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Shaoyao Gancao Decoction Mitigates Helicobacter Pylori-Induced Chronic Atrophic Gastritis by Suppressing MAOB

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Helicobacter pylori (*H. pylori*) plays an important role in chronic atrophic gastritis (CAG). Interestingly, Shaoyao Gancao decoction (SGD), a traditional Chinese analgesic prescription, has the efficacy of relaxing spasms and relieving pain. Here, we aimed to identify whether SGD alleviates CAG and the underlying mechanism. A CAG mouse model was developed using *H. pylori* colonization and a high-salt diet. Histological staining was used to study the histopathological damage changes, and RT-qPCR assays the production of inflammatory responses in the gastric mucosa of mice. *H. pylori* and a high-salt diet induced gastric mucosal damage and apoptosis of gastric mucosal epithelial cells in mice, eliciting a significant inflammatory response. Treatment with SGD alleviated CAG-induced gastric mucosal damage, reduced apoptosis of gastric mucosal epithelial cells, and inhibited the inflammatory response. Bioinformatics was then used to construct the pharmacological network of SGD to explore its potential targets. SGD inhibited inflammatory response in mice with CAG by suppressing the expression of MAOB. Overexpression of MAOB impaired the therapeutic effect of SGD on inflammation in mice with CAG. Collectively, our findings indicated that SGD has the potential to alleviate CAG via downregulating MAOB.

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Introduction

Atrophic gastritis refers to the loss of gastric glands in the context of chronic inflammation principally caused by *Helicobacter pylori* (*H. pylori*) infection or autoimmunity (Shah et al. 2021). Chronic gastritis is composed of atrophic or non-atrophic gastritis, and the complicated immune response induced by *H. pylori* is the major factor in its inflammation (Yao et al. 2023). Chronic atrophic gastritis (CAG) contributes to a great chance of gastric cancer, predominantly when there is extensive intestinal metaplasia, and *H. pylori* infection (active or past) can be found in approximately two-thirds of CAG patients (Lahner et al. 2018). Better diagnosis and management of patients with CAG are imperative to improve the quality of life and to relieve the social and economic burden caused by CAG (Lahner et al. 2019). TCM has exceptional advantages in the management of gastrointestinal disorders (Teschke et al. 2015; Wang et al. 2021).

TCM is characterized by a constant curative effect, high safety, less toxicity, and low price, which has great advantages in improving symptoms and can remarkably promote *H. pylori* eradication and reduce adverse reactions (Li et al. 2021c). Shaoyao Gancao decoction (SGD), derived from the "Typhoid Theory" proposed by Zhang Zhongjing, is composed of *Paeonia lactiflora Pall* and *Glycyrrhiza uralensis* Fisch, having the potential to relax spasms and relieve pain (Bi et al. 2014). Seven major ingredients of SGD involve paeoniflorin, albiflorin, liquiritin, isoliquiritin, liquiritin apioside, isoliquiritin apioside, as well as glycyrrhizic acid (Liu et al. 2019). SGD relieves visceral hyperalgesia in post-inflammatory irritable bowel syndrome (Shao et al. 2020), indicating its effects on gastrointestinal disorders. There are about 23 Paeonia species,

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2 subspecies, and 7 varieties, and Radix Paeoniae Rubra (Chinese name Chishao) has been used to treat gastritis (Li et al. 2021a). The *Glycyrrhiza* radix (licorice) represents the most traditionally applied medicinal plant in Asia to treat many disorders, such as cough, asthma, respiratory tract infections, as well as gastritis (Kim et al. 2021). It has been reported that special licorice extracts can attenuate *H. pylori*-induced gastritis and tumorigenesis due to their antioxidative and anti-inflammatory effects (Park et al. 2014). However, there are no clues about the efficacy of SGD on *H. pylori*-induced CAG. As a consequence, the main aim of this research was to dissect whether the administration of SGD could alleviate the CAG in mice infected with *H. pylori*. The network pharmacology study enabled us to dissect the potential targets of TCM, including SGD, thereby providing a basis for further drug development research (Li et al. 2020; Chen et al. 2022; Zhou et al. 2023). Therefore, we also explored the underlying mechanism of SGD using a pharmacological network and KEGG enrichment analysis. This study is beneficial to better understanding the possible mechanisms of SGD in the management of CAG.

Materials and Methods

Bioinformatics predictions

The expression profiles associated with chronic superficial gastritis and CAG were downloaded from the GSE163416 dataset to analyze the expression of genes. The HERB 2.0 database (http://herb.ac.cn/) was used to download the predicted targets of peony and licorice, the main components of SGD. In detail, the terms "peony" and "licorice" were used as the keywords, and the related gene targets in the search result were downloaded. The KEGG pathway analysis on the pharmacological targets of peony and licorice was conducted using Sangerbox Tools. The pharmacological networks of peony and licorice were established using Cytoscape 3.7.2 software.

H. pylori culture

H. pylori Sydney strain (SS1, Bio-091269, BioBW, Beijing, China) was cultured in tryptic soy agar medium spiked with 5% goat serum at 37°C under microaerobic conditions for 3 d. The strain was collected into fresh broth medium, centrifuged at 3,000 g for 5 min, and resuspended in the broth at 109 CFU/mL.

SGD preparation

SGD was prepared with *Paeonia lactiflora Pall* and *Glycyrrhiza uralensis* Fisch as the prescription at 1:1, and the quality of herbs met the Pharmacopoeia of the People's Republic of China 2020. In brief, *Paeonia lactiflora Pall* and *Glycyrrhiza uralensis* Fisch were mixed and powdered, followed by the addition of distilled water (1:10, w/v). After that, the samples were loaded into an ultrasonic-microwave combined reaction system at 600 W and a constant temperature of 80°C for 25 min. After the reaction termination, the supernatant was separated, and the residue was extracted twice by the same method. The supernatant was mixed, and the concentrate was dried at 55°C under reduced pressure to obtain the dried extract powder, which was stored at −20°C.

Animal handing

All animal studies were carried out following the protocols approved by the Animal Care and Use Committee of Heilongjiang University of Chinese Medicine. Thirty-six 5-week-old C57BL/6 male mice (Vital River, Beijing, China) were maintained in an SPF room with a 12 h:12 h light-dark cycle during which the mice were guaranteed food and drinking water *ad libitum.*

After one week, the mice were allocated to six groups: the control, CAG, CAG + phosphate-buffered saline (PBS), CAG + SGD, overexpression (OE)-negative control (NC), and OE-MAOB groups (n = 6). For CAG induction, the mice were injected intraperitoneally with 20 mg/kg pantoprazole, a proton pump inhibitor to reduce gastric acidity and promote colonization by *H. pylori*, three times a week. Next, 0.1 mL (10^8 CFU/mL) of *H. pylori* suspension was inoculated using a gastric intubation needle four times a week for 24 weeks (Park et al. 2021). The mice in the control group were administrated with equal doses of PBS. During this period, the mice were fed with AIN-76A high-salt diet containing 7.5% NaCl, and the control group was provided with a normal diet.

