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ORIGINAL ARTICLE

Role of *Saccharum Granorum* as a "Principal Drug" in a Traditional Chinese Medicine Formula against Chronic Atrophic Gastritis Rats

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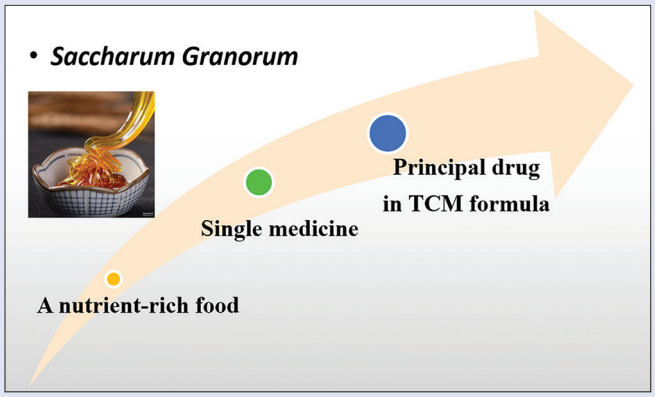
ABSTRACT

Background: *Saccharum Granorum* (YiTang) is a nutrient-rich food that has been used as traditional Chinese medicine (TCM). However, the mechanism responsible for its beneficial efficacy is poorly understood, especially for its roles involved in the TCM formulas. **Materials and Methods:** In the present work, a classical TCM formulas, Huangqi Jianzhong Tang (HQJZ), was selected to interpret the efficacy and "principal drug" role of YiTang against chronic atrophic gastritis (CAG) rats using a serum metabonomic approach. **Results:** Twelve candidate metabolites were identified to characterize the difference between CAG and normal rats, which were involved into four disturbed metabolic pathways, including glucose metabolism, amino acid metabolism, fatty acid metabolism, and choline metabolism. The further partial least square regression analysis revealed that five metabolites, including choline, acetate, alanine, a-glucose, and β-glucose, were considered as potential biomarkers related to CAG. YiTang could exert the synergistic effect with HQJZ without YiTang, where the whole formula obtained the best beneficial treatment against metabolic disturbance induced by CAG. The metabolic improvement of YiTang and HQJZ might exert the effects on the dysfunction of energy metabolism, gastric emptying, and the changes of gut microbiome, which were the important pathomechanisms induced by CAG. **Conclusion:** These findings suggested that YiTang played an indispensable role in HQJZ formula against CAG, which may provide an approach to understand the fundamental idea behind formula construction.

Key words: Chronic atrophic gastritis, Huangqi Jianzhong Tang, metabonomics, partial least square regression analysis, *Saccharum Granorum*

SUMMARY

- A study to reveal the principal contribution of *Saccharum Granorum* to HQJZ against CAG



Abbreviations used: TCM: traditional Chinese medicine; HQJZ: Huangqi Jianzhong Tang; CAG: chronic atrophic gastritis; PLS-RA: partial least square regression analysis; HE: hematoxylin-eosin; 2D: two-dimensional; PA: Pepsin activity; COSY: 1H-1H correlation spectroscopy; HSQC: ^{1}H-^{13}C heteronuclear single-quantum correlation spectroscopy; PCA; principal component analysis; OPLS-DA: orthogonal partial least squares discriminate analysis; VIP: variable importance in the projection.

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INTRODUCTION

Saccharum Granorum (YiTang), also known as gum and jelly, is a nutrient-rich food containing glucose, fructose, vitamins, and other ingredients. It has widely used to tonify qi and strengthen stomach and spleen in Chinese medicine.[1] At present, its roles as supplement material have been widely used in the pharmaceutical industry. Interestingly, it has been characterized as one principal drug in some classical traditional Chinese medicine (TCM) formulas. Huangqi Jianzhong Tang (HQJZ), one celebrated TCM formulas characterized it as "principal drug."[2] contains a mixture with six other Chinese herb medicines, including the root of *Radix astragali* mongolici (HuangQi), the bark of *Cortex cinnamomi* (GuiZhi), the root of *Radix paeoniae* alba (BaiShao), the fruit of *Fructus jujubae* (DaZao), the stem of

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Zingiberis rhizoma Recens (ShengJiang), and the root of *Glycyrrhiza uralensis* fisch (GanCao). Clinic and pharmacological researches have demonstrated that this formula could significantly treat chronic atrophic gastritis (CAG) through inhibition of inflammation, oxidative stress, and endothelial and mucosal injuries.[3,4] However, its contribution as a monarch in the prescription of HQJZ for treating CAG remains unclear.

Metabonomics has been widely developed to investigate the regulative mechanism and the compatibility of TCM due to its consistent with the systemic scientific connotations of TCM.[5,6] Our previous metabonomic study showed that the metabolic improvement might be the important contribution to the protection of HQJZ from CAG.[7,8] While differential compatible efficacy of its constitute are challenges, the application of metabonomic approach might provide new insights into its biological efficacy and the compatible regularity.[9,10]

Herein, we address a novel method to characterize the monarch role of YiTang, in which we compare the metabolic regulation of YiTang among single YiTang, the whole HQJZ formula with and without YiTang based on a metabonomics study. As we know, no study to utilize metabonomics study was applied to explore the effect of YiTang against CAG and its application in TCM formula.

