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ORIGINAL ARTICLE

Multipathway Integrated Adjustment Mechanism of *Glycyrrhiza* Triterpenes Curing Gastric Ulcer in Rats

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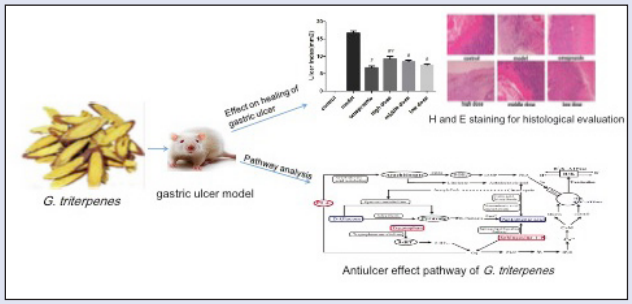
ABSTRACT

Background: Gastric ulcer is a common chronic disease in human digestive system, which is difficult to cure, easy to relapse, and endangers human health seriously. Compared with western medicine, traditional Chinese medicine has a unique advantage in improving the general situation, stablizing medical condition, and with little side effects. *Glycyrrhiza* known as "king of all the medicine", has a range of pharmacological activities and is commonly used in a variety of proprietary Chinese medicines and formulations. **Objective:** On the basis of explicit antiulcer effect of *Glycyrrhiza* triterpenes, the molecular mechanisms of its therapeutic effect on acetic acid induced gastric ulcer in rats were explored. **Materials and Methods:** Acetic acid induced gastric ulcer model in rats was established to evaluate the curing effect of *G.* triterpenes and all of the rats were randomised into six groups: Control group, model group, omeprazole group (0.8 mg/mL), triterpenes high dose group (378.0 mg/mL), triterpenes middle dose group (126.0 mg/mL), and triterpenes low dose group (42.0 mg/mL). All rats in groups were orally administered the active group solution 1.5 mL once daily (model and control groups with saline) for 7 days. HPLC-TOF-MS analysis method was performed to obtain the plasma metabolites spectrums of control group, model group, triterpenes high, middle and low dose groups. **Results:** A total of 11 differential endogenous metabolites related to the therapeutic effect of *G.* triterpenes were identified, including tryptophan, phingosine-1-phosphate, pantothenic acid, and so on, among which tryptophan and phingosine-1-phosphate are related with the calcium signaling pathway and arachidonic acid (AA) metabolism. At the same time, in order to verify the above results, quantitative real time polymerase chain reaction were performed to evaluate the expression of H^+-K^+-ATPase alpha mRNA and phospholipase a 2 mRNA in relational signaling pathways. Combined with statistical analysis of plasma metabolic spectrum and gene expression in tissue, it is suggested that *G.* triterpenes has antiulcer effect on gastric ulcer in rats. **Conclusion:** *G.* triterpenes has a certain regulating effect on the metabolism of tryptophan, AA, sphingosine, and other endogenous metabolites, and we speculated that the antiulcer potential of *G.* triterpenes can be primarily attributed to its inhibiting gastric acid secretion, reducing the release of inflammatory mediators, and protecting gastric mucosa effects to prevent the further development of gastric ulcer.

Key words: Gastric ulcer, *Glycyrrhiza* triterpenes, mechanism, metabolism, multipathway, related genes

SUMMARY

- G.triterpenes can obviously relieve the symptoms of gastric ulcer, especially the low dose group.
- *G. triterpenes* can effectively regulate the amount of small molecule metablism in gastric ulcer rats *in vivo*, including tryptophan, phingosine-1-phosphate, etc.
- *G. triterpenes* resisting gastric ulcer is probably by regulating arachidonic acid metabolism, sphingosine metabolism, etc.
- Down-regulation of H^+-K^+-ATPase alpha subunit mRNA and up-regulation of PLA_2 mRNA in gastric tissue of dose group validated the possible mechanisms of G. triterpenes for the treatment of gastric ulcer



Abbreviations used: HP: Helicobacter pylori, ECL: enterochromaffin-like,TCM: Traditional Chinese medicine; HPLC: High Performance Liquid Chromatography, HPLC/MS: High Performance Liquid chromatography Mass Spectrometry, HPLC-TOF-MS: High Performance Liquid Chromatography and Tof Mass Spectrometry, SD: Sprague Dawley, PCDL: Personal Compound Database and Library, MPP: Mass Profiler Professiona; PCA: principal component analysis, RT-PCR: real time polymerase chain reaction, PGE_2: Prostaglandin E2, COX1: cyclooxygenase 1 S1P: Sphingosine-1-phosphate, AA: Arachidonic acid, 5-HT: 5- hydroxytryptamine.