As regards mice in the CAG + SGD group, gavage treatment (5 g/kg) was started at week 10 once a day until week 24 (Park et al. 2021). MAOB expression was upregulated in mice by tail vein injection of adeno-associated viruses (AAV) harboring MAOB overexpression vectors (Shanghai Genechem Co., Ltd., Shanghai, China) 2 weeks before the start of the experiment as per the manufacturer's protocol. After 24 weeks, the mice were subjected to an intraperitoneal injection of 150 mg/kg of sodium pentobarbital for euthanasia, and the gastric mucosa was dissected and separated into two parts after washing in saline. One portion was stored at −80°C, and the other portion was fixed with 10% formalin overnight and paraffin-embedded.

Histopathologic injury

Paraffin-embedded sections of mouse gastric mucosa were dewaxed in xylene for 5 min. After rehydration using gradient concentration ethanol solution, the sections were stained with hematoxylin staining solution for 10 min, fractionated with 5% acetic acid for 30 s, washed with 95% ethanol, stained with eosin staining solution for 1 min, dehydrated with gradient concentration ethanol, treated with xylene, and sealed with neutral gum. The histopathological changes of mouse gastric mucosa were scored under the microscope by three gastroenterologists who were unaware of the grouping using defined histological damage indices. The histopathologic injury score is the sum of the inflammation score and the erosion score as previously described (Park et al. 2021).

Detection of apoptosis by TUNEL assay

Apoptosis was detected using the TUNEL Kit (C1091, Beyotime, Shanghai, China). Paraffin-embedded sections of gastric mucosa were dewaxed and dehydrated. After that, the sections were incubated with DNase-free Proteinase K and with 3% hydrogen peroxide solution (both for 20 min at room temperature). After being treated with biotin labeling solution for 1 h at 37°C in darkness, the sections were treated with reaction termination solution for 10 min and with Streptavidin-HRP working solution for 0.5 h (both at room temperature). The nuclei were counterstained with hematoxylin staining solution after a chromogenic reaction using DAB chromogenic solution. After gradient ethanol dehydration and xylene clearing, the sections were sealed with neutral gum. The epithelial cell apoptosis in gastric mucosal tissues was observed under a microscope. The apoptosis rate was counted.

Immunohistochemistry

After deparaffinization and rehydration, paraffin-embedded sections of gastric mucosa were heated with citrate for antigen retrieval, and 3% hydrogen peroxide was used to remove endogenous peroxidase, followed by sealing the sections with 5% goat serum for 1 h. The sections were then incubated with MAOB-specific primary antibody (1:1,000, ab259928, Abcam, Cambridge, UK) overnight at 4°C and with HRP-coupled goat anti-rabbit secondary antibody (1:2,000, ab6721, Abcam) at room temperature for 1 h. The sections were color-developed by DAB, nuclei counterstained using hematoxylin, cleared with xylene, and dehydrated using ethanol. The sections were sealed with neutral gum and then observed under a microscope, and the percentage of positive cells was calculated.

RT-qPCR

Total RNA was isolated from mouse gastric mucosa stored in a −80°C refrigerator using TRIzol reagent (Invitrogen Inc., Carlsbad, CA, USA). A spectrophotometer was used to examine RNA concentration and purity, and the reverse transcription reaction was performed using EasyScript Reverse Transcriptase [M-MLV, RNaseH-] (AE101-02, Transgene Biotech, Beijing, China). An ABI Prism 7900 using TransStart Tip Green qPCR SuperMix (AQ141-01, Transgene Biotech) was used for fluorescent qPCR reactions. Taking GAPDH as the reference, the relative expression of mRNAs was assessed using the $2^{-\Delta\Delta CT}$ method. The primer sequences are listed in Table 1.

qPCR for identifying H. Pylori in gastric mucosa

To assess whether SGD produces antimicrobial effects on *H. pylori* colonization in the gastric mucosa of mice, we collected RNA from the gastric mucosa and then performed qPCR by using *H. pylori* 16S rRNA-specific primers. The results represent the mean and error between groups through the relative quantitative calculation of the ΔCt (threshold cycle) value (Jung et al. 2020).

Western blot assays

Mouse gastric mucosa stored at a −80°C refrigerator was lysed using RIPA lysis buffer. Tissue homogenate was subjected to centrifugation at 12,000 g for 15 min at 4°C to harvest the supernatant, and the protein concentrations were quantified using Pierce BCA Protein Quantification Kit (23225, Thermo Fisher Scientific Inc., Waltham, MA, USA). After being heated at 100°C for 3 min, the protein was transferred to a PVDF membrane by SDS-PAGE. The membranes were blocked in 5% skim milk for 30 min and probed overnight at 4°C using specific primary antibodies to MAOB (1:1,000, ab259928, Abcam) and GAPDH (1:2,000, ab9485, Abcam). Goat anti-rabbit secondary antibody (1:2,000, ab6721, Abcam) coupled with HRP was applied for a 1-h incubation at room temperature. Immunoblots were visualized by ECL and quantified using Image J software.

Statistical analyses

GraphPad Prism software (GraphPad, San Diego, CA, USA) was applied to visualize the results. Data were presented as mean ± SD. The differences between the group means were calculated by an unpaired *t*-test or one-way ANOVA. Statistical significance was accepted at $p < 0.05$.

Results

H. Pylori-induced CAG mice are successfully induced

A CAG mouse model was induced using *H. pylori* SS1 and a high-salt diet. It was revealed by HE staining that the gastric mucosal epithelial glands of control mice were structurally intact and tightly arranged, and no significant inflammatory infiltration was observed. However, the CAG mice exhibited sparse mucosal epithelium, reduced glands, and

Table 1. Primers sequences of real-time PCR analyses for mRNA expression.

Gene	Forward Sequence (5′-3)	Reverse Sequence (5′-3)
IL-6	TACCACTTCACAAGTCGGAGGC	CTGCAAGTGCATCATCGTTGTTC
IL-1β	TGGACCTTCCAGGATGAGGACA	GTTCATCTCGGAGCCTGTAGTG
TNF-α	GGTGCCTATGTCTCAGCCTCTT	GCCATAGAACTGATGAGAGGGAG
MAOB	TACTTGGGACCGAGTGAAGCT	CCAAAGCAGGTGGAATGGCACT
GAPDH	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG

IL, interleukin; TNF-α, tumor necrosis factor alpha; MAOB, monoamine oxidase-B; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

inflammatory infiltration, while SGD treatment alleviated the production of gastritis in mice (Fig. 1A). TUNEL showed significant apoptosis in the gastric mucosal epithelial cells of CAG mice, and gavage treatment of SGD reduced the level of apoptosis (Fig. 1B). Measurement of inflammatory factors in gastric mucosa using RT-qPCR demonstrated that the *H. pylori* SS1 infection led to an inflammatory response in mice. SGD treatment alleviated the increased expression of inflammatory factors, and the mRNA expression of IL-6, IL-1β, and TNF-α was remarkably reduced (Fig. 1C-E). Finally, we analyzed the level of *H. pylori* colonization in the gastric tissues of mice, and the results showed a significant decrease in the level of *H. pylori* in the gastric tissues after the treatment of SGD (Fig. 1F).