MATERIALS AND METHODS

Materials

Te root of Astragali Radix, the bark of Cinnamomi Ramulus, the root of Paeoniae Radix Alba, the fruit of Jujubae Fructus, the stem of Zingiberis Rhizoma Recens and the root of Glycyrrhizae Radix et Rhizoma, were obtained from Taiyuan Tongren Tang pharmacy (Taiyuan, Shanxi, China). YiTang was provided by Jingchun Tianli Biotech, Co. Ltd. (Huanggang, Hubei, China). All medicinal materials were accredited by Professor Xuemei Qin from Modern research center for TCM of Shanxi University (Taiyuan, Shanxi, China). Sodium deoxycholate was purchased from Beijing Aoboxing Bio-tech, Co. Ltd. (Haiding, Beijing, China). Deuterium oxide (D_2O) with 0.05% 3-trimethylsilyl-(2, 2, 3, 3-$2H_4$)-1-propionate were supplied by Sigma-Alorich (St. Louis, USA). Pepsin activity (PA) assay kit was purchased from by Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). Others chemicals used were of analytical grade in the present study.

Drug preparation

HQJZ was prepared and monitored as our previous report,[11] of which the chemical profile is shown in Supplementary Figure 1. The preparation was processed as below: six mixed crude herbs, consisting HuangQi (9 g), GuiZhi (9 g), BaiShao (18 g), ShengJiang (12 g), GanCao (6 g), and DaZao (4 fruits), were crushed and soaked in distilled water for 30 min at room temperature. The extraction process was refluxed for 2 h twice (10:1 and 8:1, v/w). Combing and concentrating the two extracts, 30 g of Yitang was added to make the volume to 1000 mL. The preparation of HQJZ without YiTang (YY) was also carried out using the same procedure with some modification, which was not mixed with the amount of YiTang. Then, these extracts were stored at −20°C before use.

Animal treatment

The whole experiment was authorized by the Animal Ethics Committee of Shanxi University (SXU-2017-06-011). The procedures were carried out according to the National Guidelines for Experimental Animal Welfare (MOST, China, 2006), ensuring to minimize suffering. SPF-grade male Sprague-Dawley rats (180 ± 20 g) were supplied by Beijing Vital River Laboratory Animal Technology Co. Ltd. These animals were housed in a climate-controlled room with a 12 h light/dark cycle (23°C ± 1°C and 60% humidity).

After adaptation for 7 days, the animals were randomly divided into five groups (6 per group) including control group (C), model group (M), HQJZ-treated group (HQJZ), YY-treated group (YY), and YiTang-treated group (YT). Rats in the control group were free allowed for the feed and clean water in the process of experiment. CAG rats were induced by freely giving 0.1% ammonia solution and 20 mmol/L deoxycholic acids on alternate days, respectively. Moreover, these rats were treated with irregular fasting cycle of feeding freely 2 days and then fasting 1 day. The CAG rat models method was performed for 10 weeks to stimulate gastric mucosa of these rats. After CAG modeling, HQJZ, YY, and YT groups were treated intragastrically with extracts of HQJZ (10.605 g/kg), YY (7.455 g/kg), and YT (3.15 g/kg) once daily for 4 weeks, respectively. Body weights of each group were measured during the entire experiment.

Sample collection

After the last administration, the animals were fasted for 48 h with full water. Rats were anesthetized and performed the pyloric ligation by an ether overdose. Three hours later, the experimental animals were anesthetized through intraperitoneal injection with 10% urethane. Blood samples were collected from aorta abdominalis and placed in the blood vessel without anticoagulant for 40 min. After centrifugation at 4°C (3000 rpm, 15 min), serum samples were obtained and stored at −80°C. Meanwhile, their stomachs were removed with ligating the cardia after the blood collection, cut along the greater curvature of the stomach and collected the gastric contents of each stomach. Then, the stomach's tissues were washed immediately with ice-cold physiological saline. Some of the gastric tissue was cut off and placed in the phosphate-buffered 10% formalin solution for the histopathological examination. The gastric juices of gastric content were obtained by centrifugation (3000 rpm, 10 min, 4°C). These supernatants of each group were analyzed for the determination of pH and PA concentrations.

Biochemistry assays and histopathology

The changes of PA activity of gastric juice were analyzed according to the instructions of the enzymatic kit. In addition, the application of high precision acidity benchtop instruments to determination of gastric juice pH value. The formalin-fixed gastric tissues were embedded in paraffin (48 h). Hematoxylin and eosin stains were performed using the sections of 3–5 μm, and evaluated using under the microscopy (Olympus, BX53, Japan).

Nuclear magnetic resonance analysis

A volume of 300 μL serum sample was mixed with 300 μL of D_2O (20% normal saline). The mixture was centrifuged for 10 min at 4°C (13,000 rpm). 550 μL of the supernatant was individually transferred into nuclear magnetic resonance (NMR) tube (5 mm). ^{1}H-NMR spectrum analysis of each sample was performed using a Bruker 600-MHz AVANCE III NMR spectrometer (Bruker BioSpin, Bremen, Germany) at 298 K. Samples were analyzed by a one-dimensional (1D) CPMG pulse sequence with water suppression to obtain the signals of low molecular weight. For each NMR spectra of serum samples, 65536 points were collected within 64 scans, 2.654 s acquisition time and a relaxation delay of 1.0 s over a spectral width of 12345.7 Hz. Meanwhile, 2D NMR spectra, including ^{1}H-^{1}H correlation spectroscopy (COSY) and ^{1}H-^{13}C heteronuclear single-quantum COSY, were recorded to assign the followed spectra as same as our previous study.[7]

For data analysis, metabolites of serum samples NMR spectra peaks were identified and validated in-house developed NMR database

and published databases, HMDB (http://www.hmdb.ca/) and KEGG (http://www.kegg.com/).

