Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Therapeutic Assessment of Fractions of Hawthorn on Gastrointestinal Motility Disorder by UPLC-MS/MS-based Metabolomics

Kaiyang Wang
Guangdong Pharmaceutical University
Lan Luo
Guangdong Pharmaceutical University
Xiaoli Xu
Guangdong Pharmaceutical University
Xingyu Chen
Guangdong Pharmaceutical University
Qiong He
Guangdong Pharmaceutical University
Zhongjie Zou
Guangdong Pharmaceutical University
Shumei Wang
Guangdong Pharmaceutical University
Shengwang Liang (swliang371@126.com)
Guangdong Pharmaceutical University

Research

Keywords: Hawthorn, UPLC-MS/MS, Gastrointestinal motility, Biomarker, Metabolomics

Posted Date: July 30th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-47503/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Abstract

Background: Hawthorn, a commonly-used traditional Chinese medicine (TCM) for treating dyspepsia、dysmenorrhea and hyperlipidemia, etc., has been proven to improve gastrointestinal motility, avoid food retention. Due to its complex ingredients, the active fractions responsible for the treatment of improving digestion remain largely unknown. To explore the underlying material and interpret its potential mechanism, the therapeutic effect of extract from different polar parts of hawthorn on gastrointestinal motility disorder was studied based on the ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) metabolomics .

Materials and Methods: The rat model of gastrointestinal motility disorder was established by subcutaneous injecting with atropine. The modeled rats were then treated with 4 polar parts (T1-4 in descending polarity, corresponding to water, n-butanol, ethyl acetate and petroleum ether extracts, respectively) of hawthorn for 5 consecutive days. The stomach, small intestine, plasma samples were collected and then subjected to related measurement (gastric emptying rate and small intestine propulsion rate), UPLC-MS/MS metabolic profiling and multivariate/univariate statistical analysis.

Results: The results showed that T3 had the best therapeutic effect, and T1, T2 and T4 with no obvious therapeutic effect, demonstrating that the effective components of hawthorn should be compounds of medium polarity. T3 achieved good therapeutic effects due to the gastrointestinal motility promotion activity, and by rectifying the disturbed amino acid metabolism in gastrointestinal motility disorder model.

Conclusion: This integrated metabolomics approach proved the validity of the therapeutic effect of extract from different polar parts of hawthorn on gastrointestinal motility disorder, providing new insights into the underlying mechanisms, and demonstrating the feasibility of metabolomics to evaluate efficacy of herbal drug, which is often difficult by traditional means.

Background

Hawthorn is the dried and ripe fruit of Crataegus pinnatifida Bge. var. major N.E.Br. or Crataegus pinnatifida Bge [1]. In addition to being consumed as a food, it has also been commonly used as a traditional Chinese medicine (TCM) for over a thousand years. Currently, hawthorn is widely used to improve digestion, avoid food retention [2, 3], treat dysmenorrhea [4] and hyperlipidemia [5].

Gastrointestinal motility disorder (caused by gastrointestinal or systemic diseases) is characterized by slow gastrointestinal motility and delayed gastric emptying and cause decreased digestive function [6, 7]. Whether organic or functional, all gastrointestinal smooth muscle dyskinesias are included in gastrointestinal motility disorders [8, 9]. Pharmacological studies have shown that the total flavonoid extract of hawthorn can alleviate TNF-alpha-evoked intestinal epithelial barrier deficit via the NFkappaBp65-mediated MLCK-MLC signaling pathway [10]. Hawthorn can also improve gastrointestinal motility caused by atropine sulfate in rats. It contains a variety of organic acids, which increase the secretion of gastric digestive enzymes, enhance the activity of lipase and protease, and promote the peristalsis of the gastrointestinal tract [11]. These enhance mechanical and chemical processes related to digestion.

In order to further determine the active ingredient of hawthorn in the treatment of gastrointestinal motility disorder, We used multivariate statistics to analyze the effect of extracts from different polar parts of hawthorn on gastrointestinal motility (efficacy index) on model rats. In addition, the UPLC-MS/MS-based plasma metabolomics method was used to study the changes in plasma metabolites in rats with gastrointestinal dyspraxia (induced by atropine sulfate) treated by hawthorn. Thus, the possible mechanism of improvement of gastrointestinal motility (atropine sulfate-induced) in rats with hawthorn was analyzed from the perspective of metabolomics.

Methods

Materials and reagents

Hawthorn (Batch number: 170801, Guangzhou Zhixin Chinese Medicine Pieces Co., Ltd., Origin: Shandong); Domperidone tablets (Zhejiang Deend Pharmaceutical Co., Ltd.); Atropine sulfate injection (Tianjin Jinyao Pharmaceutical Co., Ltd.); Chloral hydrate (Macleans, AR > 99.0%); Petroleum ether (Tianjin Baishi Chemical Co., Ltd., analytical grade); Ethyl acetate (Tianjin Baishi Chemical Co., Ltd., analytical grade); N-butanol (Tianjin Zhiyuan Chemical Reagent Co., Ltd., analytical grade); Formic acid (Aladdin Reagent Shanghai Co., Ltd., chromatographic grade); Methanol (Germany Merck shares two companies, chromatographic grade); Acetonitrile (Germany Merck shares two companies, chromatographic grade).

Preparation of the extracts

Hawthorn was pulverized by a high speed automatic Chinese medicine grinder. Then, 500 g of the coarse powder (No. 4 sieve) of hawthorn was extracted three times with 6000 mL of water, 100 minutes each [12]. Three water extraction solutions of hawthorn were mixed and concentrated under reduced pressure at 50 ℃. The water concentrated solution of hawthorn was extracted by three different solvents (petroleum ether, ethyl acetate, and n-butanol), respectively for 3 times, and then concentrated, freeze dried into powder to obtain four different polarity parts, including water part (T1), n-butanol part (T2), ethyl acetate part (T3) and petroleum ether part (T4) [13].

