Original Article

Tibetan medicine Liuwei Muxiang pills (LWMX pills) effectively protects mice from chronic non-atrophic gastritis

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\section*{ABSTRACT}

Background: Chronic non-atrophic gastritis (CNG) is the most common type of chronic gastritis. If not actively treated, it may induce gastric cancer (GC). Western medicine is effective in CNG, but there are more adverse reactions after long-term medication, and it is easy to relapse after treatment, which affects patients’ health and life. Tibetan medicine Liuwei Muxiang Pills (LWMX pills) is a traditional Tibetan medicine compound, which has a unique curative effect in the treatment of gastric inflammation, especially chronic non-atrophic gastritis. However, the mechanisms of LWMX pills for treatment CNG still remain poor known.

Purpose: The aim of this study was to evaluate the therapeutic intervention potential of Tibetan medicine LWMX pills on CNG and explore its potential mechanisms in mice models.

Methods: The mice models was established to evaluate the therapeutic effect of LWMX pills on CNG. The main components of LWMX pills were analyzed by GC-MS. HE staining, immunohistochemistry, proteomics and Western Blot were used to analyze the potential mechanism of LWMX pills for CNG treatment.

Results: In the present study, LWMX pills containing costunolide, dehydrocostuslactone and antioxidants were found. IF results showed that the expression of ALDH1B1 in the control group was significantly lower than that in the model group in the gastric mucosa tissue, and the expression of ALDH1B1 was significantly lower in the 25 mg/ml LWMX pills group (one month) and 25 mg/ml LWMX pills group (two months) than in the model group. Western blotting showed that the protein expression levels of Furin, AMY2A, CPA3, ALDH1B1, Cam1, COXII, IL-6, IL-1β were decreased in 25 mg/mL LWMX pills. Meanwhile, we found that the CAM1 protein expression in the in 25 mg/ml LWMX pills group (two months) was increased compared to the in 25 mg/ml LWMX pills group (one months). Western blotting showed that the protein expression levels of Furin, AMY2A, CPA3, ALDH1B1, Cam1, COXII, IL-6, IL-1β were decreased in 25 mg/mL LWMX pills. Meanwhile, that the CAM1 protein expression in the in 25 mg/ml LWMX pills group (two months) was increased compared to the in 25 mg/ml LWMX pills group (one months).

Conclusion: 25mg/ml LWMX pill treatment for one month had better therapeutic effect on mice CNG. Further proteomic results showed that LWMX pills maintain gastric function by inhibiting inflammation and oxidative reactions after long-term medication, and it is easy to relapse after treatment, which affects patients’ health and life.

\section*{Abbreviations:}
LWMX, pills Liuwei Muxiang Pills; CNG, Chronic non-atrophic gastritis; GC, gastric cancer; HP, Helicobacter pylori; TTM, Traditional Tibetan medicine; IF, Immunofluorescence; IHC, Immunohistochemical; HE staining, hematoxylin-eosin staining; KEGG, Kyoto Encyclopedia of Genes and Genomes; GC-MS, Gas Chromatography-Mass Spectrometer.

\section*{Contributions:}
(I) Conception and design: R Dhondrup; (II) Administrative support: G Samdrup; (III) Provision of study materials: D Lobsang, Q Hua; (IV) Collection and assembly of data: D Geri, D C Suonan, X Feng; (V) Data analysis and interpretation: X Zhang, T Tawni, G Fan; (VI) Manuscript writing: all authors; (VII) Final approval of manuscript: all authors.