Prediction of the targets of SGD

To probe the detailed targets of SGD on CAG, we predicted the genetic targets of peony and licorice, the main components of SGD, in the HERB 2.0 database. The pharmacological networks were exhibited in Fig. 2A, B. KEGG pathway analysis showed that the pharmacological targets of both peony and licorice were enriched in the TNF signaling (Fig. 2C, D), confirming their effects on the inflammatory response.

Analysis of targets related to the impact of SGD on CAG

We analyzed the differentially expressed genes in the gastric mucosa of CAG and chronic superficial gastritis in the GSE163416 database, and 3524 differentially expressed genes were obtained (Fig. 3A). We intersected all the tar-

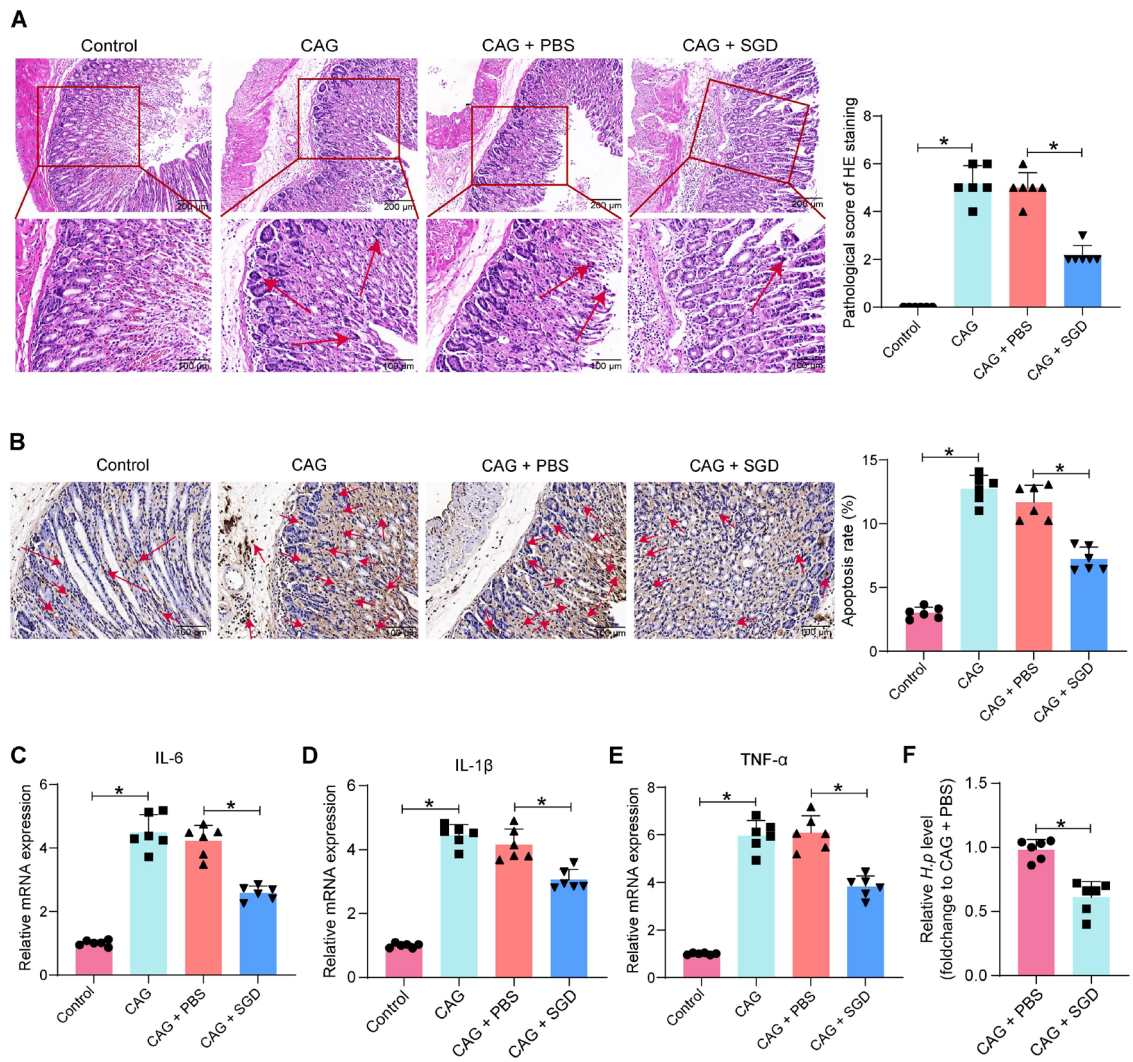

Fig. 1. *H. pylori*-induced CAG in mice.
(A) Histopathology of gastric mucosal epithelial glands in CAG mice or treated with SGD by HE staining and histopathology score. (B) The apoptosis in epithelial cells of gastric mucosa tissues of CAG mice or treated with SGD using TUNEL assay. (C) Detection of IL-6 expression in gastric mucosal tissues of CAG mice or treated with SGD by RT-qPCR. (D) Detection of IL-1β expression in gastric mucosal tissues of CAG mice or treated with SGD by RT-qPCR. (E) Detection of TNF-α expression in gastric mucosal tissues of CAG mice or treated with SGD by RT-qPCR. Data were shown as mean ± SD. (F) *H. pylori* levels in gastric mucosal tissues of CAG mice after SGD treatment were examined using qPCR. n = 6. *$p < 0.05$ versus the control or CAG + PBS group (unpaired t-test or one-way ANOVA).

gets of SGD with the differentially expressed genes in CAG, and the results showed 11 intersecting genes (Fig. 3B). Analyzing the expression of intersecting genes in the GSE163416 dataset, we plotted a heatmap in Fig. 3C. We found that MAOB was most significantly differentially expressed in CAG (p-value = 6.40E-09), and MAOB expression was significantly elevated in CAG (LogFC = 2.230795998).

We then evaluated the expression of MAOB in mice with CAG. RT-qPCR and western blot demonstrated that MAOB expression was upregulated in the gastric mucosa of CAG mice (Fig. 3D, E). Moreover, the MAOB expression was remarkably reduced in response to SGD (Fig. 3F, G).