Drug administration and model preparation

Equal numbers of male and female SD rats (body mass: 200 ± 20 g) were provided by the Guangdong Provincial Laboratory Animal Center, China. They were housed at a temperature of 24 ± 2 ℃, a relative humidity of 50 to 60%, and a 12 h light-dark cycle was set in the SPF animal laboratory. Standard diet and water was provided. The animal protocol was approved by The Animal Ethics Committee of Guangdong Pharmaceutical University.

All the rats were acclimatized for 3 days and then randomly divided into 7 groups (each with 8 rats): a normal control group (NC group), a model group (MOD group), a domperidone, group (DOMP group), and 4 sample groups of hawthorn extracts (T1-T4 groups). Rats in the NC and Mod groups were administrated with 0.5% CMC-Na solution, and those in the DOMP group were administrated with domperidone (2.8 $mg{\cdot}kg^{-1}$). Rats in the 4 sample groups of hawthorn were administrated with T1-T4 samples (2100 $mg{\cdot}kg^{-1}$), respectively. All the groups received their administrations once a day for 5 days.

After 4 days of treatment, all eating was stopped except for water for 18 hours. After 20 min of administration on the 5th day, each group was subcutaneously injected with atropine sulfate at a dose of 1.4 $mg{\cdot}kg^{-1}$, except for the NC group that received subcutaneous normal saline injections. After 20 min, the rats in each group were intragastrically administered the semi-solid black nutrient food. The nutrient food containing 10 g of carboxymethylcellulose sodium, 16 g of milk powder, 8 g of starch, 8 g of sugar, and 2 g of activated carbon powder was added in 250 mL of distilled water. The mixture was uniformly stirred to prepare 300 g of a semi-solid black nutrient paste, prepared for animal treatments.

Plasma sample collection and preparation

After the semi-solid black nutrient food administration for 20 minutes, blood was withdrawn from the abdominal aorta using a sodium heparinized blood collection vacuum tube, and centrifuged at 4000 rpm for 10 min to obtain supernatant, which was stored at − 80 ℃.

The plasma samples were thawed at 4 ℃, and then 100 μL of plasma was transferred to a new tube and diluted with 200 μL of cold acetonitrile (4 ℃). After vortex for 1 min, the diluted plasma solution was centrifuged at 4 ℃ at 13000 g for 15 min to remove proteins. The supernatant was transferred to a new tube after passing through a 0.22 μL microporous filter membrane, and then the filtrate was transferred to vials for UPLC-MS/MS analysis.

Measurement of gastric emptying rate and small intestine propulsion rate

After blood collection, gastric emptying rate and small intestine propulsion rate were examined by the following procedure. Briefly, after ligating the gastric cardia and pylorus, the operator took the stomach, wiped it with filter paper and weighed it (labeled “G”). Then the operator cut the stomach along the greater curvature, washed its contents, and again wiped it with filter paper and weighed it (labeled “g”). The “G” and “g” values were used to calculate the gastric emptying rate (Formula 1). The total length of the small intestine was also measured (from the pylorus to the ileocecal area, labeled “H”) along with the distance from the pylorus to the black semi-solid paste front (labeled “h”). The small intestine propulsion rate was calculated based on the values of “H” and “h” (Formula 2).

Gastric emptying rate (%) = 1-(G-g)/2*100% (Formula 1)

Small intestine propulsion rate (%) = h/H*100% (Formula 2)

UPLC-MS/MS analysis

Chromatography was performed on a UPLC system (Thermo Corp., USA) with an autosampler at 4 ℃. The separation was carried out on a Hypersil GOLD™ C18 column (2.1 mm × 100 mm, 1.9 μm, Thermo Corporation, USA). The column temperature was maintained at 40 ℃. The analysis was performed with gradient elution using (A) water with 0.1% formic acid, and (B) acetonitrile as the mobile phase. Gradient elution was modifed as follows: 0–4 min, 5–30% B; 4–6 min, 40% B; 6–7 min, 60% B; 7–9 min, 95% B; and 9–12 min, 95% B. The flow rate was set to 0.3 mL/min. The sample injection volume was 2 μL. Throughout the analysis, all samples were kept at 4 ℃.

A Quadrupole-Exactive Orbitrap Mass Spectrometer (Thermo Fisher Scientific Corp., USA) was connected to a UPLC system via an electrospray ionization (ESI) interface. The MS data were collected in a centroid mode from 100 to 1000 Da. The temperature of the desolvation was 320 ℃. The collision energy was 15, 35, and 55 eV. The sheath gas flow rate was 45 arb and the auxiliary gas flow rate was 10 arb.

Aliquots of 3 μL of all the plasma samples were mixed together as the quality control (QC) sample. A QC sample was run prior to the test samples for six times to condition or equilibrate the system [14]. The replicability of the method was evaluated by analyzing 6 replicates of QC samples in one day. The post-preparative stability of the samples was tested by running 6 prepared QC samples kept in an autosampler (maintained at 4 ℃) for 12 h and the freshly prepared QC samples (n = 6) continuously in a single batch. In addition, QC samples were measured every 10 samples during the testing process in order to investigate the stability of analytical method by determining relative standard deviations (RSD) of intensity and retention time of 10 randomly selected characteristic ion peaks of QC samples.

Statistical analysis

The obtained index data were analyzed using GraphPad Prism 6.0. To evaluate the statistically signifcant differences among multiple treatments for given parameters, one-way analysis of variance with Dunnett’s multiple comparison test was used for comparison among various groups. Diferences with P values < 0.05 were considered statistically signifcant.

The raw MS data files were processed using Compound Discoverer 4.0 software (Thermo Scientific Corp., USA) for peak detection, matching, and alignment. All data including detection of mass, retention time, and intensity of the peaks eluted in each chromatogram were normalized to the sum total ion intensity per chromatogram to obtain the relative intensities of the metabolites.

Potential biomarkers had been initially identified and analyzed using the MetaboAnalyst (https://www.metaboanalyst.ca) database for related metabolic pathways. After normalization, principal component analysis(PCA) and orthogonal partial least squares discriminate analysis(OPLS-DA) analysis were performed using a SIMCA-P 14.1 software. The OPLS-DA model was evaluated according to the R^2Y and Q^2 parameters; the potential biomarkers were screened by selecting VIP > 1 and P < 0.05.

