS), electronic analytical balance, Fresco 70 refrigerated centrifuge, optical microscope, etc.

2.2 Modeling and grouping

60 SD rats of SPF grade were adaptively reared for one week. 10 rats were selected for normal feeding (NC group), and the remaining 50 rats were used for modeling (Figure 1). Rats in the model group were established by MNNG: free drinking of MNNG at a concentration of 180 μg/mL, combined with 0.003 g/mL ranitidine hydrochloride by gavage, once every three days. At the same time, the rats in group NC were given the same amount of distilled water by gavage, and the other feeding conditions were the same between the two groups.

The modeling cycle was 12 weeks. Every six weeks, a rat was randomly selected from the blank group and the model group to be dissected, and the pathological HE staining was performed to observe the histomorphological changes of the gastric mucosa to test the effect of modeling. After successful modeling, the rats were divided into five groups on average according to body weight: model group (MC group), positive drug-folate group (frolic acid, AC group), high-dose Huangqi Guizhi Decoction group (HH group), middle-dose Huangqi Guizhi Decoction group (HM group), and low-dose Huangqi Guizhi Decoction group (HL group).

2.3 Method of administration

(1) The rats in NC group and model group were given 10 mL/kg/d distilled water by gavage; (2) The folic acid group (AC group) was given 5 mg/kg/d folic acid suspension solution by gavage; (3) HH group, HM group, and HL group were given 10 mL/kg crude drug 28 g/kg, 14 g/kg and 7 g/kg by gavage respectively, 5 days a week and 2 days rest.

After 6 weeks of treatment, rats in each group were fasted with water for 48 h and then anesthetized by inhaling ether, and then the rats were sacrificed.

2.4 Specimen collection

After 6 weeks of treatment, the rats in each group were fasted and watered for two days, anesthetized by inhaling ether, and

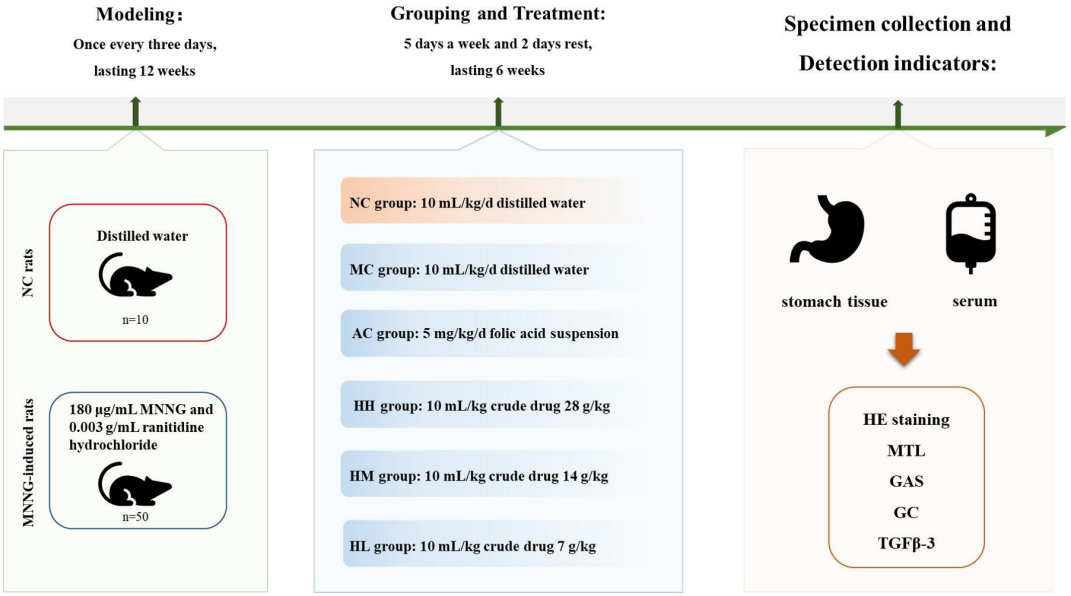

Figure 1. Experimental design.

the blood and stomach of the inferior vena cava were collected. The blood of rats in each group was centrifuged at 3500 r/min at 4°C for 15 min, and the supernatant was collected. It is used to detect the content of motilin (MTL), gastrin (GAS), glucagon (GC), and the expression of TGFβ-3. Half of the obtained rat stomach tissue was cut and fixed in formaldehyde solution, and the rest was cut into EP tubes for cryopreservation.