Overexpression of MAOB reverses the effect of SGD on CAG in mice

To confirm that the alleviating effect of SGD on CAG was elicited through the inhibition of MAOB, we overexpressed MAOB in mice and treated them with SGD. RT-qPCR (Fig. 4A), western blot assays (Fig. 4B), and immunohistochemistry (Fig. 4C) on mouse gastric mucosa confirmed the successful overexpression of MAOB. HE staining showed that MAOB overexpression contributed to increased gastric mucosal injury and inflammatory infiltration in mice (Fig. 4D), and the level of apoptotic cells in the tissue was also increased (Fig. 4E). The expression of IL-6 (Fig. 4F), IL-1β (Fig. 4G), and TNF-α (Fig. 4H) was also remarkably augmented in the gastric mucosal tissues of MABO overexpressing mice.

Discussion

CAG represents a chronic gastric mucosal inflammation related to the loss of gastric glandular cells, replaced by intestinal-type epithelium and fibrous tissue (Tong et al. 2021). *H. pylori* has a high rate of infection and antibiotic resistance and poses a threat to humans, and TCM has the

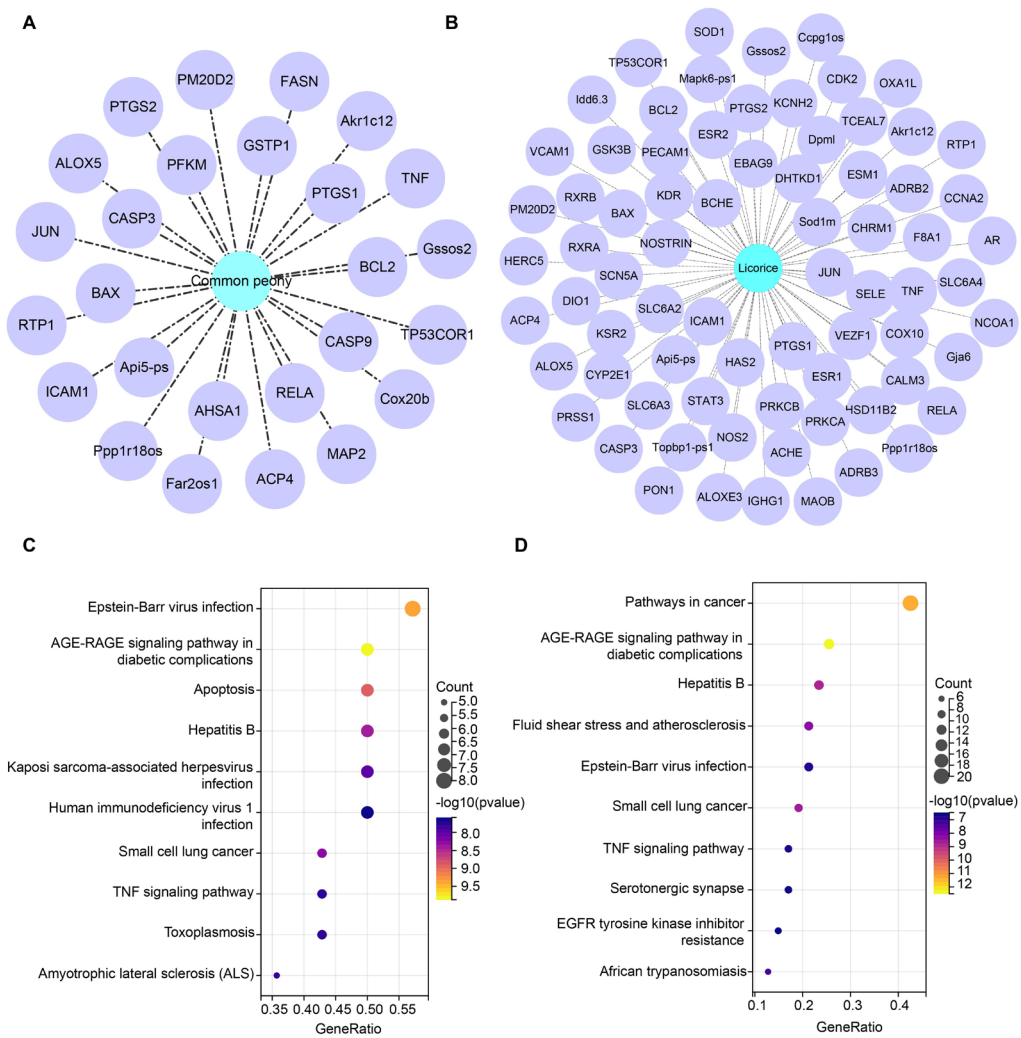

Fig. 2. Prediction of the targets of SGD.
(A) The pharmacological network of the targets of peony using HERB 2.0. (B) The pharmacological network of the targets of licorice using HERB 2.0. (C) KEGG pathway enrichment analysis of the pathways enriched by peony targets. (D) KEGG pathway enrichment analysis of the pathways enriched by licorice targets.

potential to mitigate drug resistance and facilitate the eradication of *H. pylori* (Li et al. 2021b). Even though the function of SGD has been implicated in ethanol-induced gastric ulcers in rats (Jin et al. 2022), its therapeutic effect on *H. pylori*-related diseases, particularly CAG, remains virtually unexplored. Our findings revealed that SGD can relieve CAG in mice induced by *H. pylori*, which was at least partly related to the downregulation of MAOB.

Inflammatory cell infiltration and apoptosis of gastric mucosal cells were revealed to be two prominent alterations in CAG (Zhang and Wang 2019; Kim et al. 2020). We observed consistent results in the gastric mucosa of mice induced with *H. pylori* and a high-salt diet, indicating successful modeling. The anti-inflammatory effects of SGD have been described in a substantial set of diseases, including polycystic ovary syndrome (Chang et al. 2021), cerebral ischemia-reperfusion injury (Zhang et al. 2016; Lu et al. 2022), and Parkinson's disease (Chen et al. 2021a). Meanwhile, SGD has been reported to reduce the apoptosis rate of the interstitial cells of Cajal induced by hypercholesterolemia (Zhu et al. 2021). Likewise, our *in vivo* evidence showed the anti-inflammatory (IL-6, IL-1β, and TNF-α levels) and anti-apoptotic effects of SGD on the gastric mucosa. As its name indicates, peony and licorice are the main integrates of SGD. Kwon *et al.* found that HemoHIM, an herbal preparation consisting of roots of peony, significantly alleviated gastric injury in indomethacin-induced gastric ulcers (Kwon et al. 2019). Moreover, the growth-