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INTRODUCTION

"Once an ulcer, always an ulcer" is a maxim that reveals the frequent recurrence and difficult treating characteristics of human gastric ulcer. At present, the pathogeny of the disease is believed to be caused by endogenous or exogenous stimulation.[1-4] Although many factors are considered to be involved in the pathogenesis of gastric ulcer, the mechanism of ulcer formation is not yet precisely understood.[1,2] Gastric

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acid secretion, bile regurgitation, Helicobacter pylori (HP) infection, gastric mucosal defensive ability decline are all relevant etiological factors for the development of gastric ulcer,[5,6] in which gastric acid secretion and HP infection are considered to be the most important factors leading to ulcer. At present, omeprazole, ranitidine and other western drugs are commonly used in the treatment of this disease, but the recurrence rate of ulcer is high. If people who suffered from this kind of disease stop taking drugs for 12 months, the relapse of anabrosis ulceration can reach up to 88%.[7] Nowadays, the prolonged use of non-steroidal antiinflamatory drugs plays a pivotal role in the pathogenesis of gastric ulcer and its recurrence. Furthermore, side effects such as osteoporosis, hypergastrinemia and hyperplasia of enterochromaffin-like cells (ECL) are common in the prolonged therapy with this kind of drugs.[6] Traditional Chinese medicine (TCM) is considered to be natural and harmless. Along with its increasing acceptance worldwide in recent years, clinical and experimental study about the treatment of gastric ulcer with TCM and the improvement of the healing quality have made gratifying progress.[8,9]

Glycyrrhiza is one of the most popular herbal medicines in the world, it is the dry root and rhizome of *Glycyrrhiza uralensis* Fisch., *G. inflata* Bat. or *G. glabra* L.,[10] in which *G. uralensis* Fisch. is the main medicinal species.[11] *Glycyrrhiza* was firstly cited in *Shen Nong's Herbal*, a classical masterpiece of traditional Chinese medicine[12] and was addressed as "Guolao" by Tao Hongjing, one of the most famous medical experts in the Southern Dynasties. The main chemical components of *G.* triterpenes curing gastric ulcer are *G.* triterpenes and flavonoids, and the main ingredients are glycyrrhizin, glycyrrhizic acid, liquiritin, liquiritigenin aglycone, and so on.[13-16] It is reported that *Glycyrrhiza* has certain protective and healing effect on oral ulcer, gastric ulcer and so on, but the mechanism has not understood exactly.[17-19]

Metabonomics is a technique commonly used to study metabolic pathways in biological systems by observing changes in the metabolites of the biological system with time or after stimulation or interference.[20-23] Analytical methods based on high performance liquid chromatography-mass spectrometry (HPLC/MS) have been used widely in metabolomics studies.[24] In this study, based on acetic acid induced gastric ulcer model in rats, a HPLC/MS based metabolomics approach was established to explore differential metabolites by analyzing plasma specimens collected from control group, model group, *G.* triterpenes high, middle and low dose groups;[25] in addition, the expressions of key genes involved in the altered metabolic pathways were examined, which facilitate the understanding of the action mechanism of *G.* triterpenes treating gastric ulcer and provide a broad prospect for its clinical application.

MATERIALS AND METHODS

Ethical statement

All experiments were performed in accordance with the approved animal protocols and guidelines established by Medicine Ethics Review Committee for animal experiments of Liaoning University of Traditional Chinese Medicine. The treatment protocols were approved by the Animal Care and Use Committee (HUCM-2014-03401) and handled according to NIH guidelines.

Drugs and reagents

Omeprazole was purchased from Gankang Pharmacy (Jilin, China). Mass spectrometry acetonitrile was purchased from J. T. Baker (NJ, Germany). HPLC(High Performance Liquid Chromatography) grade formic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA) and methanol was purchased from Merck (Darmstadt, Germany). Water was purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Plant material, extract preparation, and phytochemical analysis

Glycyrrhiza was purchased from Bozhou in Anhui (Inner Mongolia, located in the east longitude 97°12'–126°04', north latitude 37° 24-53°23', identified as the dry root and rhizome of *G. uralensis* Fisch. by Professor Yanjun Zhai from Liaoning University of Traditional Chinese Medicine. The content of liquiritin is 0.632% and glycyrrhizic acid is 2.14%, conform to the requirements of pharmacopoeia of the People's Republic of China.). *Glycyrrhiza* triterpenes was made in the laboratory. *Glycyrrhiza* was reflux extracted with 90% ethanol, extracted with petroleum ether and ethyl acetate, then purified with X-5 resin. The extract was again extracted with ethyl acetate and the content of *G.* triterpenes was detected with vanillin-perchloric acid colorimetric method,[26] the total content of the triterpenes exceeded 80%.