2.5 Detection indicators

HE staining was used to observe the pathological changes of the rat stomach, qRT-PCR and Western blot methods were used to detect the expression of TGFβ-3 mRNA and protein in the rat stomach.

2.6 Statistical methods

The data of each group were analyzed using GraphPad Prism 8.0.2 statistical software, and the specific experimental data were expressed in the form of $x \pm s$. When performing the normality test, if it conforms to the normal distribution, then perform the homogeneity of variance test. After the variance is homogenized, the LSD test in the one-way variance is used for analysis. $P < 0.05$ means the difference is statistically significant.

3 Results

3.1 Effect of Jiawei Huangqi Guizhi Decoction on gastric pathology

Analysis of the effect of Jiawei Huangqi Guizhi Decoction on the pathology of CAG rats induced by MNNG comprehensive method from the anatomical map and pathological section map. The stomach anatomy of each group of rats, as shown in Figure 2. According to the anatomical diagrams of the rats in the above groups (Figure 2), the gastric mucosa of the rats in the normal group was smooth and rosy, with a lot of mucus. However, the rats in the model group had verrucous protrusions and hyperplasia on the gastric mucosa, and the mucosa was dark and white. Compared with the rats in the model group, the rats in the FA group were improved, with more single-granule hyperplasia. The rats in the administration group (HH, HM, and HL) were significantly improved compared with the rats in the model group, with fewer hyperplasia and single-granule hyperplasia. The mucosa was ruddier and the improvement effect of the HH and HM groups was more obvious. According to the analysis of the pathological sections of the rats in each group (Figure 3), the columnar cells of the mucosal epithelium of the rats in the normal group were intact, the muscularis mucosa was arranged in an inner ring and an outer longitudinal, and the nuclei were distributed in an orderly manner. But the muscularis mucosa of the rats in the model group was inflammatory infiltration occurred and the arrangement was disordered. The epithelial columnar cell nuclei of the rats in the positive drug group were scattered and not closely arranged. Compared with the model group, the situation of sexual infiltration decreased. According to the Table 1, the pathological changes of gastric tissue of rats in each group, indicating that the injury score of the middle-dose group of Jiawei Huangqi Guizhi Decoction is the lowest.