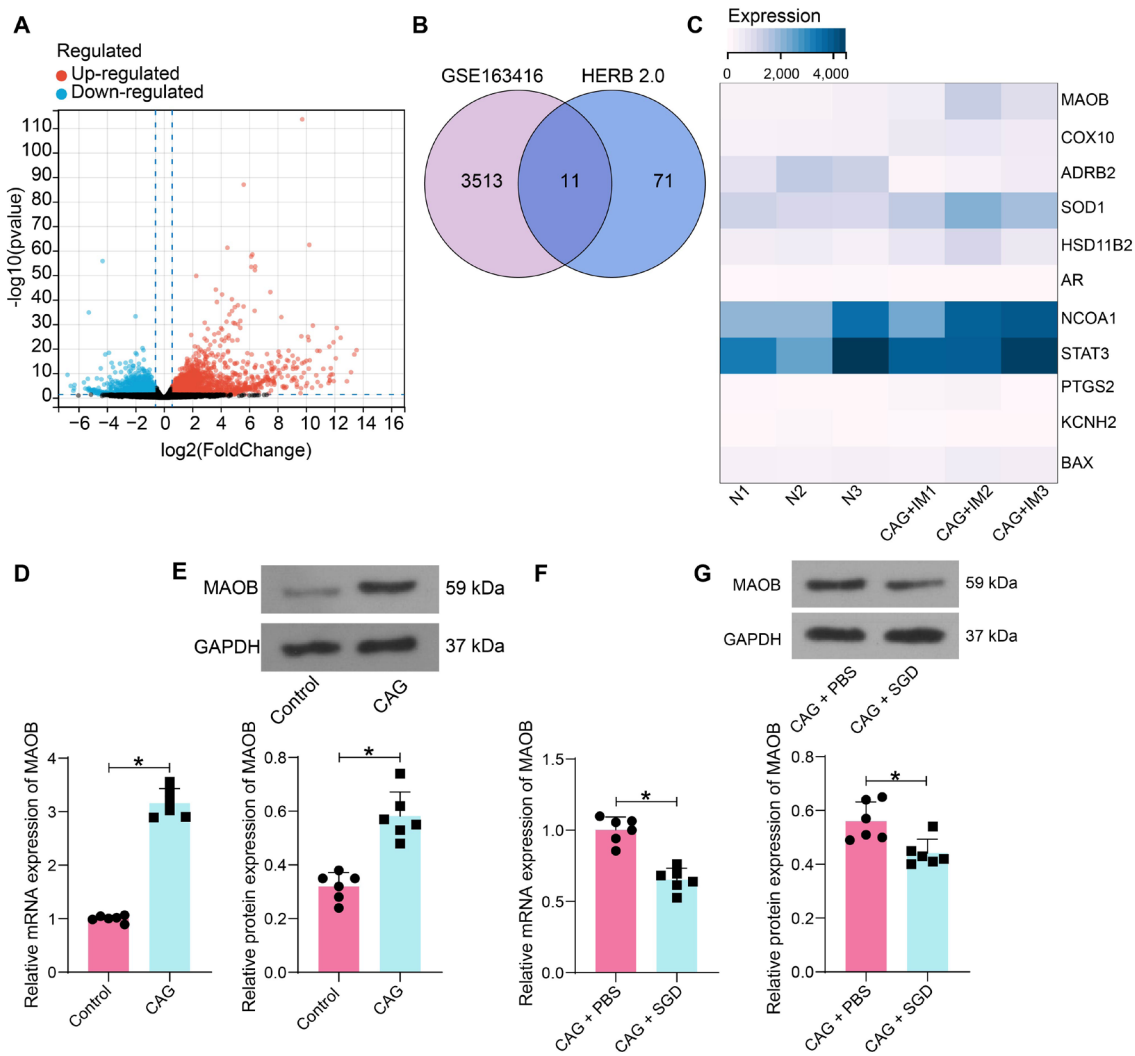

Fig. 3. MAOB is a possible target of SGD in CAG.
(A) The volcano map of differentially expressed genes in gastric mucosa of CAG and chronic superficial gastritis in GEO dataset GSE163416. (B) The intersection of differentially expressed genes in the GSE163416 dataset with the targets of SGD in HERB 2.0. (C) Heatmap showing the expression of intersecting genes in gastric mucosal tissue in the GSE163416 dataset. (D) RT-qPCR detection of MAOB expression in gastric mucosal tissues of CAG mice. (E) Western blot detection of MAOB expression in the gastric mucosa of CAG mice. (F) RT-qPCR detection of MAOB expression in gastric mucosal tissues of CAG mice treated with SGD or PBS. (G) Western blot detection of MAOB expression in the gastric mucosa of CAG mice treated with SGD or PBS. Data were shown as mean ± SD. n = 6. *$p < 0.05$ versus the control or CAG + PBS group (unpaired t test).

inhibiting and bactericidal effects of *Paeonia lactiflora* root have been verified by Ngan *et al.* (Ngan et al. 2012). Consistently, Wittschier *et al.* showed that aqueous extracts and polysaccharides from liquorice roots repressed the adhesion of *H. pylori* to the gastric mucosa (Wittschier et al. 2009). These findings were in line with our observations that SGD treatment repressed the colonization of *H. pylori* in the gastric mucosa.

