Research Article

Network Pharmacology and Molecular Docking Analysis on Pharmacological Mechanisms of Astragalus membranaceus in the Treatment of Gastric Ulcer

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Background. Astragalus membranaceus (AM, family: Leguminosae) exerts significant therapeutic effect on gastric ulcer (GU); however, there are scarce studies on its molecular mechanism against GU. This study aims to explore the key ingredients, key targets, and potential mechanisms of AM in the treatment of GU by utilizing network pharmacology and molecular docking.

Methods. Several public databases were used to predict the targets of AM and GU, respectively, and the drug and disease targets were intersected to obtain the common targets. Next, the key ingredients and key targets were identified by constructing ingredient-target network and protein-protein-interaction (PPI) network. Gene Ontology biological processes (GOBP) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were carried out on the common targets in order to ascertain the biological processes and signaling pathways involved. Finally, molecular docking was conducted to verify the binding affinity between the key ingredients and key targets.

Results. A total of 552 predicted targets were obtained from 23 screened active ingredients, of which 203 targets were the common targets with GU. Quercetin, kaempferol, and isorhamnetin were identified as the key ingredients by constructing ingredient-target network, and TP53, AKT1, VEGFA, IL6, TNF, CASP3, and EGFR were selected as the key targets by constructing PPI network. GOBP and KEGG pathway enrichment analysis suggested that the therapeutic effect of AM on GU involved multiple biological processes and signaling pathways related to inflammation, oxidative stress, apoptosis, cell proliferation, and angiogenesis. Molecular docking validation demonstrated that all key ingredients had good binding affinity with the key targets.

Conclusion. This study revealed the key ingredients, key targets, and potential mechanisms of AM against GU, and these data may provide some crucial references for subsequent research and development of drugs for treating GU.

1. Introduction

Gastric ulcer (GU) is a common disease in which gastric acid and pepsin self-digest the gastric mucosa due to the imbalance of defense mechanism and injury factors of gastric mucosa caused by multiple aggressive factors, including Helicobacter pylori (H. pylori) infection, overuse of non-steroidal anti-inflammatory drugs, excessive drinking, smoking, and stress [1, 2]. Ulcers in pyloric canal may cause spasm of pyloric smooth muscle leading to pyloric obstruction, and deep ulcers involving the blood vessels of muscular layer and even serosal layer can lead to bleeding and perforation [3, 4]. At the same time, chronic GU is considered as a precancerous lesion of gastric cancer, which can provide a favorable microenvironment for tumor transformation of gastric epithelium [5, 6]. The wide application of chemicals for H. pylori eradication and gastric acid secretion inhibition has improved the clinical cure rate of GU, but their therapeutic effect is limited and the recurrence rate of GU is still high [1]. Furthermore, long-term use of these drugs has certain severe side effects [7, 8].

Gastrointestinal diseases are the preponderant diseases in herbal therapy. A large number of studies in human and animal models have shown that the efficacy of herbal medicines in the treatment of GU is comparable or superior to that of omeprazole and cimetidine, and herbs display
fewer adverse reactions. Besides, the cost of herbal therapy in the treatment of GU is only about one-sixth of that of western medicine [9]. Therefore, herbal therapy is regarded as a valuable alternative to treat GU in humans.

*Astragalus membranaceus* (AM), also known as “Huangqi” in China, is one of the most important Chinese herbal medicines and has a medicinal history of two thousand years [10]. More than 100 ingredients have been isolated and identified from AM, mainly including flavonoids, saponins, polysaccharides, and amino acids [11]. These ingredients show a variety of biological activities *in vivo* and *in vitro*, including immunomodulatory [12], anti-inflammatory [13, 14], antiviral [15], anti-fatigue [16], anti-aging [10], and hypoglycemic [17] effects. Traditional Chinese medicine (TCM) believes that AM has the function of invigoring spleen-qi and regenerating tissue and thus can improve gastrointestinal function and promote the healing of GU. As a result, AM is commonly used in TCM prescriptions for GU treatment, such as Buzhong Yiqi Decoction and Huangqi Jianzhong Decoction.