Animals

A total of 60 male SD (Sprague Dawley) rats weighing 200 ± 20 g, were provided by the Experimental Animal Center of Dalian Medical University. All animal housed in the SPF grade Experimental Animal House in environmentally controlled conditions (22°C, RH 50%-60%), treatments were strictly in accordance with the National Institutes of Health Guide to Care and Use of Laboratory Animals.

Animal handling and sample preparation

Gastric ulcer was induced in the rats according to the method in a previous report with a slight modification.[27-29] Three days after the production of gastric ulcer, sixty healthy SD rats were randomized into six groups: Control group, model group, omeprazole group (0.8 mg/mL), triterpenes high dose group (378.0 mg/mL), triterpenes middle dose group (126.0 mg/mL), and triterpenes low dose group (42.0 mg/mL) (Not: *G.* triterpenes dosage group is calculated as, m = M × 0.018 × N/200 × n. M: Pharmacopoeia specified in the daily doses of most people; 0.018: The conversion coefficient of rat and human body; N: The weight of rats; n: extraction ratio. And this dose as low dose.). All rats in groups were orally administered the active group solution 1.5 mL once daily (model and control groups with saline) for 7 days. On the last day, rats were deprived any food for 12 h before the experiments, but free for water, 10% 0.4 mL/100g intraperitoneal injection of chloral hydrate deeply anesthetized rats, the blood was collected, plasma and serum were separated via centrifugation at 3000 rpm for 15 min at 4°C. The plasma samples were collected and stored at −80°C before metabolomics analysis. And the stomachs were cut along the greater curvature, washed with saline. The ulcer long diameter and short diameter were measured under the magnifier with vernier caliper and calculate the ulcer area. Part of the stomach with 10% formaldehyde 24 h would be well fixed, paraffin embedded, serial section, spacing of 5 μm, HE stained, and observed under light microscopy. Other gastric ulcerated tissues were rapidly removed and frozen in liquid nitrogen until the extraction of total RNA. And the general experimental procedure is shown in [Figure 1].

HPLC-TOF-MS (high performance liquid chromatography and tof mass spectrometry)

Chromatography was performed using an Agilent 1100 series HPLC system equipped with an online degasser, an autosampler, a quaternary pump, and a thermostatically controlled column compartment. Plasma samples were separated on Agilent ZORBAX SB-C18 column (4.6 × 100 mm), using water 0.1% formic acid (solvent B) and acetonitrile (solvent A), and the gradient elution program was 30-67% B at initial–5.0 min, 67%–72% B at 5.0–7.0 min, 72%–98% B at 7.0–12.0 min. The column and sample glass vials were maintained at 45 and 4°C, respectively. The