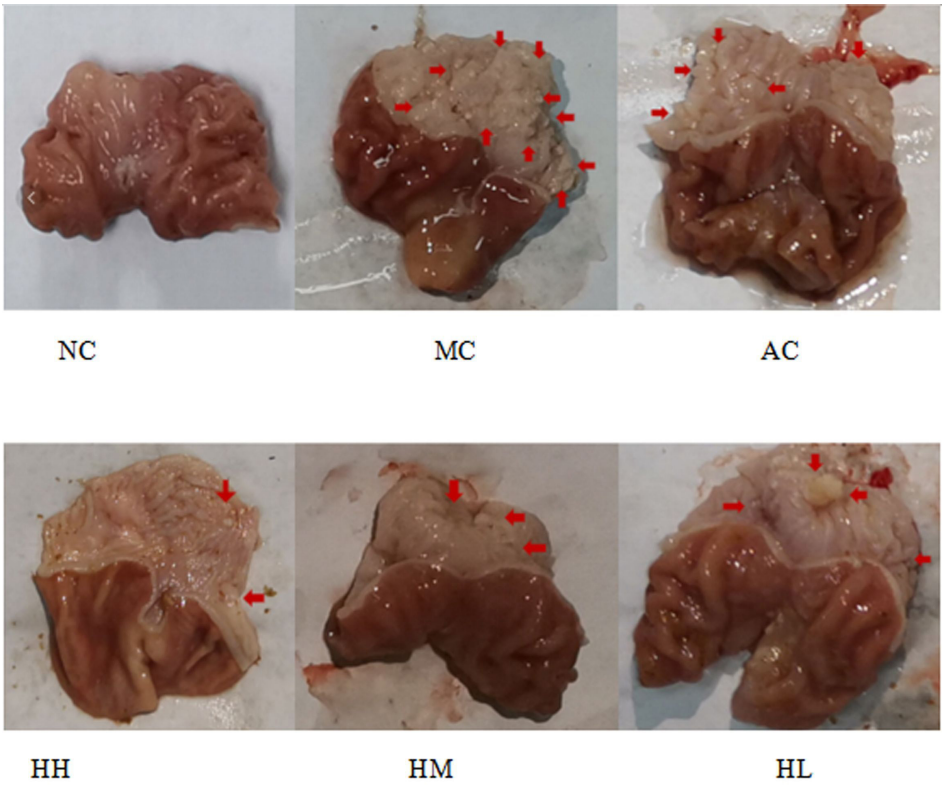

Figure 2. Effect of Jiawei Huangqi Guizhi Decoction on gastric pathology.