Results

Gastrointestinal motility ability alteration

Figure 1 showed that there had significant difference in the small intestine propulsion rate and gastric emptying rate between the MOD group and the NC group, indicating the modeling was successful. Additionally, The effect of ethyl acetate part (T3 group) was found to be particularly prominent in the model of rats with gastrointestinal motility retardation caused by atropine sulfate. The intestinal propulsion and gastric emptying rate for T3 group exceeded that for the DOMP group, indicating that its efficacy was better than that for domperidone.

Validation of UPLC-MS/MS conditions

The stability and repeatability of the UPLC-MS/MS system were evaluated by examining the relative standard deviation of the selected ion peak area and retention time in the QC sample. Table 1 showed that the UPLC-MS/MS system was relatively stable.

Table 1
RSD of the selected ion peak area and retention time of QC Sample

Ion mode	m/t-Rt	Area-RSD(%)	Rt-RSD(%)
ESI+	242.1758-1.00	10.21	0.33
	182.1065-4.18	9.4	0.27
	474.8648-6.02	10.32	0.43
	346.2158-8.08	6.62	0.06
	545.2748–10.08	8.79	0.19
ESI-	187.9367-0.93	9.32	0.23
	244.0880–3.82	7.8	0.15
	465.3088-6.49	7.52	0.13
	527.3220–8.23	6.93	0.08
	568.5431-11.40	8.41	0.17

The positive and negative mode total ion chromatogram (TIC) of the same plasma sample under the UPLC-MS/MS method had shown in Fig. 2. The results of the TIC showed that the method established in this study had strong sensitivity and a good separation effect.

Multivariate data analysis

In this study, PCA analyses were performed for the NC, MOD, DOMP, and T3 groups. Figure 3 showed that the NC and MOD groups can be clearly distinguished in the positive and negative ion modes, indicating that the modeling was successful. Additionally, the DOMP, T3 and MOD groups were also clearly distinguished in the positive and negative ion modes, indicating that the DOMP and T3 groups were effective in the model rats after administration.

Figure 4 showed the plasma samples of the NC, MOD, DOMP and T3 groups after analysis by OPLS-DA. The NC and MOD groups were clearly separated in both positive and negative ion modes in the score plot. According to the R^2X, R^2Y, and Q^2 evaluation parameters of OPLS-DA, the predictive ability and interpretation rate of the model was confirmed to be good.

To reveal the mechanism of effective hawthorn polar part and the positive drug to improve gastrointestinal motility (retardation model of rats induced by atropine sulfate), the OPLS-DA model was used to screen for potential biomarkers

associated with different effective polars and positive drug treatments. Metabolites with VIP > 1 and P < 0.05 in OPLS-DA were selected as biomarkers for potential candidates.

Identification

The exact single isotope mass was searched in the HMDB (http://www.hmdb.ca) and Metlin (http://metlin.scripps.edu) databases to match possible biomarkers. The secondary fragment mass spectrometry information of the metabolites was compared to the database of HMDB, Metlin, and literature to further screen the identification. Using the above methods, 23 candidate plasma biomarkers were initially identified to be associated with gastrointestinal dysmotility in the model rats (Table 2 is in the end of paper).The preliminary screening of 23 candidate biomarkers was input into the Meta database (https://www.metaboanalyst.ca/) for metabolic pathway analysis. If the influence value of the pathway was greater than 0, the metabolic pathway was considered to be associated with gastrointestinal motility disorder. The results showed that phenylalanine, tyrosine and tryptophan biosynthesis, glyoxylic acid and dicarboxylic acid metabolism, tryptophan metabolism, alanine, aspartic acid and glutamate metabolism, tyrosine metabolism, tricarboxylic acid cycle (TCA cycle), arginine and proline metabolism, glycine, serine and threonine metabolism, primary bile acid biosynthesis, purine metabolism, pantothenic acid and CoA biosynthetic pathways are associated with gastrointestinal motility disorders.

Table 2
List of 23 Candidate plasma biomarkers

Ion mode	No.	Metabolite	Chemical formula	M/Z	Rt	VIP
ESI+	1	Dimethylglycine	$C_4H_9NO_2$	103.0632	0.98	1.7881
	2	Pipecolinic acid	$C_6H_{11}NO_2$	129.0789	1.14	1.1577
	3	4-Hydroxyproline	$C_5H_9NO_3$	131.0585	1.62	1.1577
	4	L-Glutamine	$C_5H_{10}N_2O_3$	146.0690	1.19	1.2265
	5	L-Lysine	$C_6H_{14}N_2O_2$	146.1055	1.00	1.6856
	6	Uric acid	$C_5H_4N_4O_3$	168.0290	1.19	1.0659
	7	5-hydroxy tryptamine	$C_{10}H_{12}N_2O$	176.0290	2.65	1.2287
	8	L-tyrosine	$C_9H_{11}NO_3$	181.0738	1.65	1.3890
	9	L-Tryptophan	$C_{11}H_{12}N_2O_2$	204.0895	3.74	1.6977
	10	Pantothenic acid	$C_9H_{17}NO_5$	219.1103	1.25	1.4390
	11	Oleamide	$C_{18}H_{35}NO$	281.2715	11.99	1.6242
	12	Chenodeoxyglycocholic acid	$C_{26}H_{43}NO_5$	449.3136	9.23	1.4347
ESI-	13	Hippuric acid	$C_9H_9NO_3$	179.0581	3.81	1.3947
	14	Citric Acid	$C_6H_8O_7$	192.0278	1.19	1.4615
	15	Indolyllactic acid	$C_{11}H_{11}NO_3$	205.0737	4.59	1.2198
	16	Oleic acid	$C_{18}H_{34}O_2$	282.2557	10.46	1.4581
	17	Stearic acid	$C_{18}H_{36}O_2$	284.2714	11.39	1.0257
	18	Docosahexaenoic Acid	$C_{22}H_{32}O_2$	328.2401	9.41	1.0943
	19	Docosapentaenoic acid	$C_{22}H_{34}O_2$	330.2558	9.65	1.0279
	20	Cholic acid	$C_{24}H_{40}O_5$	408.2874	7.04	1.3247
	21	Glycocholic acid	$C_{26}H_{43}NO_6$	465.3088	6.49	1.5540
	22	Taurodeoxycholic acid	$C_{26}H_{45}NO_6S$	499.2968	5.98	1.5548
	23	Sulfolithocholylglycine	$C_{26}H_{43}NO_7S$	513.2758	5.09	1.5356

The specific analysis information of the metabolic pathway was shown in Fig. 5 and Table 3.