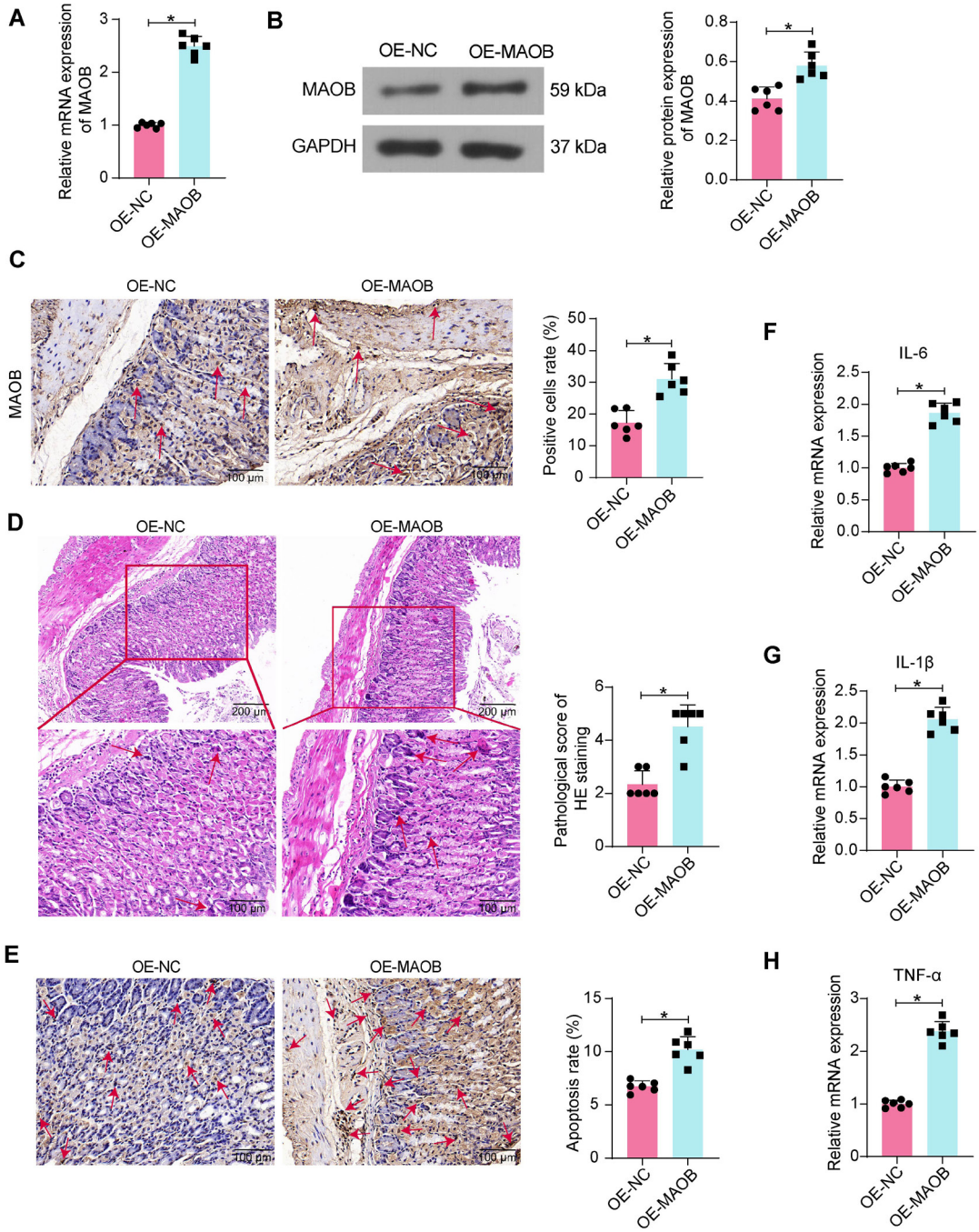

Fig. 4. Overexpression of MAOB reverses the therapeutic effect of SGD on CAG in mice. AAV containing MAOB overexpression vectors were injected into mice, followed by CAG modeling and SGD treatment.
(A) RT-qPCR detection of MAOB expression in gastric mucosal tissues of CAG mice after AAV injection. (B) Western blot detection of MAOB expression in the gastric mucosa of CAG mice after AAV injection. (C) Immunohistochemical detection of MAOB expression in the gastric mucosa of CAG mice after AAV injection. (D) Histopathology of gastric mucosal epithelial glands in mice by HE staining and histopathology score. (E) The apoptosis in epithelial cells of gastric mucosa tissues of mice using TUNEL assay. (F) Detection of IL-6 expression in gastric mucosal tissues of mice by RT-qPCR. (G) Detection of IL-1β expression in gastric mucosal tissues of mice by RT-qPCR. (H) Detection of TNF-α expression in gastric mucosal tissues of mice by RT-qPCR. Data were shown as mean ± SD. n = 6. *$p < 0.05$ versus the OE-NC group (unpaired t-test).

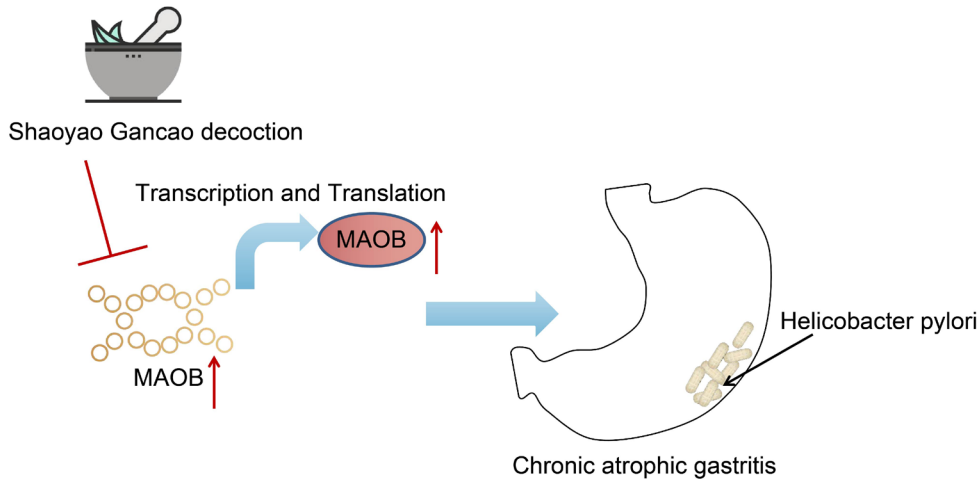

Fig. 5. Schematic presentation showing Shaoyao Gancao decoction (SGD) mitigated chronic atrophic gastritis (CAG). Inhibition of MAOB expression by SGD alleviated CAG caused by *Helicobacter pylori*.

Various methods were used to analyze the targets of SGD and to elucidate the underlying mechanism in diseases (Feng et al. 2020). We combined the network pharmacology and KEGG enrichment analysis here. The TNF signaling pathway was found to be enriched by targets of both peony and licorice, which partially corroborated the suppressing effects of SGD on the TNF-α levels. Intriguingly, monoclonal antibodies targeting TNF-α have been investigated to prevent inflammation-related gastrointestinal cancer (Lee et al. 2016). Probiotics remarkably alleviated *H. pylori*-evoked gastric pathology, including alleviated inflammatory infiltration and fewer precancerous lesions by blocking pro-inflammatory pathways, involving the TNF signaling pathway (He et al. 2022). Tsai *et al.* also found that *H. pylori*-induced sensitivity to TNF-related apoptosis-inducing ligand-mediated apoptosis in gastric epithelial cells during gastric mucosal damage (Tsai and Hsu 2017).

To further characterize the mechanism underlying the gastroprotective effects of SGD in CAG, we downloaded the data from the GSE163416 dataset to obtain differentially expressed genes and intersected them with targets of both peony and licorice. A total of 11 intersecting genes were found. MAOB whose upregulation was the most pronounced in CAG was selected. MAO with 2 isoforms, A and B, located at the outer mitochondrial membrane are flavoenzyme membranes, and MAO inhibition has emerged as a pharmacological target to mitigate oxidative stress and chronic inflammation (Sturza et al. 2019; Tripathi and Ayyannan 2019). Interestingly, in the "Paeoniflorin-targets-metabolites" interaction network, MAOB was found as one of the five key targets (Chen et al. 2021b). In the study here, we revealed that the expression of MAOB was enhanced in CAG mice and downregulated by SGD, indicating its involvement in the effects of SGD. Finally, we corroborated that the effects of SGD on CAG were achieved at least partially through the MAOB suppression using rescue experiments where MAOB overexpression overturned the anti-inflammatory properties of SGD.

As for the limitation of this study, first, only one dose of SGD has been administered to mice, and the effects of more doses are worth exploring. In addition, DUOX2 was also identified as a modifier in the preventive effects of MAO-based antidepressants on indomethacin-induced gastric lesions (Cao et al. 2020). Therefore, the deeper mechanism of MAOB in SGD needs to be fully studied. Lastly, the detailed molecular mechanisms of SGD inhibiting MAOB expression should be further explored using molecular docking.