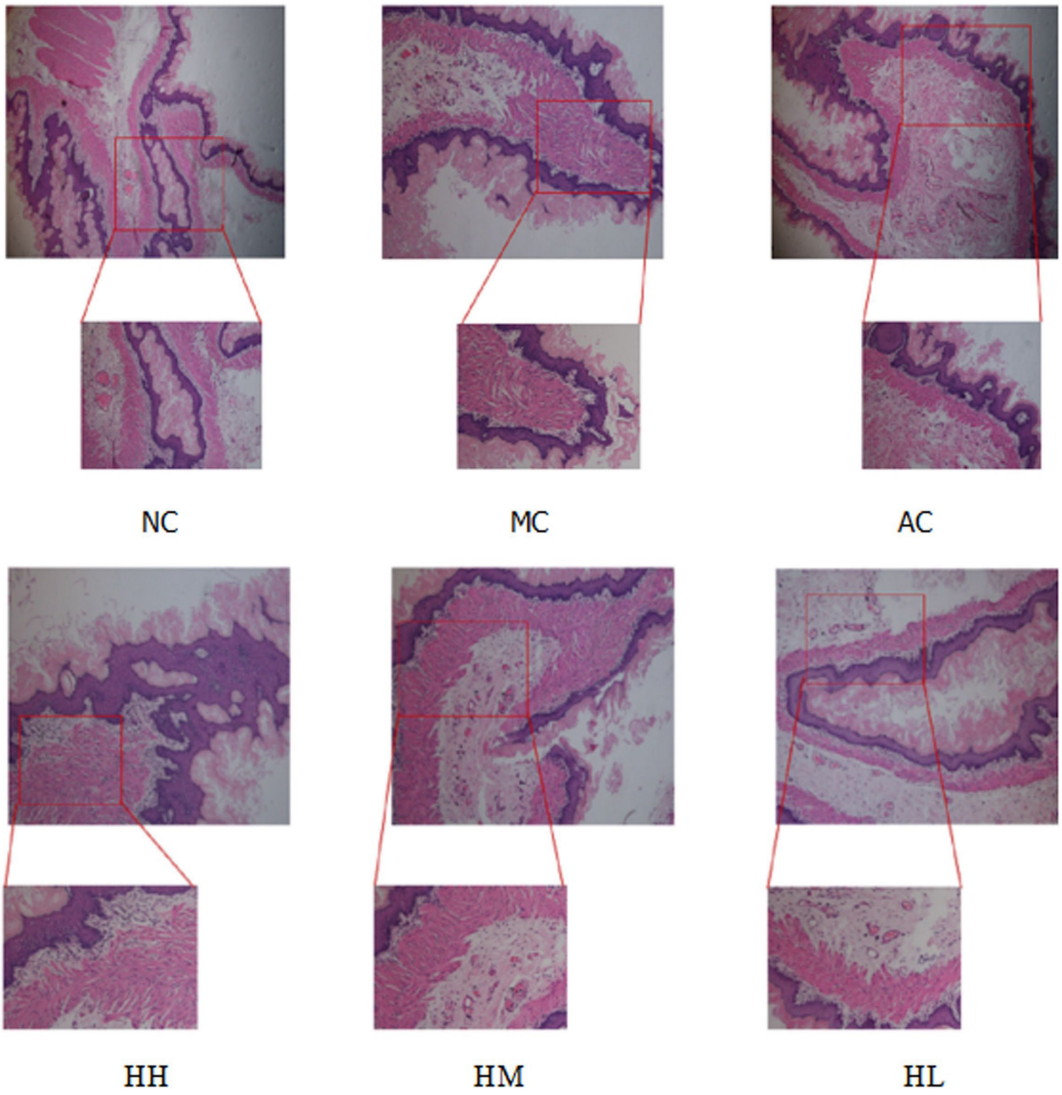

Figure 3. HE staining of stomach of rats in normal group, CAG model group, folic acid group and high, medium, and low dose groups of Jiawei Huangqi Guizhi Decoction. (Pathological sections of rats in each group (100×, 200×).

Table 1. Effects of Huangqi Guizhi Decoction on pathological changes of rat stomach tissue (x ± s, n=5).

group	Gross pathological changes	pathological changes under microscope
NC	0.0 ± 0.0	0.0 ± 0.0
MC	3.0 ± 0.5$^{\Delta\Delta}$	7.8 ± 0.7$^{\Delta\Delta}$
AC	1.6 ± 0.3*	2.8 ± 2.2*
HH	1.5 ± 0.6*	3.6 ± 1.9*
HM	1.4 ± 0.5*	2.6 ± 2.1*
HL	1.5 ± 0.5*	3.8 ± 1.7*

Note: Compared with the normal group; $^{\Delta\Delta}p<0.01$; compared with the model group; $^{*}p<0.05$.

3.2 Efficacy of Huangqi Guizhi Decoction on CAG rats induced by MNNG comprehensive method

According to the data, the content of GAS in each group of rats in Figure 4A was analyzed. Compared with the model group, the GAS content of the high-dose group, middle-dose group, and low-dose group of Huangqi Guizhi Decoction was decreased. However, the content of GAS in the folic acid group was even higher than that in the model group. Fei et al. (2006) exposed that folic acid curbed gastric carcinogenesis induced by MNNG in Wistar rats, and the repression of gastric cell proliferation may play a key role in the chemoprevention of gastric cancer by folic acid. GAS is a hormone that regulates protein synthesis in the gastric mucosa, which promotes gastrointestinal cell division and proliferation. Studies have shown that higher serum GAS levels occur in people with gastrinomas, and often accompanied by gastric mucosal hyperplasia (Schubert & Rehfeld, 2019; Waldum & Fossmark, 2018). The content of MTL in each group of rats in Figure 4B was analyzed, the high and middle dose groups of Huangqi Guizhi Decoction and folic acid all had the effect of increasing the content of MTL in the serum of rats.

MTL plays a role in promoting gastrointestinal motility, which is closely related to the transport of water and electrolytes (Kitazawa & Kaiya, 2021). The canceration of rats may lead to a decrease in MTL content, which may be the reason why the MTL content in the model group was lower than that in the normal gro up (Chen & Tsai, 2012). Jiawei Huangqi Guizhi Decoction can be used for Qi and blood deficiency syndrome, and has the effect of nourishing Qi and regulating the spleen and stomach. It may improve the pathological state of CAG rats by inhibiting the proliferation of tumor cells in the gastrointestinal tissue of rats and promoting the gastrointestinal motility of rats.

3.3 The effect of Huangqi Guizhi Decoction on serum GC in rats with CAG induced by MNNG comprehensive method

The normal adult stomach does not secrete GC. GC is the product of enteroendocrine cells, which is an embryogenic marker, and is often accompanied by intestinal metaplasia in well-differentiated intestinal-type gastric cancer. Therefore, it is believed that gastric cancers containing increased GC are mostly well-differentiated intestinal-type gastric cancers. GC also acts as a growth factor. Cancer and paracancerous tissues can produce GC through autocrine or paracrine pathways to promote tumor growth, and its receptors have also been found on the surface of gastric cancer cell membranes. It has also been found on the surface of gastric cancer cell membranes. It can be seen from Figure 4C that the GC of the model group and the modified Huangqi Guizhi decoction group showed an upward trend, indicating that the damage to the stomach caused by the modeling has reached the level of gastric cancer.