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Introduction

Chronic gastritis (CG) is a very common digestive system disease in clinical. It can be divided into three types: non-atrophic gastritis (CNG), chronic atrophic gastritis (CAG), and other special types (Rugge and Genta, 2005). CNG is characterized by chronic inflammatory cellular infiltration of the gastric mucosa, mainly plasma cells and lymphocytes, without atrophic changes of the gastric mucosa (Du et al., 2014). Further evidence suggested that without effective treatment, CAG may develop into CAG, which is an important precursor lesion in the development of gastric cancer (Ohata et al., 2004). CNG is mainly caused by Helicobacter pylori (HP) infection, and the main treatment for CNG is HP eradication (Yue et al., 2021). Western medicine mainly aims to eradicate H pylori by antibiotics, but due to the widespread use of antibiotics, many adverse effects such as antibiotic resistance and gastrointestinal side effects have emerged (Wang et al., 2022). HP eradication rate did not achieve the desired results. CNG patients urgently need drugs with ideal efficacy. Tibetan medicine has significant advantages in the treatment of avoiding the side effects of Western medicine as much as possible (Yue et al., 2021).

Traditional Tibetan medicine (TTM) has a long history of developed medical knowledge regarding the etiology, diagnostics and treatment for chronic gastritis, originating as early as the eighth century, and explicated extensively in the Tibetan medical classic known as the *Four Medical Tantras* (*Rgyud bzhi*) where it is a type of disease within the greater category of Beken (phlegm) diseases (Fu et al., 2020). As one of the great Asian medical traditions, it has a unique theory-praxis system with demonstrated clinical efficacy, particularly in treating digestive diseases. The practice of TTM is also extensively guided by cumulative empirical experience and expertise developed over decades of experience among its practitioners. Populations across China and other Asian countries frequently seek TTM for treatment of gastrointestinal diseases (Chen et al., 2022). Studies have shown that TTM can significantly improve the clinical symptoms of patients with CNG, and with fewer adverse drug reactions (Tong et al., 2021), so it is widely used in clinical practice.

LWMX Pills is a widely used classical Tibetan medicine, which was recorded in the Tibetan Medicine Standard* by the Chinese Ministry of Health in the 1995s (WS3-BC-0283-95). LWMX pills are composed of 6 Tibetan medicinal plants (*Aucklandia radix, Pomegranate, Cardamom, Piper longum, Pashia, and Phyllanthus emblica*), which can be used for diseases such as gastrointestinal diseases. It has the functions of enhancing stomach fire, slowing blood heat, relieving stomach bloating and stomach pain caused by blood-heat disorder. According to the theory of Tibetan medicine, it has a certain potential value of anti-cancer effect through the smooth flow of blood, removing blood stasis and increasing fire, slowing down blood heat and preventing the formation of gastric mucosal tumors. Clinical reports show that LWMX pills are effective in acute and chronic gastroenteritis, chronic atrophic gastritis and gastroduodenal ulcers. However, the mechanisms of LWMX pills for treatment CNG still remain poor known.

In the present study, we analyzed the main components of LWMX pills and treated mice suffering from CNG with 25 mg/ml LWMX pills. Further, we analyzed the molecular biological mechanism of LWMX pills for CNG treatment with proteomics.

Materials and methods

**Animals**

SPF grade Wistar 120 male mice (10 weeks old), purchased from Zhejiang Viton Lihua Laboratory Animal Technology Co (SCXK (Zhejiang) 2018-0001). All experiments in the current study were approved by the Institutional Animal Care and Use Committee of the Qinghai University, Xining 810,016, Qinghai (Approval No.:20,201,018).

**Chromatographic conditions**

In this study, LWMX pills were obtained from Tibetan GanLu traditional medicine Co., Ltd. (Jhassa, China, Z54020033). 1 g LWMX pills powder was placed in a 50 ml conical flask, followed by the addition of 20 ml methanol and sonication for 30 min. The samples were then centrifuged (11,000 r/min, 5 min) and the supernatant was passed through a 2 µm microporous membrane to obtain the solution to be measured. The chromatographic and mass spectrometric conditions are described as follows chromatographic column WondaSil C18 (4.6 × 250 mm, 5 µm); mobile phase: acetonitrile (A)–0.2% phosphoric acid aqueous solution (B), gradient elution program is 0–12 min, 20%~28%A; 12~15 min , 28%A; 15~20 min, 28%~55%A; 20 min~35 min, 55%~60%A; 35 min~45 min, 60%~65%A; column temperature: 30 °C; detection Wavelength: 210 nm; Flow rate: 1.0 ml/min; Injection volume: 10 µl.