Table 3
The pathway result

No	Pathway name	Match Status	P	-log(P)	Impact
a	Phenylalanine, tyrosine and tryptophan biosynthesis	1/4	0.0640	2.7470	0.5000
b	Glyoxylate and dicarboxylate metabolism	1/16	0.2336	1.4540	0.2963
c	Tryptophan metabolism	2/41	0.1435	1.9400	0.2680
d	Alanine, aspartate and glutamate metabolism	1/24	0.3298	1.1090	0.1497
e	Tyrosine metabolism	1/42	0.5059	0.6812	0.1404
f	Citrate cycle (TCA cycle)	1/20	0.2833	1.2612	0.0535
g	Arginine and proline metabolism	2/44	0.1609	1.8270	0.0441
h	Glycine, serine and threonine metabolism	1/32	0.4145	0.8806	0.0316
i	Primary bile acid biosynthesis	2/46	0.1726	1.7565	0.0297
j	Purine metabolism	2/68	0.3078	1.1781	0.0207
k	Pantothenate and CoA biosynthesis	1/15	0.2207	1.5109	0.0204

Regulation of hawthorn on the metabolic pathway in model rats with gastrointestinal motility disorder

The analyses of candidate biomarkers by metabolic pathway results was combined with MetaboAnalyst analysis and literature search. It was found that the metabolic pathways of rats with gastrointestinal dysmotility disorder model caused by atropine sulfate under the influence of hawthorn include carbohydrate metabolism, lipid metabolism, amino acid metabolism, bile acid metabolism, etc. The effects of the metabolites in the plasma of model rats are shown in Table 4. (Table 4 is in the end of paper).

Table 4
Plasma biomarkers and their changing trends in different groups (*P < 0.05,** P < 0.01)

Ion mode	No.	Chemical formula	Molecular	Identification Metabolite	Rt	MOD/NC	DOMP/MOD.	T3/MOD.
ESI+	1	$C_4H_9NO_2$	103.0632	Dimethylglycine	0.98	↑**	↓**	↓**
	2	$C_6H_{11}NO_2$	129.0789	Pipecolinic acid	1.14	↑*	–	↓*
	3	$C_5H_9NO_3$	131.0585	4-Hydroxyproline	1.62	↑**	↓**	↓**
	4	$C_5H_{10}N_2O_3$	146.069	L-Glutamine	1.19	↓**	↑**	↑**
	5	$C_6H_{14}N_2O_2$	146.1055	L-Lysine	1.00	↓*	↑*	↑*
	6	$C_5H_4N_4O_3$	168.029	Uric acid	1.19	↑**	↓**	↓**
	7	$C_{10}H_{12}N_2O$	176.029	5-hydroxy tryptamine	2.65	↓**	↑**	↑**
	8	$C_9H_{11}NO_3$	181.0738	L-tyrosine	1.65	↓**	↑**	↑**
	9	$C_{11}H_{12}N_2O_2$	204.0895	L-Tryptophan	3.74	↓**	↑**	↑**
	10	$C_9H_{17}NO_5$	219.1103	Pantothenic acid	1.25	↓*	↓**	↓**
	11	$C_{18}H_{35}NO$	281.2715	Oleamide	11.99	↑*	↓*	↓*
	12	$C_{26}H_{43}NO_5$	449.3136	Chenodeoxyglycocholic acid	9.23	↓*	↑*	↑*
ESI-	13	$C_9H_9NO_3$	179.0581	Hippuric acid	3.81	↑*	↓*	↓*
	14	$C_6H_8O_7$	192.0278	Citric Acid	1.19	↓**	↑**	↑**
	15	$C_{11}H_{11}NO_3$	205.0737	Indolyllactic acid	4.59	↑**	↓**	↓**
	16	$C_{18}H_{34}O_2$	282.2557	Oleic acid	10.46	↑*	↓*	↓**
	17	$C_{18}H_{36}O_2$	284.2714	Stearic acid	11.39	–	–	–
	18	$C_{22}H_{32}O_2$	328.2401	Docosahexaenoic Acid	9.41	–	–	–
	19	$C_{22}H_{34}O_2$	330.2558	Docosapentaenoic acid	9.65	–	–	–
	20	$C_{24}H_{40}O_5$	408.2874	Cholic acid	7.04	↓*	↑*	↑*
	21	$C_{26}H_{43}NO_6$	465.3088	Glycocholic acid	6.49	↓*	↑*	↑*
	22	$C_{26}H_{45}NO_6S$	499.2968	Taurodeoxycholic acid	5.98	↓*	↑*	↑*
	23	$C_{26}H_{43}NO_7S$	513.2758	Sulfolithocholylglycine	5.09	↓*	↑*	↑*

Discussion

Acetylcholine is the most important excitatory neurotransmitter in the gastrointestinal nervous system. It can excite the smooth muscle of the gastrointestinal tract and thus depolarize the cell membrane of the smooth muscle [15, 16]. Atropine sulfate can block the excitatory action of muscarinic receptors to inhibit acetylcholine, thereby reducing the excitability of

the gastrointestinal smooth muscle, and reducing the amplitude and frequency of gastrointestinal peristalsis [17–19]. Therefore, the muscarinic receptor pathway was blocked by atropine sulfate to establish a gastrointestinal motility disorder model of rats [20].