Conclusion

In conclusion, the work here revealed that SGD plays an alleviating role in CAG mice. In addition, its mechanism is related to the manipulation of the TNF pathway and the downregulation of MAOB (Fig. 5). This study has offered fresh insights into the mechanism of SGD in the management of CAG. Nevertheless, further clinical trials are necessary to confirm our conclusions.

Conflict of Interest

The authors declare no conflict of interest.

References

Bi, X., Gong, M. & Di, L. (2014) Review on prescription compatibility of shaoyao gancao decoction and reflection on pharmacokinetic compatibility mechanism of traditional chinese medicine prescription based on in vivo drug interaction of main efficacious components. *Evid. Based Complement. Alternat. Med.*, **2014**, 208129.

Cao, L., Li, X., Xu, R., Yao, K., Yang, W., Zhu, H., Wang, G. & Zhang, J. (2020) DUOX2, a common modulator in preventive effects of monoamine-based antidepressants on water immersion restraint stress- and indomethacin- induced gastric mucosal damage. *Eur. J. Pharmacol.*, **876**, 173058.

Chang, Z.P., Deng, G.F., Shao, Y.Y., Xu, D., Zhao, Y.N., Sun, Y.F., Zhang, S.Q., Hou, R.G. & Liu, J.J. (2021) Shaoyao-Gancao Decoction Ameliorates the Inflammation State in Polycystic Ovary Syndrome Rats via Remodeling Gut Microbiota and

Suppressing the TLR4/NF-kappaB Pathway. *Front. Pharmacol.*, **12**, 670054.

Chen, C.M., Yen, C.Y., Chen, W.L., Lin, C.H., Wu, Y.R., Chang, K.H. & Lee-Chen, G.J. (2021a) Pathomechanism Characterization and Potential Therapeutics Identification for Parkinson's Disease Targeting Neuroinflammation. *Int. J. Mol. Sci.*, **22**, 1062.

Chen, L., Zhao, X., Wei, S., Ma, X., Liu, H., Li, J., Jing, M., Wang, M. & Zhao, Y. (2021b) Mechanism of Paeoniflorin on ANIT-Induced Cholestatic Liver Injury Using Integrated Metabolomics and Network Pharmacology. *Front. Pharmacol.*, **12**, 737630.

Chen, Y., Lu, R., Wang, Y. & Gan, P. (2022) Shaoyao Gancao Decoction Ameliorates Paclitaxel-Induced Peripheral Neuropathy via Suppressing TRPV1 and TLR4 Signaling Expression in Rats. *Drug Des. Devel. Ther.*, **16**, 2067-2081.

Feng, L.M., Chen, Y.Y., Xu, D.Q., Fu, R.J., Yue, S.J., Zhao, Q., Huang, Y.X., Bai, X., Wang, M., Xing, L.M., Tang, Y.P. & Duan, J.A. (2020) An integrated strategy for discovering effective components of Shaoyao Gancao decoction for treating neuropathic pain by the combination of partial least-squares regression and multi-index comprehensive method. *J. Ethnopharmacol.*, **260**, 113050.

He, C., Peng, C., Xu, X., Li, N., Ouyang, Y., Zhu, Y. & Lu, N. (2022) Probiotics mitigate Helicobacter pylori-induced gastric inflammation and premalignant lesions in INS-GAS mice with the modulation of gastrointestinal microbiota. *Helicobacter*, **27**, e12898.

Jin, Y., Zhang, M., Wang, Y., Lu, Y., Liu, T., Yang, G., Song, S. & Liu, W. (2022) Protective Effect and Potential Mechanism of the Traditional Chinese Medicine Shaoyao-Gancao Decoction on Ethanol-Induced Gastric Ulcers in Rats. *Evid. Based Complement. Alternat. Med.*, **2022**, 3069089.

Jung, D.H., Park, M.H., Kim, C.J., Lee, J.Y., Keum, C.Y., Kim, I.S., Yun, C.H., Kim, S.K., Kim, W.H. & Lee, Y.C. (2020) Effect of beta-caryophyllene from Cloves Extract on Helicobacter pylori Eradication in Mouse Model. *Nutrients*, **12**, 1000.

Kim, D.G., Lee, J., Kim, W., An, H.J., Lee, J.H., Chang, J., Kang, S.H., Song, Y.J., Jeon, Y.D. & Jin, J.S. (2021) Assessment of General Toxicity of the Glycyrrhiza New Variety Extract in Rats. *Plants* (*Basel*), **10**, 1126.

Kim, S.E., Memon, A., Kim, B.Y., Jeon, H., Lee, W.K. & Kang, S.C. (2020) Gastroprotective effect of phytoncide extract from Pinus koraiensis pinecone in Helicobacter pylori infection. *Sci. Rep.*, **10**, 9547.

Kwon, D.A., Kim, Y.S., Baek, S.H., Kim, S.K., Kim, H.K., Jo, S. K., Jung, U., Park, H.R. & Lee, H.S. (2019) Protective effects of a standardized extract (HemoHIM) using indomethacin- and ethanol/HCl-induced gastric mucosal injury models. *Pharm. Biol.*, **57**, 543-549.

Lahner, E., Carabotti, M. & Annibale, B. (2018) Treatment of Helicobacter pylori infection in atrophic gastritis. *World J. Gastroenterol.*, **24**, 2373-2380.

Lahner, E., Zagari, R.M., Zullo, A., Di Sabatino, A., Meggio, A., Cesaro, P., Lenti, M.V., Annibale, B. & Corazza, G.R. (2019) Chronic atrophic gastritis: Natural history, diagnosis and therapeutic management. A position paper by the Italian Society of Hospital Gastroenterologists and Digestive Endoscopists [AIGO], the Italian Society of Digestive Endoscopy [SIED], the Italian Society of Gastroenterology [SIGE], and the Italian Society of Internal Medicine [SIMI]. *Dig. Liver Dis.*, **51**, 1621-1632.

Lee, H.J., Park, J.M., Han, Y.M., Gil, H.K., Kim, J., Chang, J.Y., Jeong, M., Go, E.J. & Hahm, K.B. (2016) The role of chronic inflammation in the development of gastrointestinal cancers: reviewing cancer prevention with natural anti-inflammatory intervention. *Expert Rev. Gastroenterol. Hepatol.*, **10**, 129-139.

Li, L., Qiu, H., Liu, M. & Cai, Y. (2020) A Network Pharmacology-Based Study of the Molecular Mechanisms of Shaoyao-Gancao Decoction in Treating Parkinson's Disease. *Interdiscip Sci*, **12**, 131-144.

Li, P., Shen, J., Wang, Z., Liu, S., Liu, Q., Li, Y., He, C. & Xiao, P. (2021a) Genus Paeonia: A comprehensive review on traditional uses, phytochemistry, pharmacological activities, clinical application, and toxicology. *J. Ethnopharmacol.*, **269**, 113708.