Herbs are multi-ingredient and multitarget in treating diseases, and their pharmacodynamic ingredients and molecular mechanisms are often difficult to identify. With the advancement of network technology and bioinformatics, computer-aided identification methods of drug-target interactions represented by network pharmacology and molecular docking have been developed to provide support for the research of herbs with complex action mechanism. They greatly reduce the initial time and cost of experimental determination of drug-target interaction and improve the chances of finding ideal drug candidates [18–20]. Although AM exerts significant curative effects in the clinical treatment of GU in TCM, the relevant molecular mechanism is rarely studied at present. This study aimed to elucidate the potential mechanism of AM against GU based on the network pharmacological strategy and molecular docking, and the workflow is shown in Figure 1.

## 2. Methods

### 2.1. Bioactive Ingredients and Targets Screening of AM

The chemical ingredients of AM were obtained from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://tcmsp-e.com/) by using “Huangqi” as the keyword. The chemical ingredients meeting both oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18 were screened out as the bioactive ingredients. Besides, chemical substances that do not meet the above screening criteria but have potential activity in the treatment of GU were also included by searching and reading relevant literature on PubMed (https://pubmed.ncbi.nlm.nih.gov/).

Protein targets associated with each bioactive ingredient were acquired from TCMSP and transformed into corresponding gene symbols via UniProt database (https://www.uniprot.org/) [21]. In addition, the SMILES strings of active ingredients were obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and then were used to predict potential gene targets in SwissTargetPrediction platform (http://www.swisstargetprediction.ch/) [22, 23]. The ingredient targets obtained from two databases were merged and the redundant items were deleted.

### 2.2. Disease Targets Acquisition of GU

With “gastric ulcer” as the keyword, GeneCards Database (https://www.genecards.org/) [24], DisGeNET Database (https://www.disgenet.org/) [25], Online Mendelian Inheritance in Man (OMIM) Database (https://omim.org/) [26], and Therapeutic Target Database (TTD) (http://db.idrblab.net/ttd/) [27] were used to search and screen the known disease targets. Subsequently, all the consequences were integrated and the repeated targets were removed.

### 2.3. Ingredient-Target Network Construction

The common targets of ingredient targets and disease targets were obtained by intersection and a Venn diagram was drawn to display visually. Furthermore, a network based on the interactions between drug, ingredients, common targets, and disease was constructed and visualized by Cytoscape v3.7.1 [28].

### 2.4. PPI Network Construction

The common targets were imported into STRING database (https://www.string-db.org/) to build PPI network, and the result was exported in tab-separated value (TSV) format after hiding the unconnected nodes [29]. Subsequently, Cytoscape v3.7.1 was applied to visualize the PPI data and extract the core network according to the degree, betweenness centrality (BC), and closer centrality (CC) of nodes [28].

### 2.5. GOBP and KEGG Pathway Enrichment Analysis

The previously obtained common targets were input into Metascape (https://metascape.org/) for GOBP and KEGG pathway enrichment analysis [30]. After sorting the results according to the order of *p* value from small to large, the top 25 GOBP items and the top 25 KEGG pathways were selected to draw a bar diagram and a bubble diagram, respectively.

### 2.6. Molecular Docking Validation

In order to confirm the accuracy of the network pharmacology prediction, the key ingredients were used as small molecular ligands to perform molecular docking with the key target proteins. The 2D structures of ligands were obtained from PubChem database and structurally optimized and transformed into PDB format by Chem3D v19.0 and PyMOL v2.1.0. The 3D structures of receptor proteins were downloaded from RSCB PDB database (https://www.rcsb.org/) and converted into PDB format by PyMOL v2.1.0 after removing water and original ligands [31]. Subsequently, AutoDockTools v1.5.6 were employed for molecular docking to evaluate the binding affinity [32]. According to the principle of molecular docking, the smaller the binding energy, the more stable the docking module. The minimum binding energy < −5.0 kcal/mol indicates that a good binding affinity is between receptor
and ligand, and the minimum binding energy $<-7.0\text{kcal/mol}$ represents that the binding affinity was extremely strong [33, 34]. Finally, the docking results with binding energy $<-7.0\text{kcal/mol}$ were visualized by utilizing PyMOL v2.1.0.