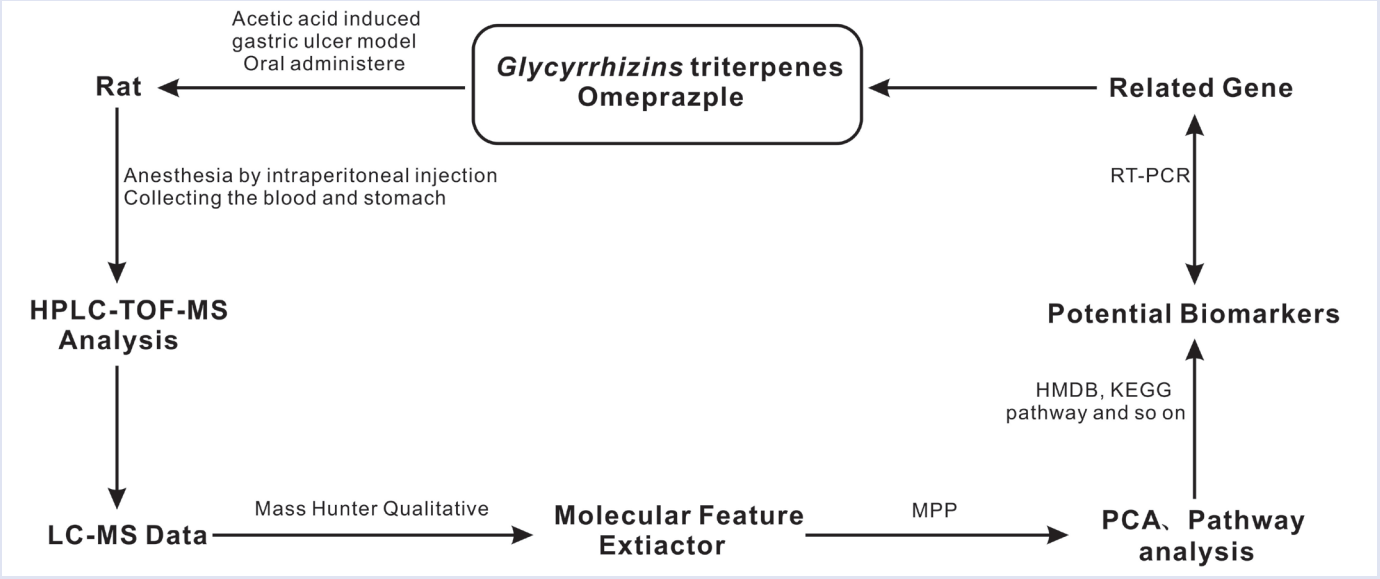

Figure 1: The experimental procedure.

mobile phase flow rate was as 1 mL/min with split ratio 1:3 and the sample injection volume was 4 μL. The eluent was introduced to the mass spectrometer directly. For mass spectrometry, the Agilent 6220 TOF-MS with an electrospray ionization source in negative mode was used. And the source parameters set as follows: Drying gas (N_2) flow rate, 9 L/min; pressure of nebulizer gas, 45 psig; gas temperature, 350°C; fragment voltage, 120 V; MS data were acquired in full-scan mode from m/z 50 to 1050 amu over 0-12 min, and the collision energy of MS/MS date acquisition was set at 20 eV and 25 eV.

Multivariate analysis

All the data were processed with the qualitative analysis B. 06.00 (Agilent, USA). The resultant data matrices were introduced to mass profiler professional (MPP) 12.6 for the principal component analysis (PCA). Variables that had significant contributions to discrimination between groups were considered as potential and subjected to further identification for the molecular formula. The mass hunter PCDL(Personal Compound Database and Library) manager program (Agilent, USA) was used to facilitate the MS/MS fragment ion analysis process by chemically intelligent peak-matching algorithms. The pathway analysis of potential biomarkers were performed with the MPP software, which was based on the database source including HMDB, KEGG, METLIN, LIPID MAPS, PUBCHEM, and other software or databases.

RNA extraction and cDNA synthesis

Each stomach tissue sample including control, model, omeprazole, and triterpenes middle groups was used for RNA extraction with TRIZOL (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized with 1 mg of total RNA, using Trans Script first-strand cDNA synthesis Super Mix kit (Beijing Trans Gen Biotech, China). Differential gene expression was evaluated by real time polymerase chain reaction (RT-PCR), using TransStart™ Top Green RT-PCR Super Mix kit (Beijing Trans Gen Biotech, China). Primers used to amplify H^+-K^+-ATPase, PLA_2 were from invitrogen and expression of these transcripts was quantified against the housekeeping gene β-actin, which was amplified using the forward 5'-ATCATTGGACGCATCGCCTCTCTGG-3', 5 -TGACAG CAGGAAGCGAACGA-3' and the reverse 5'-GTCTTCTGTGGTGTC CGCCGTGTGG-3', 5'-GACTCATACAGTGCCTT-3'. Expression levels of target genes were analyzed using the CFX manager system (BIO-RAD, USA).

Statistics processing

SPSS 19.0 statistical software was used for statistical analysis, and one-way analysis of variance (ANOVA) was used to design the group data. The log transformed values were used when the variance was not homogeneous. P values less than 0.05 were considered significant, less than 0.01 more significant.

RESULTS AND DISCUSSION

Effect of *G.* triterpenes on healing of gastric ulcer

The average ulcer area of rats in model group presents a significant increase compared with control group (P=0.000) [Figure 2]. After treating with omeprazole and *G.* triterpenes, the ulcer areas of omeprazole and triterpenes high, middle, and low dose groups decreased significantly by 63.17%, 44.95%, 49.66%, and 55.43%, respectively. And from the ulcer index results, it can be seen that the effect of *G.* triterpenes low dose group was similar to omeprazole group. Furthermore, histopathological observation was used to confirm the damage of acetic acid to the superficial layers of gastric mucosa and illustrate the mucosa protective effect of drugs. Compared with control group [Figure 3A], the stomach tissue of model group [Figure 3B] appeared as plenty of inflammatory cell infiltration, the ulcer bed emerged as granulation tissue formation, fibers were arranged in disorder. Compared with model group [Figure 3B], damages in all drug groups [Figures 3 C, D, E, F] were shallow with thicker mucous layer, less inflammatory cell infiltration, orderly arranged fibers, especially in *G.* triterpenes low dose group [Figure 3F], whose effect was equal to that of omeprazole [Figure 3C]. From the above results, it can be seen that low dose of triterpenes presented better curing effect, which may have some relationship with the dual-directional regulation efficacy of some endogenous metabolites, and needs to probe in further research.