Li, R.J., Dai, Y.Y., Qin, C., Huang, G.R., Qin, Y.C., Huang, Y.Y., Huang, Z.S., Luo, X.K. & Huang, Y.Q. (2021b) Application of traditional Chinese medicine in treatment of Helicobacter pylori infection. *World J Clin Cases*, **9**, 10781-10791.

Li, Y., Li, X. & Tan, Z. (2021c) An overview of traditional Chinese medicine therapy for Helicobacter pylori-related gastritis. *Helicobacter*, **26**, e12799.

Liu, M., Yuan, J., Hu, W.J., Ke, C.Q., Zhang, Y.F., Ye, Y., Zhong, D.F., Zhao, G.R., Yao, S. & Liu, J. (2019) Pretreatment with broad-spectrum antibiotics alters the pharmacokinetics of major constituents of Shaoyao-Gancao decoction in rats after oral administration. *Acta Pharmacol. Sin.*, **40**, 288-296.

Lu, J., Wang, J., Yu, L., Cui, R., Zhang, Y., Ding, H. & Yan, G. (2022) Shaoyao-Gancao Decoction Promoted Microglia M2 Polarization via the IL-13-Mediated JAK2/STAT6 Pathway to Alleviate Cerebral Ischemia-Reperfusion Injury. *Mediators Inflamm.*, **2022**, 1707122.

Ngan, L.T., Moon, J.K., Shibamoto, T. & Ahn, Y.J. (2012) Growth-inhibiting, bactericidal, and urease inhibitory effects of Paeonia lactiflora root constituents and related compounds on antibiotic-susceptible and -resistant strains of Helicobacter pylori. *J. Agric. Food Chem.*, **60**, 9062-9073.

Park, J.M., Han, Y.M., Park, Y.J. & Hahm, K.B. (2021) Dietary intake of walnut prevented Helicobacter pylori-associated gastric cancer through rejuvenation of chronic atrophic gastritis. *J. Clin. Biochem. Nutr.*, **68**, 37-50.

Park, J.M., Park, S.H., Hong, K.S., Han, Y.M., Jang, S.H., Kim, E.H. & Hahm, K.B. (2014) Special licorice extracts containing lowered glycyrrhizin and enhanced licochalcone A prevented Helicobacter pylori-initiated, salt diet-promoted gastric tumorigenesis. *Helicobacter*, **19**, 221-236.

Shah, S.C., Piazuelo, M.B., Kuipers, E.J. & Li, D. (2021) AGA Clinical Practice Update on the Diagnosis and Management of Atrophic Gastritis: Expert Review. *Gastroenterology*, **161**, 1325-1332 e1327.

Shao, Y.Y., Guo, Y.T., Gao, J.P., Liu, J.J., Chang, Z.P., Feng, X.J., Xu, D., Deng, G.F. & Hou, R.G. (2020) Shaoyao-Gancao Decoction Relieves Visceral Hyperalgesia in TNBS-Induced Postinflammatory Irritable Bowel Syndrome via Inactivating Transient Receptor Potential Vanilloid Type 1 and Reducing Serotonin Synthesis. *Evid. Based Complement. Alternat. Med.*, **2020**, 7830280.

Sturza, A., Popoiu, C.M., Ionica, M., Duicu, O.M., Olariu, S., Muntean, D.M. & Boia, E.S. (2019) Monoamine Oxidase-Related Vascular Oxidative Stress in Diseases Associated with Inflammatory Burden. *Oxid. Med. Cell. Longev.*, **2019**, 8954201.

Teschke, R., Wolff, A., Frenzel, C., Eickhoff, A. & Schulze, J. (2015) Herbal traditional Chinese medicine and its evidence base in gastrointestinal disorders. *World J. Gastroenterol.*, **21**, 4466-4490.

Tong, Y., Liu, L., Wang, R., Yang, T., Wen, J., Wei, S., Jing, M., Zou, W. & Zhao, Y. (2021) Berberine Attenuates Chronic Atrophic Gastritis Induced by MNNG and Its Potential Mechanism. *Front. Pharmacol.*, **12**, 644638.

Tripathi, R.K.P. & Ayyannan, S.R. (2019) Monoamine oxidase-B inhibitors as potential neurotherapeutic agents: An overview and update. *Med. Res. Rev.*, **39**, 1603-1706.

Tsai, H.F. & Hsu, P.N. (2017) Modulation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated

apoptosis by Helicobacter pylori in immune pathogenesis of gastric mucosal damage. *J. Microbiol. Immunol. Infect.*, **50**, 4-9.

Wang, X., Wang, Z.Y., Zheng, J.H. & Li, S. (2021) TCM network pharmacology: A new trend towards combining computational, experimental and clinical approaches. *Chin. J. Nat. Med.*, **19**, 1-11.

Wittschier, N., Faller, G. & Hensel, A. (2009) Aqueous extracts and polysaccharides from liquorice roots (Glycyrrhiza glabra L.) inhibit adhesion of Helicobacter pylori to human gastric mucosa. *J. Ethnopharmacol.*, **125**, 218-223.

Yao, B., Xu, X., Liu, W., Zhang, Q., Wang, W. & Huang, Z. (2023) The correlation of Th22 and regulatory T cells with Helicobacter pylori infection in patients with chronic gastritis. *Immun. Inflamm. Dis.*, **11**, e768.

Zhang, J. & Wang, H. (2019) Morroniside protects against chronic atrophic gastritis in rat via inhibiting inflammation and apoptosis. *Am. J. Transl. Res.*, **11**, 6016-6023.

Zhang, Y., Jia, X., Yang, J., Li, Q., Yan, G., Xu, Z. & Wang, J. (2016) Effects of Shaoyao-Gancao Decoction on Infarcted Cerebral Cortical Neurons: Suppression of the Inflammatory Response following Cerebral Ischemia-Reperfusion in a Rat Model. *Biomed. Res. Int.*, **2016**, 1859254.

Zhou, X., Min, J., Che, M., Yang, Y., Yang, Y., Zhang, J., Zhang, L., Zheng, X., Chen, Y., Yuan, L. & Nan, Y. (2023) Investigation on the mechanism of Shaoyao-Gancao Decoction in the treatment of gastric carcinoma based on network pharmacology and experimental verification. *Aging* (*Albany N. Y.*), **15**, 148-163.

Zhu, G.Y., Jia, D.D., Yang, Y., Miao, Y., Wang, C. & Wang, C.M. (2021) The Effect of Shaoyao Gancao Decoction on Sphincter of Oddi Dysfunction in Hypercholesterolemic Rabbits via Protecting the Enteric Nervous System-Interstitial Cells of Cajal-Smooth Muscle Cells Network. *J. Inflamm. Res.*, **14**, 4615-4628.