Multivariate pattern analysis

Data processing

All acquired serum NMR spectra were corrected of phase and baseline using MestReNovasoftware (version 8.0.1, Mestrelab Research, Santiago de Com-postella, Spain). NMR spectra peaks were referenced to internal creatinine CH_3 resonance at δ 3.04 ppm. ^{1}H-NMR spectra were segmented with fixed width of 0.001 ppm over the ranges of δ 0.50–9.00 ppm, eliminating the impact of imperfect water saturation (δ 4.68–5.19 ppm). Then, the whole data were normalized to the total spectra area to reduce differences among samples.

Metabolic profiles analysis

Multivariate analysis of NMR spectral data was analyzed using SIMCA-P software (version 13.0, Umetric, Sweden). Principal component analysis (PCA) was performed to cluster the experimental groups. Orthogonal partial least squares discriminate analysis (OPLS-DA) was utilized to further reveal the differential metabolites from S-plot constructed. Each point represents a region segment on the scores plot, where the edge was hailed as candidate metabolites related to CAG based on contribution to the differences.

Partial least square regression analysis

Partial least square regression analysis (PLS-RA) model were utilized to interpret the relationship between the differential metabolic variables and the biochemical indexes related to the pathology of CAG. Key metabolites were then selected to predict these variables with large contributions to CAG.

Statistical analysis

A two-tailed unpaired *t*-test analysis was applied to select the important variables between two groups using the SPSS software (version 16.0, Chicago, IL, USA), and the value at $P < 0.05$ was considered as the significance threshold.

RESULTS

Body weight change of chronic atrophic gastritis rats

Figure 1a shows that the bodyweight of the rats in each group. Compared with normal rats, CAG rats in the model group showed a significant variation tendency of decrease. After the treatment with HQJZ, the bodyweight change induced by CAG was markedly improved, suggesting that HQJZ had obvious therapeutic effect. Furthermore, the improvement capacities of body weight were HQJZ > YY > YT. Moreover, the pretreatment of YY showed almost similar results as HQJZ.

Biochemical indexes of chronic atrophic gastritis rats

Figure 1b and c shows that the PA and pH changes of gastric juice in the experimental groups. CAG rats in the model group showed marked reduction of PA and increasing pH value compared with normal rats, suggesting sufficient evidence of gastric lesion of CAG rats ($P < 0.01$). HQJZ could improve these dysfunction induced by CAG ($P < 0.01$). YT exerted a weaker effect on the abnormity of pH and showed negative regulation against PA. Meanwhile, YY also showed potential inhibition tendency without significant difference.

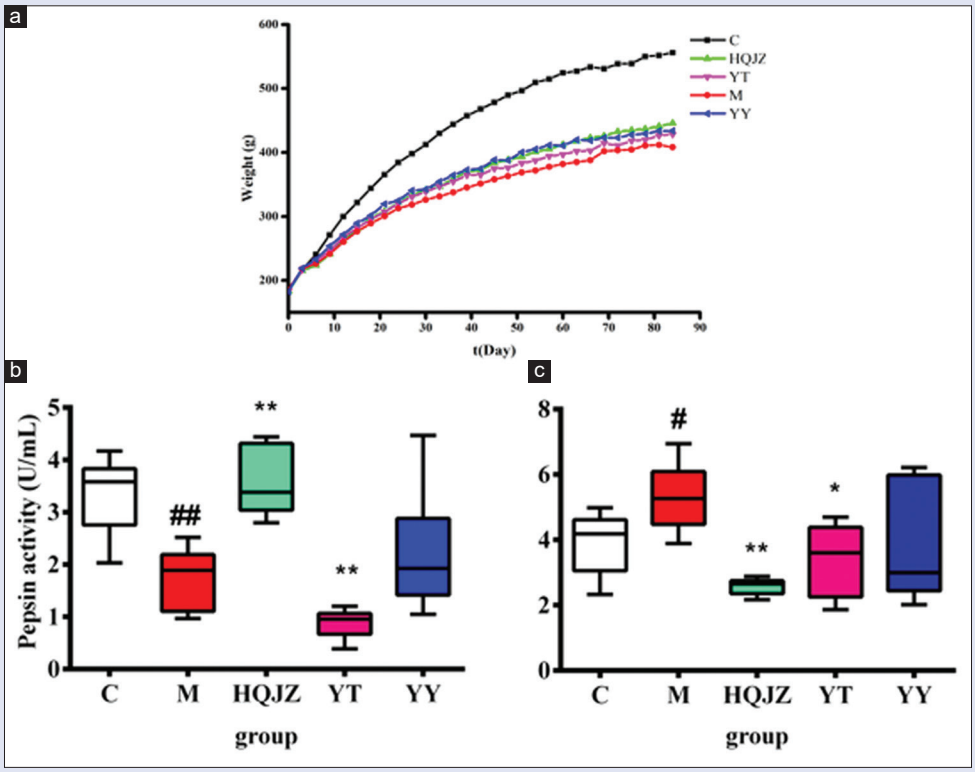

Figure 1: Plot of weight trend (a), pH (b) and pepsin activity (c) in all the experimental groups. Values are expressed as mean ± SD ($n = 6$). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with control group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with model group. (C) Control group, (M) Model group, (HQJZ) HQJZ-treated group, (YY) HQJZ without YT-treated group, (YT) YiTang-treated group