3.4 Effects of Huangqi Guizhi Decoction on TGF-β3 in serum of CAG rats induced by MNNG comprehensive method

As shown in Figure 4D, the content of TGF-β3 in the model group rats stomach tissue did not change significantly after Huangqi Guizhi Decoction treatment.

3.5 Effect of Huangqi Guizhi Decoction on TGF-β3 mRNA expression in CAG rats stomach tissue

According to the Figure 4E, the TGF-β3 mRNA expression in the model group was significantly increased by qRT-PCR detection. After administration, the mRNA expression of TGF-β3 in the positive drug group and the high, medium and low groups of Astragalus Guizhi Decoction decreased.

3.6 Effect of Huangqi Guizhi Decoction on the expression of TGF-β3 protein in CAG rats

As shown in the Figure 5, compared with the model group, the expression of TGF-β3 protein in the gastric tissue of the rats

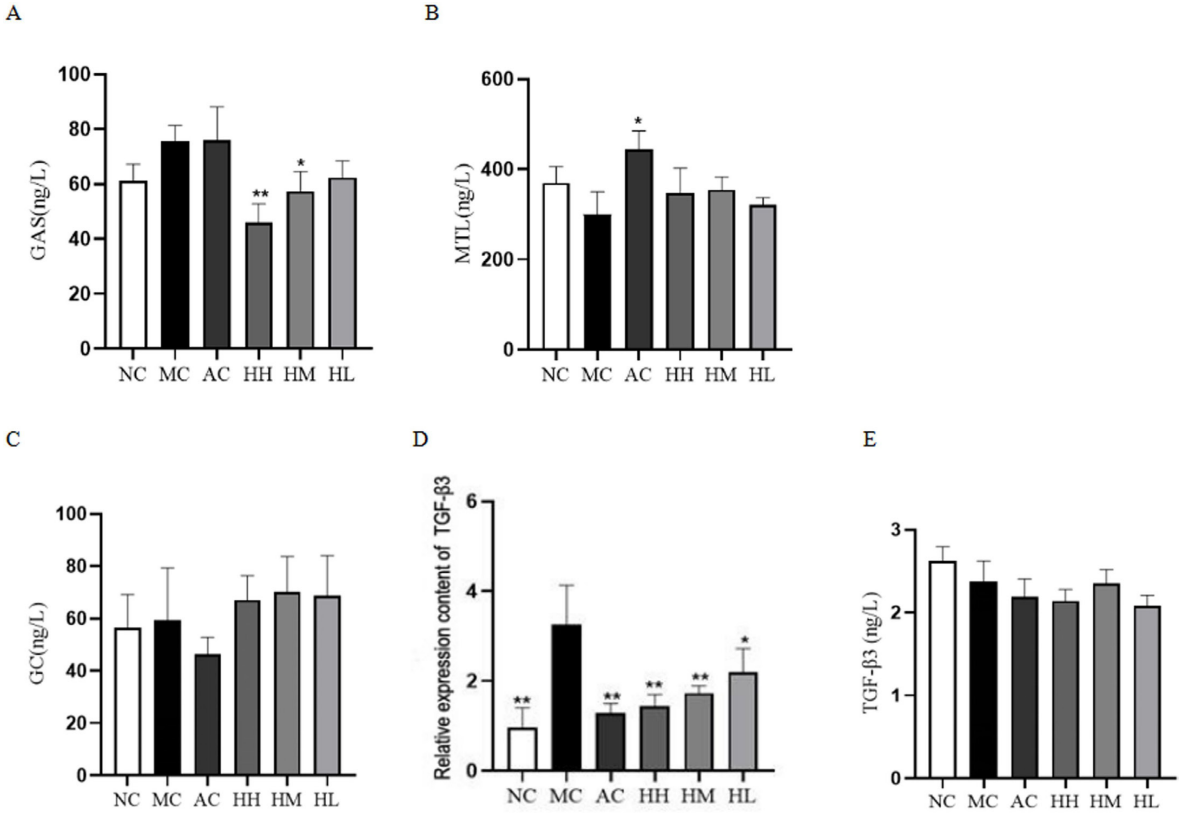

Figure 4. (A) and (B) Effects of Huangqi Guizhi Decoction on serum GAS and MTL in CAG rats induced by MNNG comprehensive method Levels of gastrin and motilin in rat serum (compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$); (C) Effects of Huangqi Guizhi Decoction on serum GC in CAG rats induced by MNNG comprehensive method Level of glucagon in rat serum (compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$); (D) Levels of TGF-β3 in rat serum. Expression of TGF-β mRNA in rat serum (compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$); (E) Levels of TGF-β3 in rat stomach tissue. Expression of TGF-β mRNA in rat stomach tissue (compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$).