### 3. Results

#### 3.1. Bioactive Ingredients and Targets Screening of AM

Among the 87 chemical ingredients of AM obtained from TCMSP, 20 ingredients with OB $\geq 30\%$ and DL $\geq 0.18$ were screened out as the bioactive ingredients. Astragaloside IV (OB $= 22.50$, DL $= 0.15$), astragaloside III (OB $= 31.83$, DL $= 0.10$), and ononin (OB $= 11.52$, DL $= 0.78$) were included together for their reported antiulcer potential in several studies though they did not meet the inclusion criteria [35–37]. Finally, a total of 23 active ingredients were included, as shown in Table 1, and after deleting the duplicates, 552 targets of these active ingredients were collected in all.

#### 3.2. Disease Targets Acquisition of GU

1226 known disease targets of GU were collected from GeneCards Database, 136 from DisGeNET, 79 from OMIM, and 3 from TTD. A total of 1320 disease targets were collected by merging the disease targets obtained from these databases and removing the duplicates.

#### 3.3. Ingredient-Target Network Construction

203 common targets of AM and GU were identified (Figure 2) and an ingredient-target network was constructed (Figure 3) to clarify how the bioactive ingredients of AM may act against GU [28]. The bioactive ingredients were sorted according to the descending order of degree value, and the top 3, including quercetin (degree $= 123$, BC $= 0.5545$, and CC $= 0.5528$), kaempferol (degree $= 65$, BC $= 0.1186$, and CC $= 0.4269$) and isorhamnetin (degree $= 52$, BC $= 0.0620$, and CC $= 0.4039$) were predicted to be the key ingredients.

#### 3.4. Protein-Protein-Interaction (PPI) Network Construction

The PPI network exported from STRING database had 201 nodes (two free nodes were hidden) and 5149 edges. The nodes with degree $\geq 84$ were selected to build a new network, but there were still too many targets. Therefore, a core network composed of 14 nodes and 91 edges was further extracted by using the median of degree, BC, and CC. The extraction process is shown in Figure 4, and the details of core targets are listed in Table 2. TP53, AKT1, VEGFA, IL6,
TNF, CASP3, and EGFR were predicted as the key targets according to the descending order of degree values.

3.5. GOBP and KEGG Pathway Enrichment Analysis. GOBP and KEGG pathway enrichment analysis was performed on 203 common targets using Metascape platform. According to the ascending order of \(p\) values, the top 25 GOBP items and the top 25 KEGG pathways were selected to draw a bar diagram (Figure 5) and a bubble diagram (Figure 6), respectively.

GOBP enrichment analysis showed the common targets involved multiple biological processes of gastric mucosa injury and repair, including apoptotic signaling pathway, cellular response to chemical stress, response to inorganic substance, response to oxidative stress, response to molecule of bacterial origin, response to wounding, response to lipopolysaccharide, wound healing, positive regulation of cell motility, positive regulation of cell migration, response to growth factor, and epithelial cell proliferation.

KEGG pathway enrichment analysis indicated that common targets were significantly enriched in pathways involving gastric mucosal injury, ulcer healing, and progression of GU to gastric cancer, such as PI3K-AKT signaling pathway, HIF-1 signaling pathway, MAPK signaling pathway, apoptosis, pathways in cancer, and MicroRNAs in cancer.

3.6. Molecular Docking Validation. Molecular docking was conducted to evaluate the binding affinity between the 3 key targets screened from PPI network and the 7 key ingredients screened from ingredient-target network. The results verified that all key ingredients had good binding affinity with the key targets. Minimum binding energy of each docking module was recorded in Table 3, and the docking modules with minimum binding energy less than \(-7.0\) kcal/mol are shown in Figure 7.