**CNG mouse model**

Wistar mice were randomly divided into control group, model group, low treatment group, medium treatment group and high treatment group, 10 mice in each group. The mice in the control group drank water freely, and the mice in the other groups dosed with 1 g/l of MNNG mother liquor in double-distilled water every week for mice to drink freely and gavaged with a dose of 2.25 g/l ranitidine salt solution, and the test mice were taken to be full for 2 days and starved for 1 day without water fasting, and the model was observed from six months onwards Two mice were taken under pathology for HE staining every two weeks to determine the success of CNG mouse model construction.

The control group and CNG mice were treated with 0.2 ml of saline by gavage, while the treatment group was given 12.5 mg/ ml, 25 mg/ml and 50 mg/ml of LWMX pills by gavage in the low, medium and high doses, respectively, once a day in the morning and once a day in the afternoon.

**HE staining of mouse gastric tissues**

At seven month, three mice in the modeling group, three mice in the treatment group and three mice in the control group were randomly taken, and the whole stomachs were washed with pre-cooled PBS at 4 °C to clean the blood stains, loaded into lyophilization tubes, snap-frozen in liquid nitrogen for 10 min, removed and stored at −80 °C. Finally, 1 mm of gastric mucosal tissue at the junction of the body-sinus of the gastric lesser curvature was cut along the gastric greater curvature, and the lesions were taken at 5 mm intervals, fixed with 4% formaldehyde solution, dehydrated, paraffin-embedded, and sectioned at a thickness of 4 µm. The pathological changes of gastric mucosal tissue in mice with heterogeneous hyperplastic lesions were observed by HE and Alcian blue-periodic acid Schiff’s (AB-PAS) staining. Suitable samples were selected for proteomic analysis using the results based on gastric mucosal histopathology.

**Proteomics analysis**

The sample was grinded with liquid nitrogen into cell powder and then transferred to a 5-ml centrifuge tube. After that, four volumes of lysis buffer (8 M urea, 1% protease inhibitor cocktail) was added to the
cell powder, followed by sonication three times on ice using a high intensity ultrasonic processor (Scientz). (Note: For PTM experiments, inhibitors were also added to the lysis buffer, e.g. 3 μM TSA and 50 mM NAM for acetylation, 1% phosphatase inhibitor for phosphorylation). The remaining debris was removed by centrifugation at 12,000 g at 4 °C for 10 min. Finally, the supernatant was collected and the protein concentration was determined with BCA kit according to the manufacturer’s instructions.

For digestion, the protein solution was reduced with 5 mM dithiothreitol for 30 min at 56 °C and alkylated with 11 mM iodoacetamide for 15 min at room temperature in darkness. The protein sample was then diluted by adding 100 mM TEAB to urea concentration less than 2 M. Finally, trypsin was added at 1:50 trypsin-to-protein mass ratio for the first digestion overnight and 1:100 trypsin-to-protein mass ratio for a second 4-h digestion. Finally, the peptides were desalted by C18 SPE column.

Tryptic peptides were firstly dissolved in 0.5 M TEAB. Each channel of peptide was labeled with their respective TMT reagent (based on manufacturer’s protocol, ThermoFisher Scientific), and incubated for 2 h at room temperature. Five microliters of each sample were pooled, desalted and dried by vacuum centrifugation. The tryptic peptides were fractionated into fractions by high pH reverse-phase HPLC using Thermo Betasil C18 column (5 μm particles, 10 mm ID, 250 mm length). The tryptic peptides were treated and subjected to NSI source followed by tandem mass spectrometry (MS/MS) in Q Exactive™Plus (Thermo Scientific, Waltham, MA, USA) coupled online to the UPLC. A data-dependent procedure alternated between one MS scan followed by 20 MS/MS scans with 15.0 s dynamic exclusion. Raw data were analyzed by MaxQuant software version 1.5.3.8. The FDR (False discovery rate) was set to 1% for all peptides and proteins. Protein expression more than 1-log fold changes were found to be associated with liver cancer.