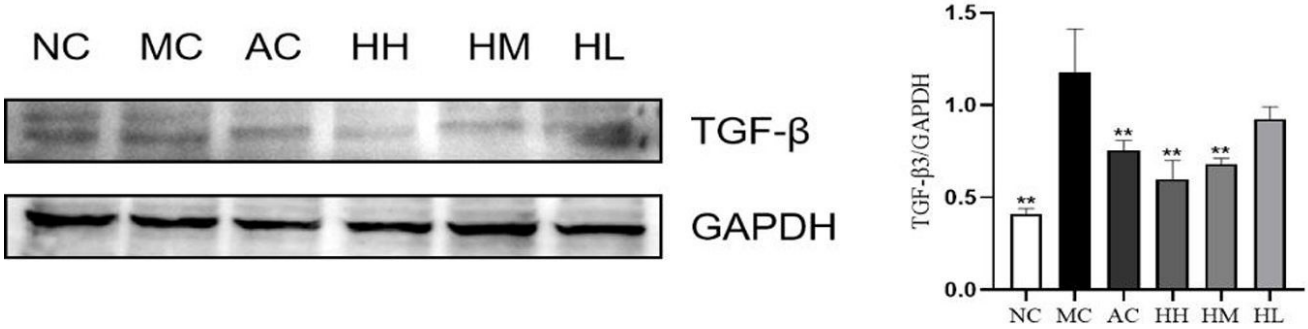

Figure 5. Effect of Huangqi Guizhi Decoction on the expression of TGF-β3 protein in CAG rats. Protein expression level of TGF-β3 in rat gastric tissue (compared with model group, $*P < 0.05$, $**P < 0.01$).

in the compound high, medium and low dose groups and the positive drug group was down-regulated ($P<0.05$).The expression level of compound high and medium dose groups was significantly down-regulated, and TGF-β3 played a promoting role in the occurrence and development of rat gastric tissue ($P <0.01$).

4 Discussion

It has been reported in the literature that *Helicobacter pylori* causes gastric cancer, which is more likely to be related to hypergastrinemia (Waldum & Fossmark, 2021). In addition, some other related influencing factors include people's smoking, alcoholism, heavy oil and heavy salt eating habits in daily life, as well as the expression of genetic mutations (Li et al., 2022b). Traditional Chinese medicine believes that the occurrence of gastric cancer is the result of the combined action of multiple factors, and the deficiency of righteousness and the disorder of the viscera are the internal causes of the disease. In Western medicine, gastric cancer is the result of the joint regulation of proto-cancer, tumor suppressor genes, and some growth factors. The balance between proliferation and apoptosis of gastric mucosa is destroyed, oncogenes are activated, tumor suppressor factors are inhibited, and gastric mucosal epithelium is destroyed. Excessive cell proliferation without initiating apoptosis may lead to gastric cancer (Guan et al., 2020).

The pathogenesis of CAG is still unclear in modern medicine, and its treatment methods mainly include drug therapy and surgery. Drug treatment is mainly to enhance gastrointestinal motility, promote gastric emptying, reduce gastric acid secretion, protect gastric mucosa, and use antibiotics to treat Hp-positive CAG. Surgical treatment mainly includes endoscopic minimally invasive treatment and general surgery (Zhou et al., 2018). Drug therapy mainly includes eradication of *H. pylori* therapy, acid suppression therapy, protection of gastric mucosa, promotion of gastric motility and folic acid therapy, etc., which are usually used as routine treatments (Wang et al., 2019).