4. Discussion

In this study, the material basis and molecular mechanism of the anti-GU action of AM were explored by using network pharmacology and molecular docking. Through the construction of ingredient-target network and PPI network, three flavonoids (quercetin, kaempferol, and isorhamnetin)
Figure 3: Ingredient-target network. The yellow diamonds represent bioactive ingredients, and the green circles represent the common targets. The edges represent the interaction between the ingredients and targets. Node size is proportional to its degree value. The larger the node, the more important the target in the network.

Figure 4: PPI core network screening flowchart. The node size is proportional to its degree value. The larger the node, the more important the target in the network.
were predicted to be the key ingredients of AM in the treatment of GU, and TP53, AKT1, VEGFA, IL6, TNF, CASP3, and EGFR were identified as the key targets. Molecular docking also verified that all key ingredients had good binding affinity with the key targets. GOBP and KEGG pathway enrichment analysis suggested that the therapeutic effect of AM on GU involved multiple signaling pathways and biological processes related to the occurrence and healing of GU, such as inflammation, oxidative stress, apoptosis, cell proliferation, and angiogenesis.

4.1. AM May Improve GU via Its Antibacterial and Antiviral Activities. H. pylori is the leading cause of GU[38].

| Number | Uniprot ID | Gene name | Protein name | Degree |
|--------|------------|-----------|--------------|--------|
| 1      | P04637     | TP53      | Cellular tumor antigen p53 | 157    |
| 2      | P31749     | AKT1      | RAC-alpha serine/threonine-protein kinase | 154    |
| 3      | P15692     | VEGFA     | Vascular endothelial growth factor A | 146    |
| 4      | P05231     | IL6       | Interleukin-6 | 145    |
| 5      | P01375     | TNF       | Tumor necrosis factor | 143    |
| 6      | P42574     | CASP3     | Caspase-3 | 134    |
| 7      | P00533     | EGFR      | Epidermal growth factor receptor | 132    |
| 8      | P40763     | STAT3     | Signal transducer and activator of transcription 3 | 130    |
| 9      | P01584     | IL1B      | Interleukin-1 beta | 130    |
| 10     | P05412     | JUN       | Transcription factor AP-1 | 129    |
| 11     | P0106      | MYC       | Myc proto-oncogene protein | 128    |
| 12     | P03372     | ESR1      | Estrogen receptor | 124    |
| 13     | P12931     | SRC       | Proto-oncogene tyrosine-protein kinase Src | 119    |
| 14     | P60484     | PTEN      | Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN | 118    |

Figure 5: Top 25 GOBP items. The y-axis represents GOBP item. The x-axis indicates the number of genes enriched in the item. The redder the color, the smaller the p value and the more important the GOBP items.
### Figure 6: Top 25 KEGG pathways. The y-axis represents the KEGG pathway. The x-axis shows the number of genes enriched in the pathway. The size of bubbles represents the number of targets in the pathway and the color represents the p value. The bigger the bubble size, the more the targets in the pathway. The redder the color, the smaller the p value.