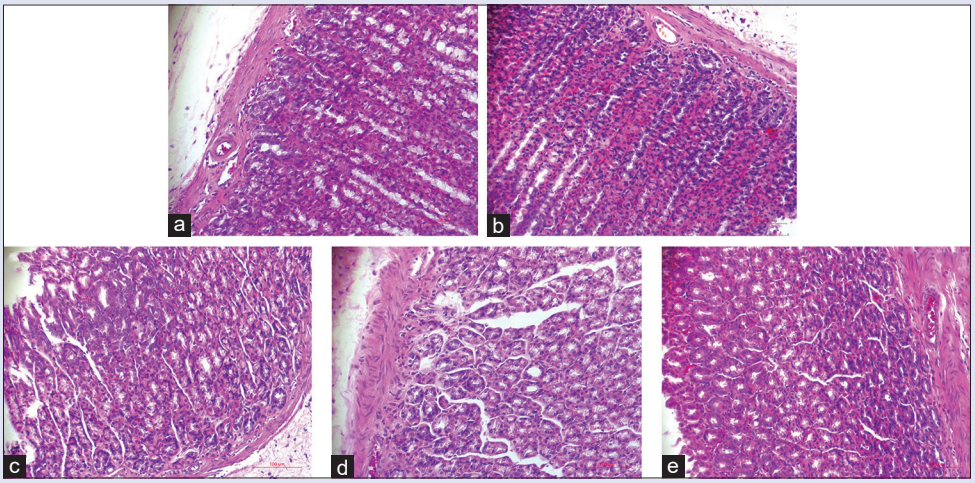

Figure 2: Histological examination of gastric tissues from all the experimental groups. (a) Control group, (b) Model group, (c) Huangqi Jianzhong Tang-treated group, (d) Huangqi Jianzhong Tang without YT-treated group, (e) YiTang-treated group

Gastric injury of chronic atrophic gastritis rats

The analysis of gastric pathological injury of CAG showed that the gastric changes, including the thinned of gastric mucosa, disordered glands arrangement, inflammatory cell infiltration in the interstitium were observed in the CAG rats, which were the typical pathological characterizations of CAG [Figure 2]. These CAG pathological abnormalities were significantly improved by the treatment of them. HQJZ exerted best effect against CAG, which the thickness of gastric mucosa and the eosinophilic infiltration were improved significantly. Meanwhile, the pathological injury of CAG was also be retrieved by YT, which was stronger than YY and weaker.

Serum metabolic profiles of chronic atrophic gastritis rats

The obtained NMR data set with aligning peaks and their peak areas were combined into a single matrix to perform multivariate pattern analysis. PCA was then first applied to district the metabolic profiles based on the preprocessed raw data from the spectra [Figure 3a]. The established PCA model presented satisfactory explanation and prediction with well cross-validations ($R^2X_{(cum)}$ = 0.809 and $Q^2_{(cum)}$ = 0.673) [Figure 3b]. As a result, a clear deviation was observed between the control group and CAG group. The metabolic profile affected by the whole HQZJ-treated group deviated farthest from model group but closed to the control group. Similarly, YY and YT groups were much closer to model group than the whole HQJZ group, indicating that HQJZ obtained the best performance against the deviations induced by CAG.

Differential variables responsible for chronic atrophic gastritis rats

A clear separation between the two groups could be observed in the OPLS-DA score plot [Figure 3c], which was a supervised statistical analysis to arrive maximum separation for two groups. The corresponding S-plots [Figure 3d] were applied to screen differential variables. Combining with VIP (variable importance in the projection) >1.0 and significant difference ($P < 0.05$), 12 differential metabolites were screened as the candidate biomarkers associated with CAG [Table 1].

Table 1: Potential biomarkers identified in rat serum sample based on ^{1}H-NMR

Number	Metabolites	Moieties	Chemical shifts	VIP
1	Isoleucine	CH_3	1.013 (doublet)	1.42
2	Lactate	αCH, βCH_3	1.331 (doublet)	4.21
3	Alanine	βCH_3	1.478 (doublet)	2.27
4	Acetate	CH_3	1.92 (singlet)	1.14
5	Acetoacetate	CH_3	2.278 (singlet)	1.70
6	Glutamate	CH_2, CH_2	2.350 (multiplet), 2.103 (multiplet)	1.43
7	Pyruvate	CH_3	2.371 (singlet)	1.41
8	Glutamine	βCH_2, γCH_2	2.45 (multiplet), 3.77 (multiplet)	2.09
9	Choline	$N(CH_3)_3$	3.233 (singlet)	3.40
10	Serine	NH_2	3.830 (doublet of doublet)	1.12
11	β-glucose	1-CH	4.650 (doublet)	10.08
12	α-glucose	1-CH	5.237 (doublet)	2.22

VIP: Variable importance in projection

Potential biomarkers responsible for chronic atrophic gastritis rats

Restless legs syndrome-RA was further utilized to excavate the potential biomarkers associated with the pathological injury of CAG (PA, pH, and weight change). The relative peak areas of 12 identified metabolites and the corresponding change rates of the pathological indexes were considered as X variables and Y variables, respectively. The cumulate R^2Y and Q^2Y values of the established model related to PA were 0.842 and 0.492, which suggested that this model owned good predictive abilities and fitness of PA. Meanwhile, PLS-RA models between NMR data and pH and weight change showed that the analysis could predict 87.8% and 94.2% of the change of pH and weight change and explain accordingly 65.2% and 78.6% of the information of Y variables. The t1/u1 plots of PLS-RA were also indicated the established model showed a good fitness with an *R*2 value of 0.7066, 0.545, and 0.8684, where *u*[1] (the corresponding pathological indexes scores values) significantly correlated with *t*[1] (the variables score values of each rat).