PCA analysis of metabolites

PCA is a non-targeted statistical method used to define nonobvious differences between samples.[30] It was performed with the quality analyzer 12.6 of MPP. On the observation of three-dimensional plots [Figure 4], samples in the same

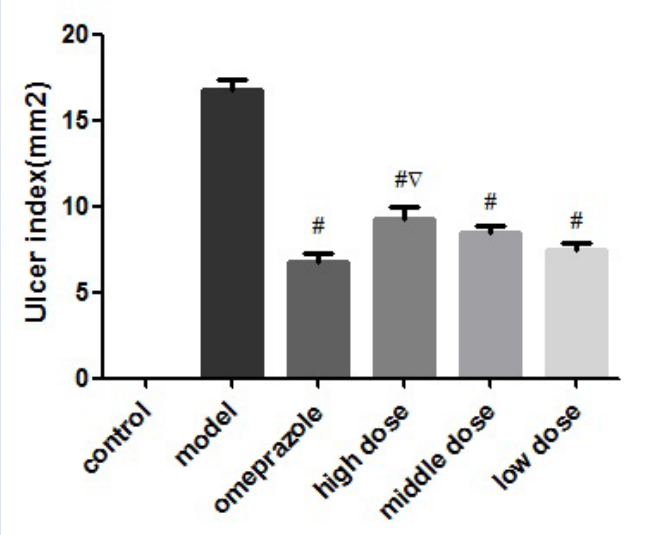

Figure 2: Ulcer index calculation result. Note: Ulcer area = ∏ × length (mm) × width (mm) / 4. The values are the means ± SD. Significant difference $^{\#\#}P < 0.01$ compared with the model group. And triterpenes dosage group was different ($\nabla P < 0.05$) from the low dose group.

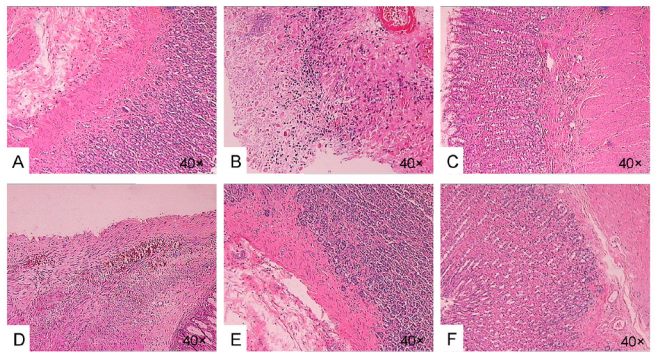

Figure 3: H and E staining for histological evaluation. Note: Typical photographs of control group (A), model group (B), omeprazole group (C), high dose group (D), middle dose group (E), low dose group (F) sections stained with H and E.

group is clustered and the model group differed significantly from others, indicating that the model of acetic acid induced gastric ulcer was successfully reproduced. Moreover, the spatial position of *G.* triterpenes groups was close to control group, argued that the plasma metabolite composition of gastric ulcer rats has a tendency to back to normal after treatment.

Identification of endogenous metabolites with difference

PCA combined with T test, ANOVA, multiple test and other statistical analysis methods, the compounds with significantly difference between groups ($P < 0.05$, $f > 2$) were selected. MPP 12.6 software was used to match the ID Browsers Mentlin (Agilent) and the molecular formula, the possible results, and compounds were obtained. Using Agilent TOF-MS analysis of compounds with significant difference and with the help of containing 23000 metabolite information database search function, according to the KEGG, HMDB metabolite databases the potential structures of endogenous metabolites with difference were identified

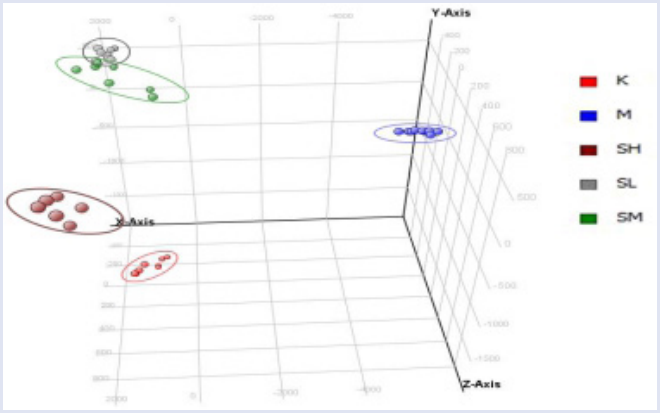

Figure 4: Principal component analysis of each groups. Note: Each colored point represents a sample. The first, second, and third principal components are displayed on the X, Y, and Z-axis, respectively. These three components represent the largest fraction of the overall variability. Red ball: Control group; blue ball: Model group; brown ball: Triterpenes high dose group; green ball: Triterpenes middle dose group; gray ball: Triterpenes low dose group.