### Table 3: Minimum binding energy between the key targets and key ingredients.

| Key targets (PDB ID) | Key ingredients | Binding energy (kcal/mol) |
|----------------------|-----------------|--------------------------|
| TP53 (3TG5)          | Quercetin       | −8.33                    |
|                      | Kaempferol      | −8.05                    |
|                      | Isorhamnetin    | −8.72                    |
|                      | Quercetin       | −7.58                    |
| AKT1 (4EJN)          | Kaempferol      | −7.39                    |
|                      | Isorhamnetin    | −7.12                    |
|                      | Quercetin       | −6.07                    |
| VEGFA (4QAF)         | Kaempferol      | −5.95                    |
|                      | Isorhamnetin    | −6.53                    |
|                      | Quercetin       | −5.81                    |
| IL6 (5FUC)           | Kaempferol      | −5.85                    |
|                      | Isorhamnetin    | −5.74                    |
|                      | Quercetin       | −7.50                    |
| TNF (6X83)           | Kaempferol      | −7.56                    |
|                      | Isorhamnetin    | −7.65                    |
|                      | Quercetin       | −6.85                    |
| CASP3 (1NME)         | Kaempferol      | −7.03                    |
|                      | Isorhamnetin    | −6.61                    |
|                      | Quercetin       | −7.23                    |
| EGFR (2RGP)          | Kaempferol      | −7.25                    |
|                      | Isorhamnetin    | −6.74                    |
biological activities against *H. pylori* of the screened key ingredients have been reported previously. Quercetin was found to exert excellent inhibitory activity on urease, an enzyme which enables *H. pylori* to survive in acidic gastric juice and infect gastric mucosa [39]. Kaempferol significantly reduced *H. pylori* colonies in a dose-dependent manner both *in vitro* and *in vivo* [40]. GOBP enrichment analysis also revealed that the treatment of AM on GU was related to the response to molecule of bacterial origin and response to lipopolysaccharide, suggesting that AM can alleviate gastric mucosal damage of virulence factors produced by *H. pylori*. Furthermore, KEGG pathway enrichment analysis showed that EBV infection was associated with the antiulcer effect of AM, and Epstein-Barr virus (EBV) was proved to promote gastrointestinal mucosal inflammation, indicating that AM may be considered for the treatment of GU caused by EBV [41, 42].

4.2. AM May Improve GU via Its Anti-Inflammatory Effect. Gastric mucosal inflammation caused by increased expression of proinflammatory factors is an important reason for the occurrence, aggravation, and long-term nonhealing of GU [43, 44]. TNF-α and IL-6 are regarded as the major contributors to gastric inflammation and mucosal injury, and their serum levels are positively correlated with the severity of GU [45–47]. Our study found that they were both the key targets of AM in the treatment of GU and had good binding affinity with all key ingredients. Meanwhile, evidence from existing studies has also confirmed that the screened key ingredients attenuated gastric mucosal inflammation by downregulating the expression of these two proinflammatory factors [48–50]. Furthermore, MAPK signaling pathway and PI3K-AKT signaling pathway have been already verified to ameliorate GU by regulating proinflammatory cytokines, and our study revealed that the common targets between AM and GU are enriched in these two pathways [51, 52].

4.3. AM May Improve GU via Its Antioxidant Effect. Oxidative stress (OS) is a state of imbalance between oxidation and antioxidation in the body, under which excessive reactive oxygen (ROS) produces oxidative damage to lipids,
proteins, and DNA of cells [53]. Gastrointestinal tract is the main source of ROS, and oxidative stress plays a crucial role in gastric mucosal injury [54, 55]. Flavonoids are known as potent antioxidants, and quercetin has been proved to alleviate GU by increasing the activity of antioxidant enzymes [56, 57]. AKT is a powerful inhibitory signal of apoptosis, and the activation of PI3K-AKT pathway is crucial to reduce oxidative stress-induced damage and apoptosis of gastric mucosal cells [51]. KEGG pathway enrichment analysis revealed that the therapeutic effect of AM on GU was closely related to PI3K-AKT signaling pathway, and GOBP enrichment analysis also indicated that the antilucre effect of AM was associated with cellular response to oxidative stress.

4.4. AM May Improve GU via Cell Apoptosis and Proliferation Regulation. The balance of proliferation and apoptosis of gastric epithelial cells maintains the integrity of gastric mucosa. When this balance is destroyed by various pathogenic factors and apoptosis is dominant, gastric ulcers can be induced [58]. Studies have demonstrated that quercetin promoted the normal proliferation of gastric mucosa cells through inhibiting apoptosis and cell cycle arrest induced by H. pylori infection [59, 60]. Among the key targets screened from PPI network, TP53, AKT1, and CASP3 are closely related to the regulation of cell cycle arrest and apoptosis, while the activation of EGFR can provide continuous intracellular division signal to induce the proliferation of epithelial cells at ulcer edge and promote their migration to the ulcer surface to repair damaged gastric mucosa [61]. GOBP and KEGG pathway enrichment analysis also suggested that the common targets were significantly enriched in the pathways and biological process involving apoptosis, response to growth factor, epithelial cell proliferation, wound healing, positive regulation of cell motility, and positive regulation of cell migration.