Furthermore, the important biomarkers related to CAG were screened using the followed VIP plots, correlation coefficient plots and loading

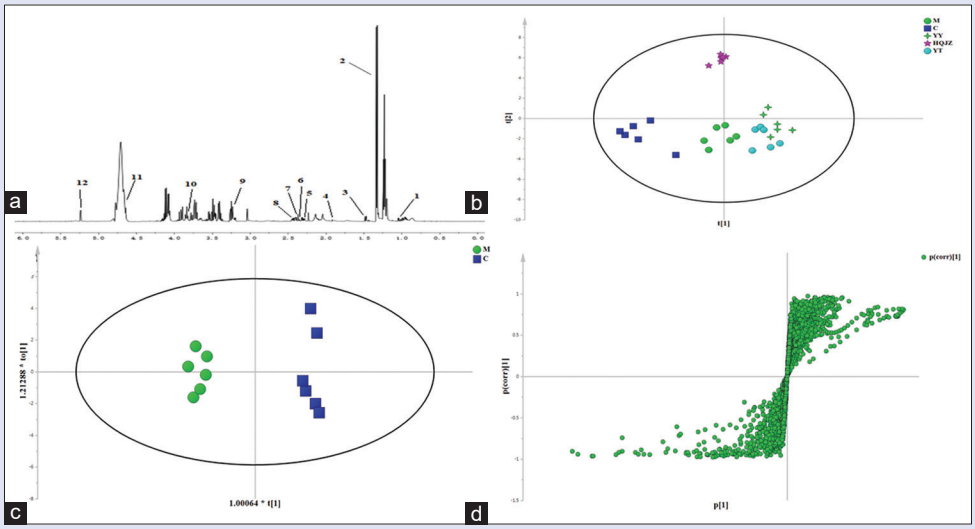

Figure 3: (a) Typical serum samples ^{1}H CPMG NMR spectroscopy; (b) PCA score plot of serum samples collected from different treatment groups; (c) OPLS-DA score plot of serum samples collected from the control and CAG group; (d) OPLS-DA S-plot of serum samples collected from the control and CAG group. (c) Control group, (m) Model group, (Huangqi Jianzhong Tang) Huangqi Jianzhong Tang -treated group, (YY) Huangqi Jianzhong Tang without YT-treated group, (YT) YiTang-treated group