Studies had shown that in rats with gastrointestinal motility disorders, there were some changes in the amino acids levels [21, 22]. In this study, L-tyrosine, L-tryptophan, and L-glutamine decreased in different degrees in the gastrointestinal motility disorder model of rats (caused by atropine sulfate). After intervention at different polars of hawthorn, their content increased. It is hypothesized that the mechanism may involve increasing the in-vivo content of certain essential amino acids so that these amino acids can be better utilized. For example, tryptophan metabolism by oxidative denucleation to produce 5-hydroxy tryptamine (5-HT) can activate the gastrointestinal motility reflex, regulate gastrointestinal motility, and play a role in gastrointestinal inflammation [23–25]. L-glutamine is involved in the biosynthesis of glucosamine, a component of mucin in the digestive tract mucosa, and maintain the normal function of the intestinal mucosal barrier [26–28].

In this study, citric acid content in the gastrointestinal dysmotility model rats (induced by atropine sulfate) decreased when compared with the NC group, indicating that the TCA cycle metabolic pathway weakened [29–31]. After treatment of different polar parts of the products of hawthorn in the model rats, citric acid content and TCA cycle metabolism increased. N, N-Dimethylglycine rapidly absorbs metabolism in-vivo. After emulsification, the particles of fat droplets in the nutrient are reduced, thereby improving the ability of digestive enzymes in the intestine; the intestinal epithelial villi also came in contact with nutrients to promote digestion and absorption of nutrients [32, 33]. At the same time, the content of (R)-pantothenic acid decreased, indicating that the synthesis of CoA increased and the amount of the intermediate, acetyl-CoA, also increased [34–36]. This indicated that the metabolism of the 3 major nutrients returned to normal and improved the nutritional status of the model rats [37–39].

Cholic acid is the main bile acid produced in the liver. It is usually combined with Glycine or Taurine to form Taurocholic acid and Glycocholic acid, respectively, the major bile acids in humans and animals [40–44]. Glycocholic acid belongs to the conjugated bile acid and is mainly distributed in the bile in the form of sodium salts [45–48]. In this study, the amount of cholic acid and glycocholic acid decreased in the model rats. After intervention at different polars of hawthorn, there was a definite increase which indicated that the different polars of hawthorn regulate metabolism of bile acids to a certain extent. Therefore, it returned to normal, enhanced digestion of hydrophobic nutrients and the absorption of fat in rats, and helped cholesterol excretion.

Conclusions

In this study, we prepared different polar parts of hawthorn using equal volumes of petroleum ether, ethyl acetate, and n-butanol solvent to extract a decoction and obtain 4 different polar parts. We used a variety of multivariate statistical methods to analyze the efficacy index of gastrointestinal motility in different sites of hawthorn, and identify the site with significant efficacy. The results showed that the ethyl acetate part of hawthorn had the best effect in improving gastrointestinal motility disorders.

Based on the UPLC-MS/MS metabolomics method, 23 potential biomarkers such as L-tryptophan, related to gastrointestinal motility model rats were screened. The data showed that hawthorn improved gastrointestinal motility and related to phenylalanine, tyrosine and tryptophan biosynthesis, glyoxylic acid and dicarboxylic acid metabolism, tryptophan metabolism, alanine, aspartic acid and glutamate metabolism, tyrosine metabolism, citric acid circulation (TCA cycle), arginine and proline metabolism, glycine, serine and threonine metabolism, primary bile acid biosynthesis, purine metabolism, pantothenic acid and CoA biosynthetic pathway.

Abbreviations

TCM: traditional Chinese medicine; UPLC-MS/MS: ultra-high performance liquid chromatography-tandem mass spectrometry; NC: normal control; MOD: model; DOMP: domperidone control; T1: water part; T2: n-butanol part; T3: ethyl acetate part; T4: petroleum ether part; UPLC: ultra-high performance liquid chromatography; TIC: total ion chromatogram; ESI: electrospray ionization; MS: mass spectrometry; QC : quality control; RSD: relative standard deviations; PCA: principal component analysis; OPLS-DA: orthogonal to partial least-squares discriminate analysis; VIP:variable importance in the projection; ANOVA: one-way analysis of variance; HMDB: Human Metabolome Database; TCA: tricarboxylic acid.

Declarations

Ethics approval and consent to participate

Institutional animal ethics committee approval for the experimental protocol was obtained from Animal Ethics Committee of Guangdong Pharmaceutical University (Guangdong, China) before initiation of the study.

Consent for publication

All authors have provided consent for publication in the Journal of Chinese Medicine.

Data availability statement

The datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors have no conflicts of interest to declare.

Funding

This work was supported by a grant from the National Natural Science Foundation of China [grant number:81773905, 81803728].

Authors’ contributions

KYW and LL were responsible for the experimental setup, measurements and analysis of data, interpretation of data, and manuscript writing. XLX□XYC□ZJZ and QH helped with the pharmacological study design and edited the manuscript. SMW and SWL contributed to the experimental design and manuscript editing. All the authors participated equally and approved the final version of the manuscript.

Acknowledgments

Not applicable.