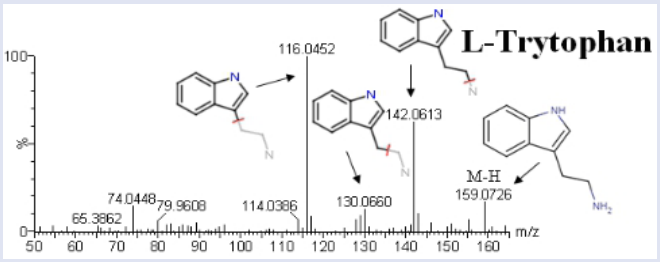

Figure 5: Mass spectromertry spectra of L-tryptophan

according to the MS/MS data [Table 1]. L-tryptophan was taken as an example to illustrate fragments of the structure and the appraisal process. The primary and secondary mass spectrometry information was analyzed by Masslynx (vision 4.1, waters) software, compared with database, and ion fragments of 204.0899 ($C_{11}H_{12}N_2O_2$) was shown in [Figure 5]. The main fragment ions analyzed by MS/MS screening of L-tryptophan was m/z 116.0452 (C_8H_7N), 142.0613 ($C_{10}H_9N$), 159.0726 ($C_{10}H_{12}N_2$), and 130.0660 (C_9H_9N), which was speculated as L-rryptophan after referring and according to its polarity size.

Pathway analysis

According to the formation mechanism of gastric ulcer, related compounds with differences were determined preliminarily, then after further screening and confirmation with Agilent-TOF-MS, combined with the query and analysis of KEGG, HMDB, CHEMICBOOK, METLIN and other databases, the relationship between endogenous metabolites with difference in plasma of *G.* triterpenes and relevant pathways was as follows [Figure 6].

Tryptophan metabolism disorders can cause the dysfunction of the nervous system and then affects the function of the gastrointestinal tract.[31-35] In the presence of tryptophan hydroxylase, tryptophan can generate 5-hydroxytryptamine (5-HT), more than 95% of which present in the gastrointestinal tract, among which 90% is contained in enterochromaffin cell, function as sensory transducers that respond to mechanical or chemical stimulation of the mucosa by releasing

Table 1: Identified endogenous metabolites with difference in plasma of *Glycyrrhiza* triterpenes

No.	RT (min)	Exact Mass (Da)	Fragment ions	Molecular formula	Identity	Regulation
1	1.030	102.0317	55.01, 57.03(20V)	$C_4H_6O_3$	Succinic acid semialdehyde	up
2	1.084	168.0283	41.99, 124.01, 69.01(20V)	$C_5H_4N_4O_3$	Uric acid	down
3	1.302	219.1107	88.04, 146.08, 71.05(20V)	$C_9H_{17}NO_5$	Pantothenic acid	down
4	1.320	204.0899	116.04, 142.06,159.07(25V)	$C_{11}H_{12}N_2O_2$	L-tryptophan	up
5	2.569	513.276	512.26, 79.95, 124.00(25V)	$C_{26}H_{43}NO_7S$	Sulfolithocholylglycine	down
6	3.499	515.2917	514.28, 498.28, 124.00, 79.95(25V)	$C_{26}H_{45}NO_7S$	Taurallocholic acid	down
7	1.033	180.0634	59.01, 71.01(20V)	$C_6H_{12}O_6$	D-Glucose	down
8	5.186	381.2647	224.06, 82.02, 165.12, 379.24(25V)	$C_{18}H_{40}NO_5P$	Sphingosine-1-phosphate	down
9	8.025	320.2351	319.22, 255.23, 179.09, 135.11(25V)	$C_{20}H_{32}O_3$	(+/-)14,15-EpETrE	down
10	3.535	499.2968	498.28, 79.97, 496.26, 124.04(25V)	$C_{26}H_{45}NO_6S$	Tauroursodeoxycholic acid	down
11	10.115	284.2715	212.24, 143.13, 117.09(25V)	$C_{18}H_{36}O_2$	Stearic acid	down