In modern clinical practice, traditional Chinese medicine prescriptions are often chosen by physicians as adjuvant therapy drugs for cancer. By adding and subtracting compatible prescriptions, physicians can stimulate the body's immunity and at the same time play a role in stabilizing hormone levels in the human body. And compatibility can reduce drug toxicity, reduce side effects, and at the same time play a role in conditioning the whole body (Song et al., 2019). Its main function is to reduce the inflammatory response, delay the progress of inflammation, reduce the tumor and balance the levels of various hormones in the body. Among the traditional Chinese medicine compounds, such as Sijunzi Decoction, Shenling Baizhu Powder, etc., can improve the immunity of patients and improve their gastrointestinal function (Li et al., 2016, 2020). The TGF-β family is involved in the regulation of cell growth, differentiation, apoptosis and other processes (Mou et al., 2014; Yi et al., 2015). In terms of biological functions, the low expression of TGF-β may be related to factors such as the involvement of traditional Chinese medicine in tissue repair and the preparation of cellular inflammatory responses; Fibroblasts or other mesenchymal-derived cells may have stimulatory effects that promote tumor mesenchyme generation (Li et al., 2022a).

The original recipe of Astragalus Guizhi Decoction comes from Zhang Zhongjing's "Golden Chamber Synopsis". Hu Xiaohang and others used Huangqi Guizhi Decoction combined with western medicine to treat peptic ulcer and found that Huangqi Guizhi Decoction combined with western medicine treatment scheme can optimize the efficacy of gastroscope and symptoms, reduce the incidence of adverse reactions in patients, and improve the safety of treatment (Hu, 2015). In clinical treatment of patients with CAG, it has achieved good clinical effects, and comprehensively adjusts the balance of yin and yang in the human body, which is better than Western medicines that simply improve gastric motility.

Therefore, the prevention and treatment of CAG and the development of gastric precancerous lesions are important issues. With the advantages of traditional Chinese medicine culture in our country, we explored the improvement effect of Huangqi Guizhi Decoction on CAG model rats through animal experiments. The results showed that the high-dose and middle-dose groups of Huangqi Guizhi Decoction had better improvement effect on CAG rats. Figure 2 shown that a large number of hyperplasia and verrucous protrusions appear on the gastric mucosa of gastric cancer model rats, and the mucosa is white, dull, and slightly eroded. After treatment, the hyperplasia on the gastric mucosa of the rats in the high and middle dose groups of Huangqi Guizhi Decoction was significantly reduced, the protrusions were mostly single, and the mucosa was also rosier than the model group; while the positive drug-folic acid

group and Astragalus Guizhi. The treatment effect of the low-dose decoction group was slightly worse, with more hyperplasia on the mucosa.

After HE staining, the histopathological sections were analyzed, and the rats in the model group had infiltration of inflammatory factors and disordered arrangement of mucosal epithelial cells, which were improved to a certain extent after administration. According to the analysis and scoring of the pathological pictures of the rats in each group, the gastric tissue damage of the rats in each administration group was improved to varying degrees compared with the model group. After the application of traditional Chinese medicine intervention, the expression of TGF-β3 was decreased, which may be related to tissue repair and inflammatory response of simulated cells. According to the test results of ELISA assay, Huangqi Guizhi Decoction can reduce the levels of GAS and TGF-β3 in serum of rats, increase the content of MTL, accelerate the apoptosis of tumor cells, and thus play a role in suppressing tumors.

5 Conclusion

Modified Astragalus Guizhi Decoction can effectively prevent the occurrence and progression of gastric cancer, can reduce the concentration of GAS and TGF-β3 in serum, and increase the concentration of MLT in serum, and has the effect of treating gastric cancer.The formula provides some basis for the research on the prevention and treatment of gastric cancer by Chinese herbal medicine.

Ethical approval

The experimental protocol was approved by the Animal Experimentation Ethics Committee of Panyu District Traditional Chinese Medicine Hospital. Experimental animals underwent all procedures under anesthesia, and every effort was made to minimize their pain, suffering, and death.

Conflict of interest

All of the authors had no any personal, financial, commercial, or academic conflicts of interest separately.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

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Author contributions

Study conception and design: Yun An, Weigang Chen, Yong Cao, Data collection: Hongdong Duo, Yuan Fang, Boshen Chen, Data analysis and interpretation: Qiangbin Li, Fang Huang, Siyu Chen, Weihan Huang, Drafting of the article: All authors. Critical revision of the article: All authors.

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