4.5. AM May Improve GU via Angiogenesis Promotion. Angiogenesis is essential for the healing of gastric mucosal injury because it contributes to delivering oxygen and nutrients to the healing site. Angiogenesis is initiated and regulated by angiogenic factors, among which VEGF is the most effective and basic regulator [62]. The fact that stimulation of angiogenesis in granulation tissues by upregulation of VEGF expression or exogenous VEGF supplementation can significantly accelerate experimental GU healing in rats indicates the important role of this target in GU treatment [63, 64]. VEGFA is one of the screened key targets of AM in the treatment of GU, and KEGG pathway enrichment analysis found some pathways in which common targets were enriched, such as PI3K-AKT signaling pathway, MAPK signaling pathway, and HIF-1 signaling pathway, can also regulate the angiogenesis of gastric mucosa through VEGF [65–67].

5. Conclusion

To sum up, our study has revealed that the therapeutic effect of AM on GU is multi-ingredient, multitarget, and multimechanism. The key ingredients of the curative effect are mainly flavonoids, and the molecular mechanism is closely related to inhibition of H. pylori and virus, anti-inflammation, antioxidation, regulation of gastric mucosal cell proliferation and apoptosis, and promotion of angiogenesis. These data can provide some crucial references for the research and development of new anti-GU drugs in the future.

Data Availability

The figures and tables supporting the results of this study are included in the article, and the original datasets are available from the first author or corresponding author upon request.

Conflicts of Interest

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

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References

[1] R.-Q. Yang, H. Mao, L.-Y. Huang, P.-Z. Su, and M. Lu, “Effects of hydroxylcine combined with esomeprazole on gastric ulcer healing quality: a clinical observation study,” World Journal of Gastroenterology, vol. 23, no. 7, pp. 1268–1277, 2017.
[2] C. Harsha, K. Banik, D. Bordoloi, and A. B. Kunnumakkara, “Antilucre properties of fruits and vegetables: a mechanism based perspective,” Food and Chemical Toxicology, vol. 108, no. Pt A, pp. 104–119, 2017.
[3] K. S. Gurusamy and E. Pallari, “Medical versus surgical treatment for refractory or recurrent peptic ulcer,” Cochrane Database of Systematic Reviews, vol. 3, no. 3, Article ID Cd011523, 2016.
[4] T. E. Yusuf and W. R. Brugge, “Endoscopic therapy of benign pyloric stenosis and gastric outlet obstruction,” Current Opinion in Gastroenterology, vol. 22, no. 5, pp. 570–573, 2006.
[5] S. X. Lv, J. H. Gan, C. C. Wang et al., “Biopsy from the base of gastric ulcer may find gastric cancer earlier,” Medical Hypotheses, vol. 76, no. 2, pp. 249–250, 2010.
[6] P. Correa and B. A. Schmidt, “The relationship between gastric cancer frequency and the ratio of gastric to duodenal ulcer,” Alimentary Pharmacology & Therapeutics, vol. 9, no. Suppl 2, pp. 13–19, 1995.
[7] L. Kuná, J. Jakab, R. Smolík, N. Raguz-Lucic, A. Vc?ev, and M. Smolic, “Peptic ulcer disease: a brief review of conventional therapy and herbal treatment options,” Journal of Clinical Medicine, vol. 8, no. 2, 2019.
[8] K. E. McColl, “Acid inhibitory medication and risk of gastric and oesophageal cancer,” Gut, vol. 55, no. 11, pp. 1532-1533, 2008.
[9] W. P. Bi, H. B. Man, and M. Q. Man, “Efficacy and safety of herbal medicines in treating gastric ulcer: a review,” World Journal of Gastroenterology, vol. 20, no. 45, pp. 17020–17028, 2014.
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[41] Z. M. Chen, R. Shah, G. R. Zuckerman, and H. L. Wang, "Epstein-Barr virus gastritis: an underrecognized form of severe gastritis simulating gastric lymphoma," The American Journal of Surgical Pathology, vol. 31, no. 9, pp. 1446–1451, 2007.