References

1. Committee CP. Chinese pharmacopoeia (2015 edition). Beijing China. p.31.

2. Wang Y, Lv M, Wang T, Sun J, Wang Y, Xia M, et al. Research on mechanism of charred hawthorn on digestive through modulating "brain-gut" axis and gut flora. J Ethnopharmacol. 2019;245:112166.
3. Wei Z, Ai L, Chen X, Li L, Wang L, Fan W, et al. Comparative studies on the regulatory effects of raw and charred hawthorn on functional dyspepsia and intestinal flora. Tropical Journal of Pharmaceutical Research. 2019;18(2).
4. McEwen BJ. The influence of herbal medicine on platelet function and coagulation: a narrative review. Semin Thromb Hemost. 2015;41(3):300-14.
5. Zhang Z, Ho WK, Huang Y, James AE, Lam LW, Chen ZY. Hawthorn fruit is hypolipidemic in rabbits fed a high cholesterol diet. J Nutr. 2002;132(1):5-10.
6. Broberg B, Madsen JL, Fuglsang S, Holst JJ, Christensen KB, Rydahl C, et al. Gastrointestinal motility in patients with end-stage renal disease on chronic hemodialysis. Neurogastroenterol Motil. 2019;31(4):e13554.
7. Fox MR, Kahrilas PJ, Roman S, Gyawali CP, Scott SM, Rao SS, et al. Clinical measurement of gastrointestinal motility and function: who, when and which test? Nat Rev Gastroenterol Hepatol. 2018;15(9):568-79.
8. Chen JD, Yin J, Wei W. Electrical therapies for gastrointestinal motility disorders. Expert Rev Gastroenterol Hepatol. 2017;11(5):407-18.
9. Deane AM, Chapman MJ, Reintam Blaser A, McClave SA, Emmanuel A. Pathophysiology and Treatment of Gastrointestinal Motility Disorders in the Acutely Ill. Nutr Clin Pract. 2019;34(1):23-36.
10. Liu F, Zhang X, Ji Y. Total Flavonoid Extract from Hawthorn (Crataegus pinnatifida) Improves Inflammatory Cytokines-Evoked Epithelial Barrier Deficit. Med Sci Monit. 2020;26:e920170.
11. Chen ZY, Zhang ZS, Kwan KY, Zhu M, Ho WK, Huang Y. Endothelium-dependent relaxation induced by hawthorn extract in rat mesenteric artery. Life Sci. 1998;63(22):1983-91.
12. Luo JQ, Xu B, Chen Y, Liang SW. Extraction and HPLC Determination of Eight Hypolipidaemic Constituents in Crateagus. Guangdong Chemical Industry, 2017;44:17-19.
13. Xu H, Ruan LY, Chen C, Fan JT, Chen JF, Zhao WL, et al. Therapeutic assessment of fractions of Gastrodiae Rhizoma on chronic atrophic gastritis by (1)H NMR-based metabolomics. J Ethnopharmacol. 2020;254:112403.
14. Filippos M, Lindsay L, Helen G ,Georgios T, Ian W. UPLC-MS-based analysis of human plasma for metabonomics using solvent precipitation or solid phase extraction. Journal of Proteome Research. 2009;8:2114-2121.
15. Konishi M, Hayakawa Y, Koike K. Role of Muscarinic Acetylcholine Signaling in Gastrointestinal Cancers. Biomedicines. 2019;7(3).
16. Li C, Micci M-A, Mehta KR, Pasricha PJ. Substance P Maintains Gastrointestinal Smooth Muscle Sensitivity to Acetylcholine: A Novel Role for Co-Neurotransmitters in the Enteric Nervous System. Gastroenterology. 2011;140(5).
17. Chernish S, Brunelle R, Rosenak B, Ahmadzai S. Comparison of the effects of glucagon and atropine sulfate on gastric emptying. The American journal of gastroenterology. 1978;70(6):581-6.
18. Zhang RX, Wang XY, Chen D, Huizinga JD. Role of interstitial cells of Cajal in the generation and modulation of motor activity induced by cholinergic neurotransmission in the stomach. Neurogastroenterol Motil. 2011;23(9):e356-71.
19. Greenwood B, Read NW, Hardcastle PT, Hardcastle J. Effect of secoverine and atropine on intestinal secretion and motor activity in the rat small intestine in-vivo. J Pharm Pharmacol. 1984;36(2):100-6.
20. Ducharme N, Fubini S. Gastrointestinal complications associated with the use of atropine in horses. Journal of the American Veterinary Medical Association. 1983;182(3):229-31.
21. De Filippis A, Ullah H, Baldi A, Dacrema M, Esposito C, Garzarella EU, et al. Gastrointestinal Disorders and Metabolic Syndrome: Dysbiosis as a Key Link and Common Bioactive Dietary Components Useful for their Treatment. Int J Mol Sci. 2020;21(14).
22. Liu Y, Wang X, Hou Y, Yin Y, Qiu Y, Wu G, et al. Roles of amino acids in preventing and treating intestinal diseases: recent studies with pig models. Amino Acids. 2017;49(8):1277-91.