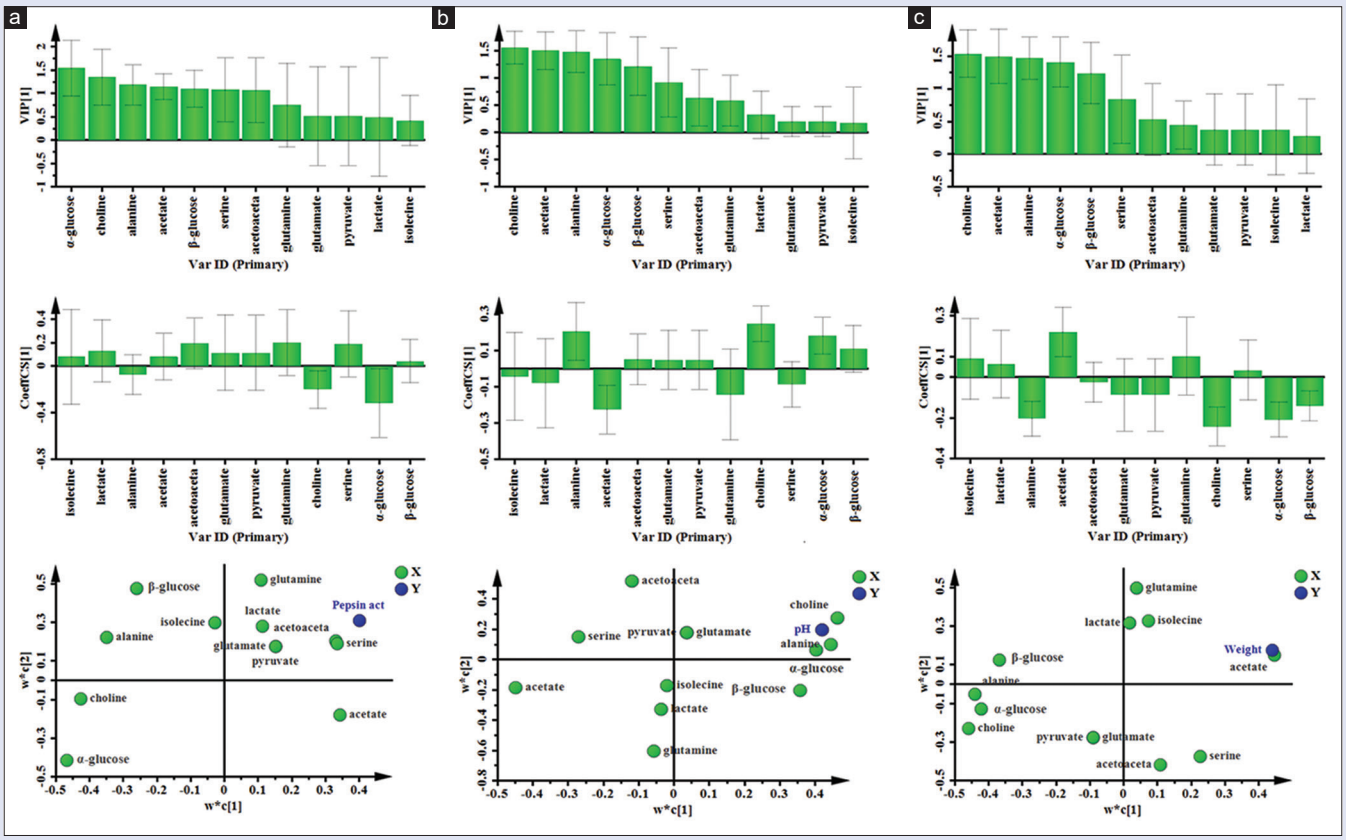

Figure 4: PLS-RA analysis between serum differential metabolites and different pathological indexes (a: PA; b: pH and c: weight change) with from CAG and control groups. The plots were the relative correlation coefficient plot, variable importance in projection plot and loading weights plot from top to bottom, respectively

weight plots from PLS-RA [Figure 4]. The VI*P* values [Figure 4a] were characterized as the contributions of the variables to the change of Y. The larger the VI*P* value, the greater the contribution to the model. VIP >1 of the metabolites indicated that it markedly contribute to CAG development. The correlation coefficients [Figure 4b] indicated basically how strongly Y variables are related to the systematic part of each X

variable. If the confidence interval has not cross zero (displayed by bars in the Figure), it means that variable is significant. The loading weights plot [Figure 4c] showed the relationship among variables. In addition, X variables which contributed more to Y variable were located farther from the origin in the loading weights plots. Hence, those metabolites which had the characteristic of VIP >1, significant coefficients and locating farther from the origin of the loading weights plot were screened as the potential biomarkers related to CAG.

As results, 2 (α-glucose and choline), 4 (choline, acetate, alanine, and a-glucose), and 5 (choline, acetate, alanine, α-glucose, and β-glucose) metabolites were screened as potential biomarkers based on their significant correlation with PA, pH, and weight change, respectively. The various pathological indexes were all used to depict the differential features of CAG. Hence, these indexes were all selected to screen the potential biomarkers. Five metabolites, including choline, acetate, a-glucose, β-glucose, and alanine, were considered as potential biomarkers related to CAG.

Metabolic change of the potential biomarkers

Figure 5 shows the varied tendencies of nine potential markers associated with CAG in each group. Serum concentrations of acetate, acetoacetate, alanine, choline, isoleucine, serine, lactate, pyruvate, glutamine, and glutamate in control group were obviously higher than in CAG group, whereas α-glucose, β-glucose, and serine were significantly reduced in CAG rats. With HQJZ treatment, these abnormalities could be markedly regulated excepting for alanine, lactate, choline, and β-glucose. Treatment of YT exerted the regulative effect of six metabolites (glutamate, pyruvate, alanine, serine, lactate, and α-glucose), while HQJZ with the absence of YT (YY) corrected the variations of acetate, serine, lactate, pyruvate, and α-glucose. Obviously, the combination of YT to YY generated a synergistic reaction as a whole formula, which showed the best meditative actions on these metabolic disturbances. Alternatively, the exclusive use of YT or YY appears to have a relative weaker gastroprotective effect on most of various biomarkers.

DISCUSSION

TCM has recently gained worldwide popularity being due to its overall therapeutic effect in the form of combination drug formulas. Their constitutions range from medicinal plants (herbs), animals, food or mineral substance (such as mirabilite), which acted as the roles of principal drug, ministerial drug, adjunctive drug, and guide drug according to the theory of TCM. As a celebrated TCM formula, HQJZ has been usually used to treat CAG. Previous researches were mainly focused on the action mechanisms responsible for the whole formula or their compounds. Special biological activities were reported for each single component, their exact, and compounds. Astragalus, as a principal drug, could exert some pharmaceutical effect, including anti-oxidation properties, the immune regulative function and the treatment of cardiovascular diseases, inhibition of liver fibrosis, and others.[12,13] The polysaccharides from Astragalus had a beneficial effect