Functional enrichment

Proteins were classified by GO annotation into three categories: biological process, cellular compartment and molecular function. For each category, a two-tailed Fisher’s exact test was employed to test the enrichment of the differentially expressed protein against all identified proteins. The GO with a corrected P-value < 0.05 was considered significant. Encyclopedia of Genes and Genomes (KEGG) database was used to identify enriched pathways by a two-tailed Fisher’s exact test to test the enrichment of the differentially expressed protein against all identified proteins. The pathway with a corrected P-value < 0.05 was considered significant. These pathways were classified into hierarchical categories according to the KEGG website.

Western blot analyses

All samples were lysed with RIPA buffer to obtain protein extracts.
Protein samples were separated on 5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel. After electrophoresis, proteins were transferred from PAGE onto a nitrocellulose membrane (Millipore Corporation, Billerica, MA). The membranes were blocked for 2 h at room temperature with 5% powdered skim milk and then were incubated with primary antibodies (1:1000; Cell Signalling Technology, Boston, MA, USA) at 4°C overnight. Subsequently, the membranes were washed with TBST three times (ten minutes each time) and incubated with horseradish peroxidase-conjugated secondary antibody (1:10,000; Bioss, Beijing, China) for 1 h at 37°C. The membrane was washed with TBST three times (ten minutes each time), and then the reactive proteins on the membrane were detected by chemiluminescent detection kit (Beyotime, Shanghai, China). Bands on the blots were quantified by Image J software (Media Cybernetics, Rockville, MD). The intensity of the β-actin bands was used for normalization.

Immunofluorescence (IF) of gastric tissues

Gastric tissues were collected for the preparation of tissue sections in 4% (w/v) paraformaldehyde solution. Tissue sections were cut out and paraffin-embedded. Subsequently, the sections were dewaxed with xylene and then hydrated with a gradient of alcohol. Next, the slices were incubated with 3% H2O2 in the dark at room temperature (18–22 min). Tissue sections were incubated with the primary anti-ALDH1B1 (1:2000; bs-6601; Bioss Antibodies, Woburn, MA, USA), anti-cpa3 (1:2000; bs-6601; Bioss Antibodies, Woburn, MA, USA), anti-cma1 (1:2000; ab180610; Abcam, Cambridge, MA, USA) monoclonal antibodies at 4°C overnight. Antibody diluent (Cat No.: G2025–100, Servicebio) was used to incubate the samples. FITC-PNA (100 μg/ml in PBS; a marker of acrosome in round spermatid and sperm) and DAPI (cell nuclear staining) were used to visualize antigens by an epifluorescence microscope (Nikon Eclipse C1; Nikon, Tokyo, Japan). The sample was observed by a phase-contrast microscope (Nikon Eclipse C1).

Immunohistochemical (IHC) staining

Paraffin-embedded rat stomach tissues were deparaffinized and subjected to antigen retrieval. Then, the samples were incubated with a primary rabbit monoclonal anti-amyl2 antibody (Cat No.: ab191434, Abcam, 1: 2000), rabbit polyclonal anti-Furin antibody (Cat No.: 12,375–1-AP, Proteintech, 1:100), rabbit polyclonal anti-Zurin antibody at 4°C overnight. Antibody diluent (Cat No.: G2025–100, Servicebio) was used to incubate the samples. After incubation, the slices were washed with Phosphate-Buffered Saline (PBS) and then incubated with Cy3-conjugated goat anti-rabbit IgG (1:2000). Subsequently, the slices were stained with DAPI (cell nuclear staining) and observed using an epifluorescence microscope (Nikon Eclipse C1; Nikon, Tokyo, Japan). The sample was observed by a phase-contrast microscope (Nikon Eclipse C1).