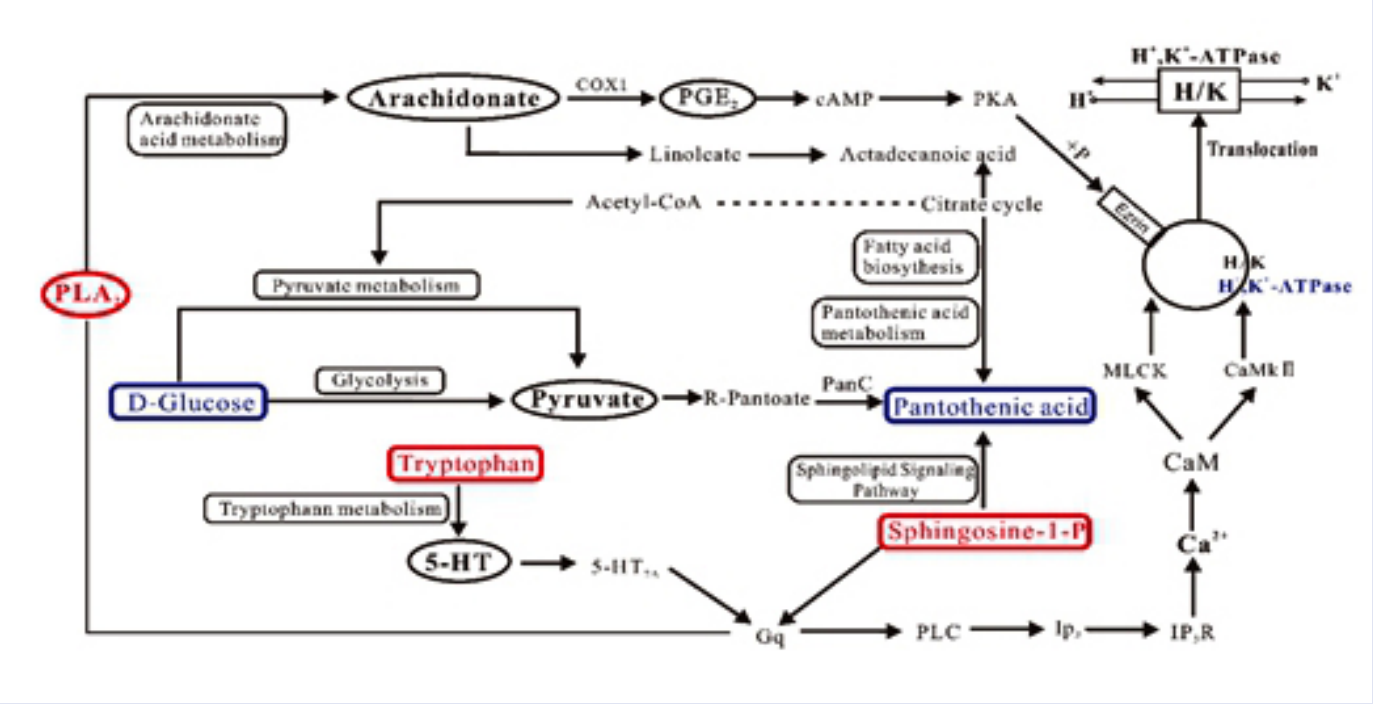

Figure 6: Antiulcer effect pathway of *G.* triterpenes

5-HT.[36-39] 5-HT is important neurotransmitter and adjacent secreted signaling molecule involves in both regulation of intestinal peristalsis and secretion function. In this experiment, *G.* triterpenes, through regulating tryptophan metabolism, can indirectly increase the content of 5-HT *in vivo*, when it is binding specifically with 5-HT_{2A} receptor, Gq can be generated and combine with phospholipase C β receptor, then phosphatidylinositol-3 (IP3) is produced and IP3 can stimulate internal Ca^{2+} release through acting on specific receptor on the endoplasmic reticulum. Extracellular Ca^{2+} influx and intracellular luminal Ca^{2+} release from the ER can evoke a Ca^{2+}-reliant sequential activation of Ca^{2+}/calmodulin-dependent protein kinase II and myosin light chain kinase, which contribute to carry H^+-K^+-ATP enzyme to the apical membrane of transitional cell membrane through the activation of protein kinase, streptokinase, and eventually make the enzyme embedded in top. Eventually induce the exchange of extracellular K^+ and H^+ to reduce gastric acid secretion,[40] and present the effect of antigastric ulcer. The action mechanism is similar to omeprazole.