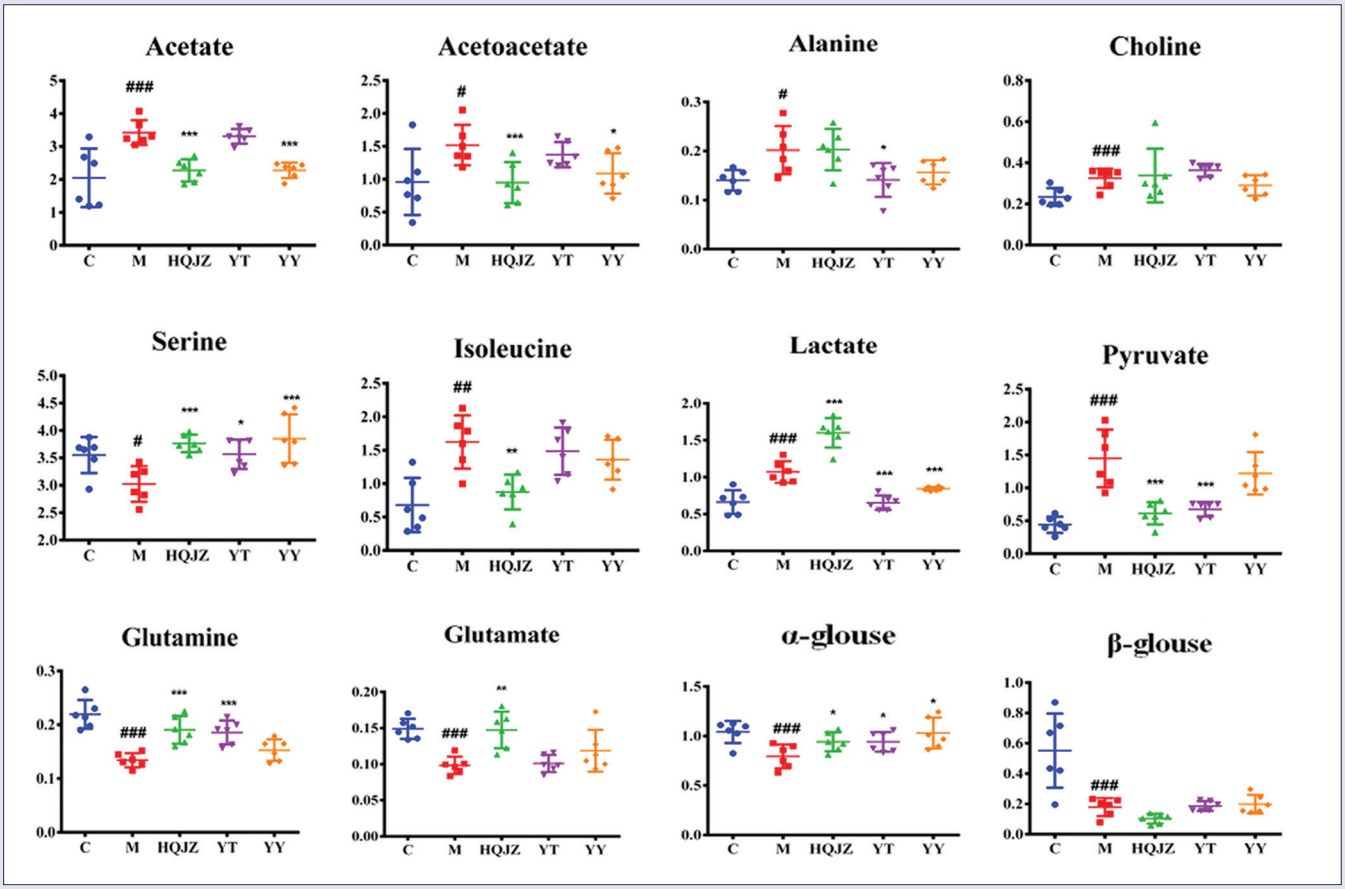

Figure 5: Comparison of the relative intensity of putative potential biomarkers in the CAG rats associated with the experimental drug treatment. $^{\#}P$ <0.05, $^{\#\#}P$ <0.01 compared with control group; $^{*}P$ <0.05, $^{**}P$ <0.01 compared with model group; $^{\#\#\#}P$ < 0.001 compared with control group; $^{***}P$ < 0.001 compared with model group. (C) Control group, (M) Model group, (Huangqi Jianzhong Tang) Huangqi Jianzhong Tang-treated group, (YY) Huangqi Jianzhong Tang without YT-treated group, (YT) YiTang-treated group

on CAG rats by deregulating EGFR at its downstream effectors COX-2 and MMP-2.[14] While astragaloside IV also showed the treatment of CAG through improving the body elimination of oxygen free radicals.[15] A larger number of compounds from *Radix paeoniae* alba (as ministerial drugs in HQJZ) have greater potential for anti-inflammatory, antiviral, antibacterial, and antioxidant activities.[16] The anti-inflammatory effect of volatile oil from *Cinnamomum cassia* might be contributed to anther "ministerial drugs" effect in HQJZ. The other three herbs, *Rhizoma zingiberis*, *Fructus jujubae and Glycyrrhi zauralensis* fisch (liquorice), were all used for their "guide" effects, which might be due to the immunoregulation of *Rhizoma zingiberis and F. jujubae,* the regulation of CYPs and P-gp of liquorice.[17,18] However, the role and compatibility of YiTang (another principal drug) are still poorly understood, especially in the whole formula.

In our study, NMR-based metabonomic approach was introduced to define its "principal drug" role in HQJZ formula against CAG. Consistent with the results of traditional pharmacodynamic indexes studies, the metabonomic study revealed that YT exerted a medium regulation on the metabolic alterations induced by CAG, compared with the whole formula HQJZ with or without YT. Among them, HQJZ showed the best efficacy. The results suggested that the metabolic contribution of YT responsible for the gastroprotective effect of the whole formula might be due to its synergistic action with the other constitutes. Four metabolic pathways were disturbed by CAG, including glucose metabolism, amino acid metabolism, fatty acid metabolism, and choline metabolism. Compared with YT and YY, the whole HQJZ pretreatment could almost mediate all the metabolic alterations induced by CAG.