Table 1

<table>
<thead>
<tr>
<th>Peak No</th>
<th>tR (min)</th>
<th>Chemical formula</th>
<th>Ionic mode</th>
<th>Measured value (m/z)</th>
<th>Theoretical value (m/z)</th>
<th>Error (ppm)</th>
<th>Secondary fragment ion information</th>
<th>Identification results</th>
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<tr>
<td>1</td>
<td>36.60</td>
<td>C15H23O2</td>
<td>+</td>
<td>231.1379</td>
<td>231.1380</td>
<td>0.43</td>
<td>231.1379(90); 213.1271(16); 203.1429(10); 185.1324(100); 175.0752(16); 157.1011(25); 143.0855(39); 131.0856(27); 128.0624(9)</td>
<td>Dehydrocostuslactone</td>
</tr>
<tr>
<td>2</td>
<td>42.52</td>
<td>C14H6O3</td>
<td>–</td>
<td>300.9992</td>
<td>300.9979</td>
<td>4.32</td>
<td>300.9992(100); 283.9962(7); 257.0103(3); 229.0147(6); 201.0189(4); 185.0237(4)</td>
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<tr>
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<td>–</td>
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<td>301.0343</td>
<td>0.66</td>
<td>301.0351(100); 273.0407(5); 151.0200(94); 121.0286(31); 107.0130(27)</td>
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</tr>
<tr>
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<td>47.51</td>
<td>C12H20N4</td>
<td>+</td>
<td>274.1438</td>
<td>274.1438</td>
<td>0.00</td>
<td>274.1438(100); 189.0547(73); 171.0440(11); 143.0493(49); 115.0545(16)</td>
<td>Piperlongumine</td>
</tr>
<tr>
<td>5</td>
<td>47.78</td>
<td>C15H20O6</td>
<td>–</td>
<td>285.0405</td>
<td>285.0394</td>
<td>3.86</td>
<td>285.0405(100); 241.0501(1); 199.0397(5); 151.0032(10)</td>
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<tr>
<td>6</td>
<td>49.22</td>
<td>C14H10O6</td>
<td>–</td>
<td>285.0409</td>
<td>285.0394</td>
<td>5.26</td>
<td>285.0409(100); 239.0354(2); 229.0506(2); 211.0938(2); 185.0604(3); 169.0653(1); 149.0285(1); 133.0373(1); 82.0549(2); 115.0545(29)</td>
<td>Kaempferol</td>
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<td>286.1438</td>
<td>0.00</td>
<td>286.1438(70); 201.0547(100); 171.0441(16); 143.0492(21); 115.0545(29)</td>
<td>Piperine</td>
</tr>
<tr>
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<td>58.07</td>
<td>C15H20O2</td>
<td>+</td>
<td>233.1536</td>
<td>233.1536</td>
<td>0.00</td>
<td>233.1536(63); 215.1431(29); 187.1481(100); 159.1168(16); 145.1012(33); 131.0856(34); 119.0858(17); 105.0703(33); 91.0547(16); 81.0705(31)</td>
<td>Costunolide</td>
</tr>
</tbody>
</table>

Control Group

Model Group

Low dose Medium dose High dose

Fig. 2. HE staining to observe the pathological changes of mice gastric mucosa in each group (X200).
was purchased from Wuhan Servicebio Biotechnology Co., Ltd. The sections were then incubated with a horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (Cat No.: GB23303, Servicebio, 1:5000) and counterstained with hematoxylin. The sections were imaged by light microscopy (Olympus, Japan) at 200 × and 400 × magnification was applied to photograph images.