[42] M. G. Cárdenas-Mondragón, J. Torres, L. Flores-Luna, R. Carreón-Talavera, M. Camorlinga-Ponce, and E. M. Fuentes-Pananá, "Epstein-Barr virus association with peptic ulcer disease," Analytical Cellular Pathology, vol. 2015, Article ID 164840, 7 pages, 2015.

[43] M. Sugimoto, Y. Yamaoka, and T. Furuta, "Influence of interleukin polymorphisms on development of gastric cancer and peptic ulcer," World Journal of Gastroenterology, vol. 16, no. 10, pp. 1188–1200, 2010.

[44] T. Arakawa, T. Watanabe, T. Tanigawa, K. Tominaga, Y. Fujiwara, and K. Morimoto, "Quality of ulcer healing in gastrointestinal tract: its pathophysiology and clinical relevance," World Journal of Gastroenterology, vol. 18, no. 35, pp. 4811–4822, 2012.

[45] M. Tourani, M. Habibzadeh, A. Karkhah, J. Shokri-Shirvani, L. Barari, and H. R. Nouri, "Association of TNF-α but not IL-1β levels with the presence of Helicobacter pylori infection increased the risk of peptic ulcer development," Cytokine, vol. 110, pp. 232–236, 2018.

[46] M. C. de Souza, A. J. Vieira, F. P. Beserra, C. H. Pellizzon, R. H. Nóbrega, and A. L. Rozza, "Gastroprotective effect of limonene in rats: influence on oxidative stress, inflammation and gene expression," Phytomedicine, vol. 53, pp. 37–42, 2019.

[47] J. C. Brown, J. Wang, L. Kasman, X. Jiang, and V. Haley-Zitlin, "Activities of muscadine grape skin and quercetin against Helicobacter pylori infection in mice," Journal of Applied Microbiology, vol. 110, no. 1, pp. 139–146, 2010.

[48] M. J. Yeon, M. H. Lee, D. H. Kim et al., "Anti-inflammatory effects of Kaempferol on Helicobacter pylori-induced inflammation," Bioscience Biotechnology & Biochemistry, vol. 83, no. 1, pp. 166–173, 2018.

[49] G. Liu, C. Jiang, D. Li et al., "Isorhamnetin alleviates esophageal mucosal injury in a chronic model of reflux esophagitis," European Journal of Pharmacology, vol. 864, Article ID 172720, 2019.

[50] S. Ren, Y. Wei, R. Wang et al., "Rutaecarpine ameliorates ethanol-induced gastric mucosal injury in mice by modulating genes related to inflammation, oxidative stress and apoptosis," Frontiers in Pharmacology, vol. 11, Article ID 600295, 2020.

[51] M. R. Akanda, I. S. Kim, D. Ahn et al., "Anti-inflammatory and gastroprotective roles of Rabdosia inflexa through downregulation of pro-inflammatory cytokines and MAPK/NF-κB signaling pathways," International Journal of Molecular Sciences, vol. 19, no. 2, 2019.

[52] K. Daenen, A. Andries, D. Mekehl, A. Van Schepdael, F. Jouret, and B. Bammens, "Oxidative stress in chronic kidney disease," Pediatric Nephrology, vol. 34, no. 6, pp. 975–991, 2019.