Glucose metabolism

Increasing works have showed that malignant transformation was involved into an increase in glycolytic flux and lactate excretion, which named "Warburg effect."[19,20] In this study, the decreasing of α-glucose and β-glucose and increasing of lactate, pyruvate and acetoacetate were observed in model rats, suggesting glucose metabolism was promoted to ameliorate gastric injury. In addition, significant correlations of α-glucose and β-glucose with pH and weight change also indicated that this metabolism was the key pathway under CAG condition. The treatment of YT showed the potential regulations on the altered lactate, pyruvate and α-glucose, while acetoacetate, lactate, and α-glucose could be significantly ameliorated by HQJZ without YT. Moreover, the whole HQJZ exerted the best improvement on them. All these results suggested that YT played an important role in the form of HQJZ.

Amino acid metabolism

Alanine, serine, and isoleucine had different inhibition and delay of gastric emptying, which was usually a symptom of human gastritis.[21,22] Glutamine is also to be reported as a potent stimulus for releasing glucagon-like peptide-1 which could increase postprandial insulin and slow gastric emptying. The drink of glutamine could slow the gastric emptying and attenuate the rise blood glucose in healthy controls.[23] The decreasing of serine and glutamine in our study might be conducive to explain the rise gastric emptying to aggravate the development of CAG. The other two metabolites, alanine, and isoleucine might perform other regulative mechanism in CAG. Meanwhile, the microinjections of glutamate into the NA could inhibit the gastric motility by activating the cholinergic preganglionic neurons, partially through the NMDA receptor-NO pathway.[24] The reduction of glutamate in the present work might also contribute to the promotion of gastric motility. HQJZ could effectively ameliorate the alterations of serine, isoleucine, glutamine, and glutamate, while YT regulated alanine, serine, and glutamine. These results further demonstrated that they might inhibit the gastric emptying to attenuate the pathological changes induced by CAG.

Fatty acid metabolism

The augment of acetate in CAG serum samples indicated fatty acid β-oxidation was significantly promoted.[25] In addition, the abnormity of acetate might be licked to change of gut microbiome. HQJZ showed the significant treatment on its dysfunction, as same as HQJZ formula without YT (YY). The result indicated that YT showed little effect on fatty acid *β*-oxidation and gut microbiome.

Choline metabolism

Increased choline was observed in model group compared with control group. Furthermore, altered choline could lead to degrade fatty acids.[26] The reduction of acetate was also evidence of it. PLS-RA also indicated that the metabolism of choline was closely correlative with PA, pH and loss weight. Administration of the tested drug showed no effect on them.

Through metabonomic analysis, an unbiased view of 12 differential metabolites was obtained to characterize the pathological states regulated by CAG and experimental drugs. However, some of these differential metabolites might be not relevant to the factors of biological reasons, but affected by other inevitable experimental factors. To screen the candidate biomarkers related to CAG, PLS-RA was utilized to analyze the link of the specific disease status with metabolic varieties.[27] In addition, various pathological indexes might characterize the different aspect of the disease to a certain degree. Hence, these indexes were all selected to screen the potential biomarkers. Their correlations might reveal the intrinsic linkage with the formation of CAG. As results, 2 (α-glucose and choline), 4 (choline, acetate, alanine, and a-glucose) and 5 (choline, acetate, alanine, a-glucose, and β-glucose) metabolites were selected as potential biomarkers based on their significant correlation with PA, pH, and weight change, respectively. Five metabolites, including choline, acetate, alanine, a-glucose and β-glucose, were considered as candidate biomarkers related to CAG.

Moreover, the underlying mechanisms of YiTang against CAG were involved in four disturbed metabolic pathways, including glucose metabolism, fatty acid metabolism, amino acid metabolism, and choline metabolism, which may benefit us to understand its gastro-protective role. In particular, the investigation of the compatibility of one single herb in a classical TCM formula was also matched the theory of TCM compared with the conventional analysis into one single herb, which could understand the synergistic action of TCM formula against single herbs.

CONCLUSION

We performed serum metabonomics to reveal the role of YT in the HQJZ formula against CAG. 12 candidate metabolites were identified to characterize the difference between CAG and normal rats, which were involved in four disturbed metabolic pathways, including glucose metabolism, fatty acid metabolism, amino acid metabolism, and choline metabolism. The further PLS-RA was conducted to screen the potential biomarkers closely related with pathological indexes induced by CAG. As a result, 2 (α-glucose and choline), 4 (choline, acetate, alanine and α-glucose), and 5 (choline, acetate, alanine, α-glucose and β-glucose) metabolites were screened to be candidate biomarkers based on their significant correlation with PA, pH and weight change, respectively. Five metabolites including choline, acetate, alanine, α-glucose, and β-glucose were considered as potential biomarkers related to CAG. YT could exert the synergistic effect with HQJZ without YT, where the whole formula obtain the best beneficial treatment against metabolic disturbance induced by CAG. The metabolic improvement

of HQJZ was mainly effected on the dysfunction of energy metabolism, gastric emptying and the changes of gut microbiome, which were the important pathomechanism induce by CAG. However, in this work, we only measured the metabolite change to characterize the contribution of YT toward HQJZ. A systemic analysis integrating metabonomics, transcriptomics, and proteomics, should be implemented to illustrate its role in the whole TCM formula.

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Conflicts of interest

There are no conflicts of interest.

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