Data analysis
In this study, protein quantification were presented as mean ± SEM. Data were compared using Student’s t-test by SPSS version 20 for Windows. \( p < 0.05 \) between groups were considered statistically significant.

Results
Analysis of the chemical components of the extract of Liuwei Muxiang pills
The extract of LWMX pills was analyzed by UPLC-Q/TOF-MS. As shown in the Fig. 1 and Table 1, it mainly contains dehydrocostus lactone, ellagic acid, quercetin, piperlonguminine, luteolin, kaempferol, piperine, costunolide and other ingredients (Fig. 1, Table 1).

Histopathological analysis of gastric mucosa in mice
HE staining showed that the gastric mucosa of control mice had a...
small area of heavy edema, loose arrangement of connective tissue, increased number of blood vessels, and a small amount of lymphocyte infiltration, no obvious intestinal hyperplasia, and no obvious atrophy of gastric glands. The submucosal layer was edematous, with loose arrangement of connective tissue and lymphocyte infiltration, no obvious intestinal hyperplasia and mucosal gland atrophy were observed. In the low dose group, the submucosa was moderately edematous with loose connective tissue arrangement and increased number of blood vessels, accompanied by lymphocyte infiltration (Fig. 2). In the middle dose group, the epithelial cells in the mucosal layer were normal in shape, and the gastric glands were abundant, evenly distributed and closely arranged, with no obvious intestinalization or glandular atrophy. The gastric mucosa in the high-dose group did not show any obvious abnormalities, and a small amount of inflammatory cell infiltration was seen in the mucosal muscle layer, mucosal layer and submucosal layer.

Proteomic analysis

To further understand the protective mechanism of gastric mucosa protection by LWMX pills, we performed proteomic analysis on gastric mucosa tissues of mice in groups (control group, model group, 25 mg/ml LWMX pills group). The results of differential proteins showed that 209 proteins were up-regulated and 225 proteins were down-regulated in control group and CNG group (Fig. 3). Such as, the expression of CELA1, Muc6, Tgbi, Pnliprp1, Lamb2 proteins were down-regulated. The expression of Obp2a, Aldh1b1, H2-Ea, Mgst1, Nolc1 proteins was upregulated. The up-regulated proteins were mainly involved in biological Processes including metabolic process, positive regulation of gene expression, regulation of RNA metabolic process, etc. The down-regulated proteins were mainly involved in biological Processes including extracellular matrix organization, negative regulation of cell motility, leukocyte migration (Fig. 4). KEGG analysis revealed that up-regulated proteins were mainly included in biological Processes including metabolic process, positive regulation of gene expression, regulation of RNA metabolic process, etc. The down-regulated proteins were mainly involved in biological Processes including extracellular matrix organization, negative regulation of cell motility, leukocyte migration. Proteins were down-regulated were primarily involved in signaling pathways such as protein digestion and absorption, pancreatic secretion, focal adhesion (Fig. 5). There were 519 differential proteins in the control and 25 mg/ml LWMX pills groups, of which 216 proteins were up-regulated and 303 proteins were down-regulated (Fig. 3). For instance, the expression of Chil4, Bpiib1, Cma1 Mcpt2, and Bca1 proteins was up-regulated, and the expression of Ca3, Amy2, Maoa, and Purin Ywhae proteins was downregulated. The results of protein enrichment showed that the up-regulated proteins were mainly involved in biological processes including regulation of defense response, positive

<table>
<thead>
<tr>
<th>Biological Processes</th>
<th>Differential Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic process</td>
<td>CELA1, Muc6, Tgbi</td>
</tr>
<tr>
<td>Positive regulation of gene expression</td>
<td>Obp2a, Aldh1b1, H2-Ea</td>
</tr>
<tr>
<td>Regulation of RNA metabolic process</td>
<td>Mgst1, Nolc1</td>
</tr>
<tr>
<td>Extracellular matrix organization</td>
<td>Down-regulated proteins</td>
</tr>
<tr>
<td>Negative regulation of cell motility</td>
<td>Up-regulated proteins</td>
</tr>
</tbody>
</table>