[53] A. Bhattacharyya, R. Chattopadhyay, S. Mitra, and S. E. Crowe, "Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases," Physiological Reviews, vol. 94, no. 2, pp. 329–354, 2014.

[54] D. Bandopadhyay and A. Chattopadhyay, "Reactive oxygen species-induced gastric ulceration: protection by melatonin," Current Medicinal Chemistry, vol. 13, no. 10, pp. 1187–1202, 2006.

[55] S. J. Maleki, J. F. Crespo, and B. Cabanillas, "Anti-inflammatory effects of flavonoids," Food Chemistry, vol. 299, Article ID 125124, 2019.

[56] S. S. Khan, N. Erkasap, T. Koken, M. Serteser, F. Aktepe, and S. Erk, "The antioxidative and antihistaminic properties of quercetin in ethanol-induced gastric lesions," Toxicology, vol. 183, no. 1–3, pp. 133–142, 2003.

[57] I. Szabó and A. S. Tarnawski, "Apoptosis in the gastric mucosa: molecular mechanisms, basic and clinical implications," Journal of Physiology & Pharmacology, vol. 51, no. 1, pp. 3–15, 2000.

[58] S. Zhang, J. Huang, X. Xie, Y. He, F. Mo, and Z. Luo, "Quercetin from polygonum capitatum protects against gastric inflammation and apoptosis associated with Helicobacter pylori infection by affecting the levels of p38MAPK, BCL-2 and BAX," Molecules, vol. 22, no. 5, 2017.

[59] J. E. Shim, J. M. Kim, E. A. Bae, Y. J. Hyun, and D. H. Kim, "In vitro inhibitory effect of flavonoids on growth, inflammation and vacuolization of Helicobacter pylori," Planta Medica, vol. 71, no. 3, pp. 197–201, 2021.

[60] P. C. Konturek, S. J. Konturek, T. Brzozowski, and H. Ernst, "Epidermal growth factor and transforming growth factor-alpha: role in protection and healing of gastric mucosal lesions," European Journal of Gastroenterology and Hepatology, vol. 7, no. 10, pp. 933–937, 1995.

[61] A. S. Tarnawski, A. Ahluwalia, and M. K. Jones, "Angiogenesis in gastric mucosa: an important component of gastric erosion and ulcer healing and its impairment in aging," Journal of Gastroenterology and Hepatology, vol. 29, no. Suppl 4, pp. 112–123, 2014.

[62] M. K. Jones, H. Kawanaka, D. Baatar et al., "Gene therapy for gastric ulcers with single local injection of naked DNA encoding VEGF and angiopoietin-1," Gastroenterology, vol. 121, no. 5, pp. 1040–1047, 2001.

[63] A. V. Sharma, K. Ganguly, S. Paul, N. Maulik, and S. Swarnakar, "Curcumin heals indomethacin-induced gastric ulceration by stimulation of angiogenesis and restitution of collagen fibers via VEGF and MMP-2 mediated signaling," Antioxidants and Redox Signaling, vol. 16, no. 4, pp. 351–362, 2012.

[64] A. Ahluwalia, M. K. Jones, N. Hoa, E. Zhu, T. Brzozowski, and A. S. Tarnawski, "Reduced NGF in gastric endothelial cells is one of the main causes of impaired angiogenesis in aging gastric mucosa," Cellular and Molecular Gastroenterology and Hepatology, vol. 6, no. 2, pp. 199–213, 2018.

[65] M. Yeo, D. K. Kim, S. U. Han et al., "Novel action of gastric proton pump inhibitor on suppression of Helicobacter pylori induced angiogenesis," Gut, vol. 55, no. 1, pp. 26–33, 2006.

[66] H. Hashimoto, M. Akimoto, A. Maeda, M. Shigemoto, and K. Yamashito, "Relation of hypoxia-inducible factor-1alpha to vascular endothelial growth factor and vasoactive factors during healing of gastric ulcers," Journal of Cardiovascular Pharmacology, vol. 44, no. Suppl 1, pp. S407–S409, 2004.