Arachidonic acid (AA), a component of the cell membrane, is known to be metabolized to prostaglandins, thromboxane A_2, leukotrienes, and other bioactive substances.[41,42] Prostaglandin E_2 (PGE_2) is the first discovered

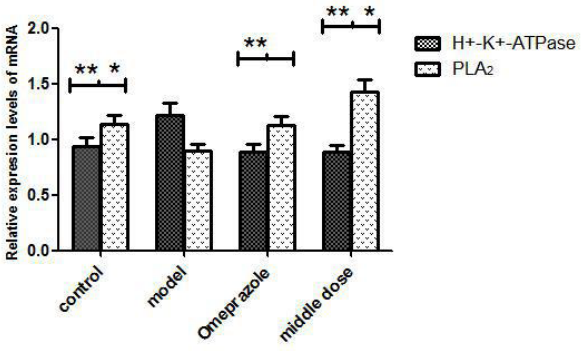

Figure 7: The expression level of mRNAs. Note: The ordinate represents the relative expression levels of mRNAs in the basis of control group and abscissa represents the mRNAs of H^+-K^+-ATPase and phospholipase a2. Values are expressed as mean ± standard deviation. The method of relative quantitative analysis was used to compare the gene expression in each group. Significant difference $^{**}P < 0.01$ compared with the model group.

endogenous substance which is known to have a cell protective effect and regulates the secretions of pepsinogen and mucus and the motility of gastric smooth muscle.[43,44] Cell membrane phospholipids will transform into AA by the stimuli of PLA_2 after suffering external irritation, and then under the effect of cyclooxygenase 1 (COX1), PGE_2 is generated and inhibit H^+-K^+-ATPase activity to exert its antisecretory effect by inhibiting the cAMP pathway, and promote carbonate and gastric mucus secretion to protect the gastric mucosa. PLA_2, as the rate limiting enzyme in the synthesis of PGE_2, has a great influence on the mucosal protective effect of PGE_2. After orally admininstrated *G.* triterpenes, the expression of PLA_2 mRNA was increased and H^+-K^+-ATPase alpha subunit mRNA decreased significantly in gastric tissue of rats compared with model group [Figure 7], which demonstrated that *G.* triterpenes could promote the release of PGE_2 by promoting the synthesis of PLA_2 *in vivo* and through the above mechanism to protect the gastric mucosa and inhibit the development of gastric ulcer.

Sphingosine-1-phosphate (S1P) and ceramide are two important bioactive metabolites derived from metabolic pathway of sphingosine. S1P is now recognized as an important intracellular messenger and extracellular media, participating in various cellular functions such as antiapoptotic and inflammatory signaling pathway.[45-47] When it accumulates in the gastrointestinal mucosa, it will cause ulcers.[48] From the experimental results we can infer that the *G.* triterpenes not only reduce the content of S1P by adjusting the sphingosine metabolism, but also reduce inflammatory mediators releasing and apoptosis of gastric mucosa cells, so as to promote the recovery of gastric mucosa and exert the effect of antiulcer. In addition, the content of uric acid in plasma was decreased by the administration of *G.* triterpenes, which released the inflammatory response induced by abnormal metabolism of purine.[49,50] Besides, the metabolic processes of pantothenic acid and D-glucose can generate pyruvate, whose metabolism is associated with the morbidity of gastric ulcer. *G.* triterpenes can indirectly influence the development of gastric lesions by regulating glucose and pantothenic acid metabolism.[50,51]

Combined with the above discoveries, it is certificated that *G.* triterpenes possesses the same mechanism of inhibiting the proton pump to limit the secretion of gastric acid with omeprazole, besides that it can also reduce the release of inflammatory mediators and protect gastric mucosa to prevent the development of peptic ulcer disease, which revealed the multiple pathway integrated regulation function of *G.* triterpenes.

CONCLUSION

Acetic acid induced gastric ulcer model in rats was established to evaluate the curing effect of *G.* triterpenes. Combined with the ulcer area, ulcer inhibition rate and pathological results of stomach tissue, it is demonstrated that *G.* triterpenes can obviously relieve the symptoms of gastric ulcer, especially the low dose group. Furthermore, HPLC-TOF-MS based metabolomic study was applied to investigate the mechanism of action. It is determined that *G.* triterpenes can effectively regulate the amount of small molecule metablism in gastric ulcer rats *in vivo*, including tryptophan, phingosine-1-phosphate, and other *G.* triterpenes' important, sensitive potential biomarkers, involving tryptophan metabolism, AA metabolism, sphingosine metabolism, and other metabolic pathways. The down-regulation of H^+-K^+-ATPase alpha subunit mRNA and up-regulation of PLA_2 mRNA in gastric tissue of dose group validated the possible mechanisms of *G.* triterpenes for the treatment of gastric ulcer in a molecular level by RT-PCR. In summary, we speculate that *G.* triterpenes might through inhibiting gastric acid secretion, reducing the release of inflammatory mediators and protecting the gastric mucosa to cure gastric ulcer, which revealed the multipathway integrated adjustment mechanism of *G.* triterpenes and laid a foundation for seeking and developing new, high potential, and less side effects drugs for treating gastric ulcer.

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Conflicts of interest

There are no conflicts of interest.

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