**Fig. 4.** Biological process analyses of the differentially expressed proteins by GO database in CK group, Model group, Drug1 groups and Drug2 groups.
regulation of immune response, response to bacterium, animal organ development, etc. The down-regulated proteins were mainly involved in biological Processes including protein-containing complex assembly, nucleoside phosphate metabolic process, ribose phosphate (Fig. 4). KEGG analysis revealed that the up-regulated proteins were mainly involved in signaling pathways including phagosome, complement and coagulation cascades, natural killer cell mediated cytoxicity. The signaling pathways of the down-regulated proteins were mainly in Oxidative phosphorylation, chemical carcinogenesis - reactive oxygen species, thermogenesis (Fig. 5). There were 794 differential proteins in the CNG group and 25 mg/ml LWMX pills group, of which 287 proteins were upregulated and 507 proteins were down-regulated (Fig. 3). For example, the expression of Chil4, Mybpb1a, Bpib1, and Cpa3 proteins was upregulated, and the expression of Muc6, Amy2, Furin, and Atp5mf proteins was downregulated. The enrichment of differential proteins showed up-regulated proteins involved in biological Processes including RNA metabolic process, nucleic acid metabolic process. The down-regulated proteins were mainly involved in biological Processes including mitochondrion organization, nucleoside phosphate metabolic process, carboxylic acid metabolic process (Figs. 4 and 6). KEGG analysis revealed that the up-regulated proteins were mainly involved in signaling pathways including Ribosome biogenesis in eukaryotes. The

Fig. 5. Functional classification of differential proteins by GOG database in CK group, Model group, Drug1 groups and Drug2 groups.
signaling pathways of the down-regulated proteins mainly included in Chemical carcinogenesis - reactive oxygen species, Oxidative phosphorylation (Fig. 5).

**IF analysis of gastric mucosa in mice**

IF analysis was presented in Fig. 7. The results showed that the expression of ALDH1B1 in the control group was significantly lower than that in the model group in the gastric mucosa tissue, and the expression of ALDH1B1 was significantly lower in the 25 mg/ml LWMX Pills group (one month) and 25 mg/ml LWMX Pills group (two months) than in the model group, which indicated that the treatment with LWMX pills could reduce the expression of ALDH1B1. The expression of Cma1 in the control group was higher than that in the LWMX pills and model groups. The expression of Cma1 in the model group was higher than that in the LWMX pills groups, while we found that the staining of Cma1 was weakest in 25 mg/ml LWMX Pills. The expression of Cpa3 was the highest among all groups, while the expression of Cpa3 in the 25 mg/ml LWMX Pills group (one mouth) was higher than that in the 25 mg/ml LWMX Pills (two months) group and the model group, however interestingly the staining of Cpa3 in the 25 mg/ml LWMX Pills group (two months) was weaker than in the model group, these suggest that 25 mg/ml LWMX pills treated for 1 month have a therapeutic effect on chronic gastritis, but the same concentration treated for 2 months may accelerate the inflammation of gastric mucosal tissue.

**IHC analysis of gastric mucosa in mice**

IHC revealed that model group samples expressed higher levels of Furin than 25 mg/ml LWMX Pills group samples, as evidenced by very strong staining of Furin in gastric mucosal cells. However, AMY2 staining in gastric mucosal cells did not differ significantly between the treated and control groups (Fig. 8).

**The relative expression of proteins in gastric mucosa**

Western blotting was used to explore the effect of LWMX pills intervention on the chronic gastritis (Fig. 9). Compared with the control
group, chronic gastritis could increase protein expression of Furin, AMY2A, CPA3, ALDH1B1, Cam1, COXII, IL-6, IL-1β. However, the protein expression levels of these proteins were decreased in 25 mg/ml LWMX pills. Meanwhile, we found that the CAM1 protein expression in the 25 mg/ml LWMX pills group (two mouths) was increased compared to the in 25 mg/ml LWMX pills group (one mouths).