23. Ledochowski M, Widner B, Murr C, Sperner-Unterweger B, Fuchs D. Fructose malabsorption is associated with decreased plasma tryptophan. Scandinavian journal of gastroenterology. 2001;36(4):367-71.
24. Fernstrom JD. A Perspective on the Safety of Supplemental Tryptophan Based on Its Metabolic Fates. J Nutr. 2016;146(12):2601S-8S.
25. Swami T, Weber HC. Updates on the biology of serotonin and tryptophan hydroxylase. Curr Opin Endocrinol Diabetes Obes. 2018;25(1):12-21.
26. Steinert RE, Landrock MF, Horowitz M, Feinle-Bisset C. Effects of Intraduodenal Infusions of L-phenylalanine and L-glutamine on Antropyloroduodenal Motility and Plasma Cholecystokinin in Healthy Men. J Neurogastroenterol Motil. 2015;21(3):404-13.
27. Dechelotte P, Hasselmann M, Cynober L, Allaouchiche B, Coeffier M, Hecketsweiler B, et al. L-alanyl-L-glutamine dipeptide-supplemented total parenteral nutrition reduces infectious complications and glucose intolerance in critically ill patients: the French controlled, randomized, double-blind, multicenter study. Crit Care Med. 2006;34(3):598-604.
28. Marc R, Robert A, Chen WN, Richard A, John K, Adrienne D, et al. L-glutamine stimulates intestinal cell proliferation and activates mitogen-activated protein kinases. American Physiological Society. 1997;272(1):943-953.
29. Luo L, Hu M, Li Y, Chen Y, Zhang S, Chen J, et al. Association between metabolic profile and microbiomic changes in rats with functional dyspepsia. RSC Advances. 2018;8(36):20166-81.
30. Wang D, Ma X, Guo S, Wang Y, Li T, Zou D, et al. Effect of Huangqin Tang on Urine Metabolic Profile in Rats with Ulcerative Colitis Based on UPLC-Q-Exactive Orbitrap MS. Evid Based Complement Alternat Med. 2020;2020:1874065.
31. Dong G, He X, Chen Y, Cao H, Wang J, Liu X, et al. Genetic variations in genes of metabolic enzymes predict postoperational prognosis of patients with colorectal cancer. Mol Cancer. 2015;14:171.
32. Chalvatzi S, Papadopoulos GA, Tsiouris V, Giannenas I, Karapanagiotidis IT, Theodoridis A, et al. Dimethylglycine Supplementation in Reduced Energy Broilers' Diets Restores Performance by Improving Nutrient Digestibility. Animals (Basel). 2020;10(5).
33. Yamamoto M, Pinto-Sanchez MI, Bercik P, Britz-McKibbin P. Metabolomics reveals elevated urinary excretion of collagen degradation and epithelial cell turnover products in irritable bowel syndrome patients. Metabolomics. 2019;15(6):82.
34. Cagin YF, Atayan Y, Sahin N, Parlakpinar H, Polat A, Vardi N, et al. Beneficial effects of dexpanthenol on mesenteric ischemia and reperfusion injury in experimental rat model. Free Radic Res. 2016;50(3):354-65.
35. Sabui S, Kapadia R, Ghosal A, Schneider M, Lambrecht NWG, Said HM. Biotin and pantothenic acid oversupplementation to conditional SLC5A6 KO mice prevents the development of intestinal mucosal abnormalities and growth defects. Am J Physiol Cell Physiol. 2018;315(1):C73-C9.
36. Bly C, Heggeness F, Nasset E. The Effects of Pantothenic Acid and Inositol Added to Whole Wheat Bread on Evacuation Time, Digestion and Absorption in the Upper Gastro-Intestinal Tract of Dogs: Two Frigure. 1943;(2):2411-2414.
37. Ali A, Zhang Y, Fu M, Pei Y, Wu L, Wang R, et al. Cystathionine gamma-lyase/H2S system suppresses hepatic acetyl-CoA accumulation and nonalcoholic fatty liver disease in mice. Life Sci. 2020;252:117661.
38. Belew GD, Silva J, Rito J, Tavares L, Viegas I, Teixeira J, et al. Transfer of glucose hydrogens via acetyl-CoA, malonyl-CoA, and NADPH to fatty acids during de novo lipogenesis. J Lipid Res. 2019;60(12):2050-6.
39. Zhang S, Yang W, Chen H, Liu B, Lin B, Tao Y. Metabolic engineering for efficient supply of acetyl-CoA from different carbon sources in Escherichia coli. Microb Cell Fact. 2019;18(1):130.
40. Sun W, Guo Y, Zhang S, Chen Z, Wu K, Liu Q, et al. Fecal Microbiota Transplantation Can Alleviate Gastrointestinal Transit in Rats with High-Fat Diet-Induced Obesity via Regulation of Serotonin Biosynthesis. Biomed Res Int. 2018;2018:8308671.
41. Oddsson. E, Rask M, Krag. E. A secretory epithelium of the small intestine with increased sensitivity to bile acids in irritablc bowel syndrome associated with diarrhoea. Scand. J Gatroetit. 1978;13:409-416. .

42. Flynn M, Darby C, Hyland J, Hammond P, Taylor I. The effect of bile acids on colonic myoelectrical activity. Br J Surg. 1979;66(11):776-9.
43. Taylor I, Basu P, Hammond P, Darby C, Flynn M. Effect of bile acid perfusion on colonic motor function in patients with the irritable colon syndrome. Gut. 1980;21(10):843-7.
44. Zhang Y, Li H, Hu T, Li H, Jin G, Zhang Y. Metabonomic profiling in study hepatoprotective effect of polysaccharides from Flammulina velutipes on carbon tetrachloride-induced acute liver injury rats using GC-MS. Int J Biol Macromol. 2018;110:285-93.
45. Penagini R, Spiller RC, Misiewicz JJ, Frost PG, Silk DB. Effect of cholecystectomy on mouth-to-cecum transit of a liquid meal. Dig Dis Sci. 1988;33(1):19-22.
46. Pasternak A, Matyja A, Gil K, Gajda M, Tomaszewski KA, Gajda M, et al. Interstitial cajal-like cells and bile lithogenicity in the pathogenesis of gall-stone disease. Pol Przegl Chir. 2013;85(6):311-6.
47. Spiller RC, Frost PF, Stewart JS, Bloom SR, Silk DB. Delayed postprandial plasma bile acid response in coeliac patients with slow mouth-caecum transit. Clin Sci (Lond). 1987;72(2):217-23.
48. Feurle GE. Pathophysiology of diarrhea in patients with familial amyloid neuropathy. Digestion. 1987;36(1):13-7.

Figures

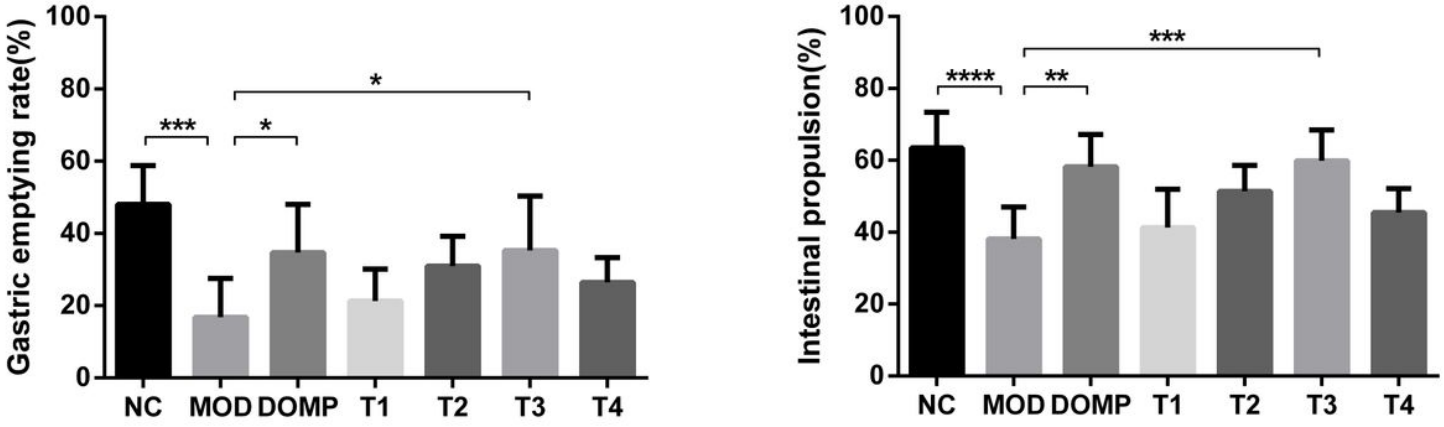


Figure 1

The effect of extracts from different polar parts of hawthorn and domperidone on gastrointestinal motility in rat. (A) Gastric emptying rate. (B) Intestinal propulsion rate. (Data were analyzed by one-way ANOVA (mean SD, n = 6). GraphPad Prism 6.0 was used for statistical analyses. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.)