Discussion

CNG was the most common type of CG. Without effective treatment, patients may suffer from upper gastrointestinal symptoms, influencing their normal life and work, and it may develop into CAG, a precursor lesion of gastric carcinoma (GC) (Ohata et al., 2004). There is no ideal treatment option for CNG, and previous studies have found that herbal medicine has a good effect in treating CNG (Hwang et al., 2018). LWMX pills, a classic prescription of TTM, has been widely used for centuries in the treatment of gastrointestinal disorders. At present, the underlying mechanisms of these results were unclear. In this study, we found that LWMX pills have a good therapeutic effect on CNG, and further we found that LWMX pills can be used mainly by increasing resistance to inflammation and downregulating genes associated with cancer development through proteomic analysis. To the best of our knowledge, this is the first proteomic analysis of the possible molecular mechanisms of LWMX pills in the treatment of CNG, and our study provides a scientific reference for the promotion of TTM.

The results of liquid chromatography analysis showed that...
Fig. 9. The expression of ALDH1B1, CPA3, CAM1, COXII, IL-6, IL-1β was analyzed by Western blotting.
costunolide and dehydrocostuslactone were found in the extract of LWXM pills, a large number of antioxidants (Piperine glycosides, quercetin, luteolin, catechin, β-sitosterol, kaempferol). Previous studies have shown that costunolide and dehydrocostuslactone have significant protective effects against gastric ulcers (Zheng et al., 2016) and that H. pylori causes excessive oxidative stress in the gastrointestinal tract inducing chronic gastritis and even gastric cancer. Antioxidants are able to maintain gastric function by inhibiting the inflammatory response and oxidative stress (Fu et al., 2018). HE staining showed better results with 25 mg/ml LWXM pills (one mouth, two mouths) for CNG. The efficacy of drugs is mostly dose-related, so in this trial we chose the optimal concentration of 25 mg/ml for LWXM pills treatment for subsequent proteomic analysis.

CELA1 can act as a protein marker of tumor progression in colorectal cancer (Xie et al., 2016). Muc6 is associated with cancer migration and invasion (Giraldi et al., 2018), and previous studies found that MUC6 expression was significantly lower in gastric cancers with lymph node metastasis and poor pathological staging than in gastric cancers without lymph node metastasis and good pathological staging, respectively (Lee et al., 2001). TGFBI protein has an important role in tumor growth, metastasis and immunity. TGFBI knockdown inhibits tumor growth and metastasis in vivo (Poulsen et al., 2018; Wang et al., 2019). These lipase genes Pnliprp1 expression was significantly reduced in pancreatic tumor metastasis and immunity. TGFBI knockdown inhibits tumor growth and metastasis in vivo (Poulsen et al., 2018; Wang et al., 2019). These results suggest that LWXM pills maintain gastric function by suppressing inflammation and oxidative stress, and we also found that LWXM pills regulate the expression of protein related to cancer development (Amy2, Furin).

Conclusion

In this study, we found that LWXM pills contain costunolide, dehydrocostuslactone and antioxidants. Further study found that 25 mg/ml LWXM pill treatment for one month had better therapeutic effect on CNG. Further proteomic results showed that LWXM pills maintain gastric function by inhibiting inflammation and oxidative stress, and we also found that LWXM pills regulate the expression of proteins associated with cancer development (Amy2, Furin). Our study is the first to explain the possible mechanism of LWXM pills through proteomics, providing a theoretical basis for promoting TTM.

References


Note

The authors have no competing financial interests to declare.

CRediT authorship contribution statement


Declaration of Competing Interest

The authors declare that there are no conflicts of interest in this article.

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