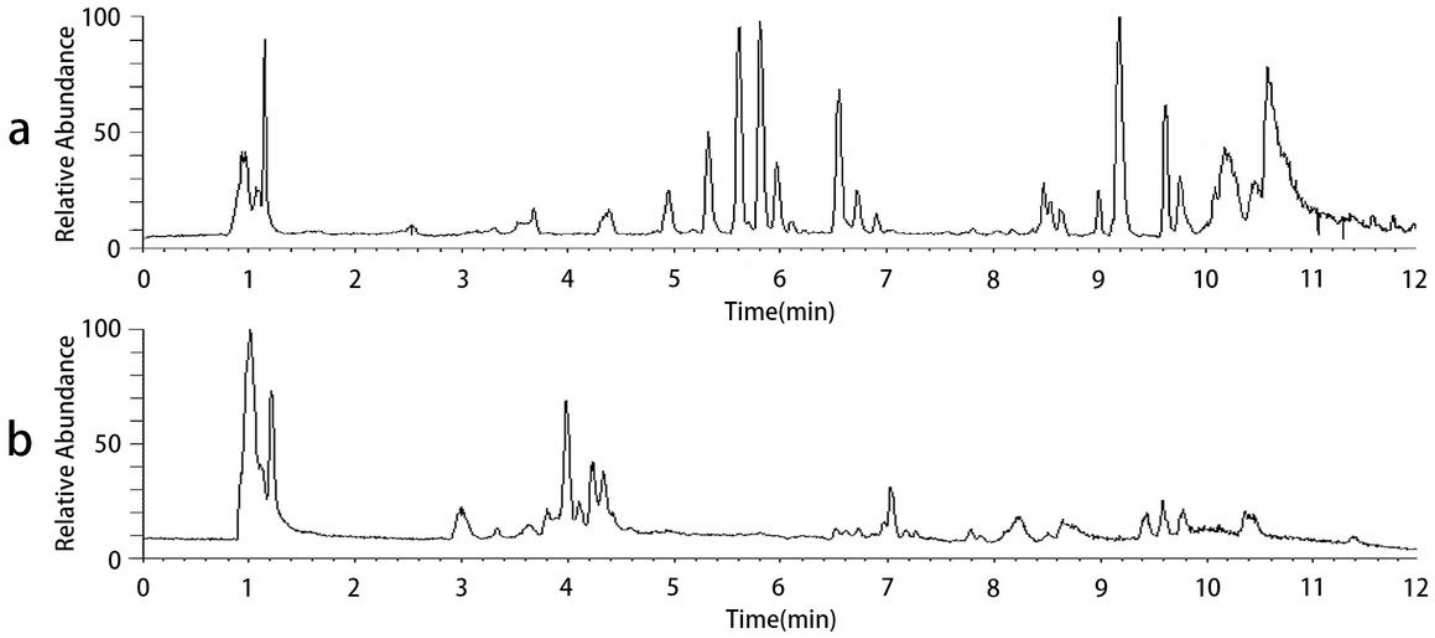


Figure 2

TIC of the same plasma sample (a) Positive ion mode (b) Negative mode

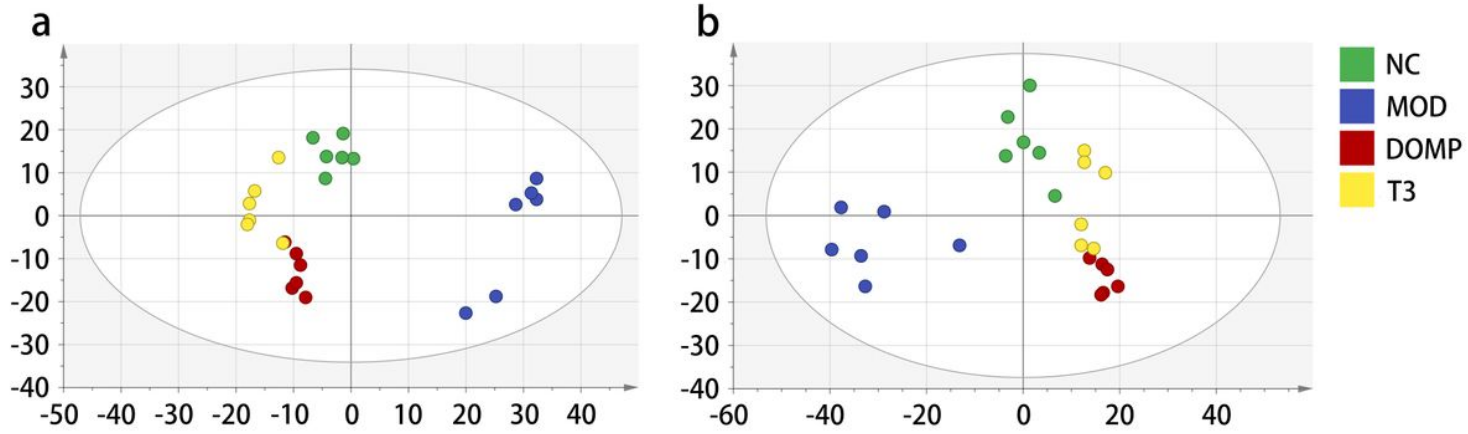


Figure 3

Score plot of PCA of the different groups; (a) positive ion mode (b) negative ion mode

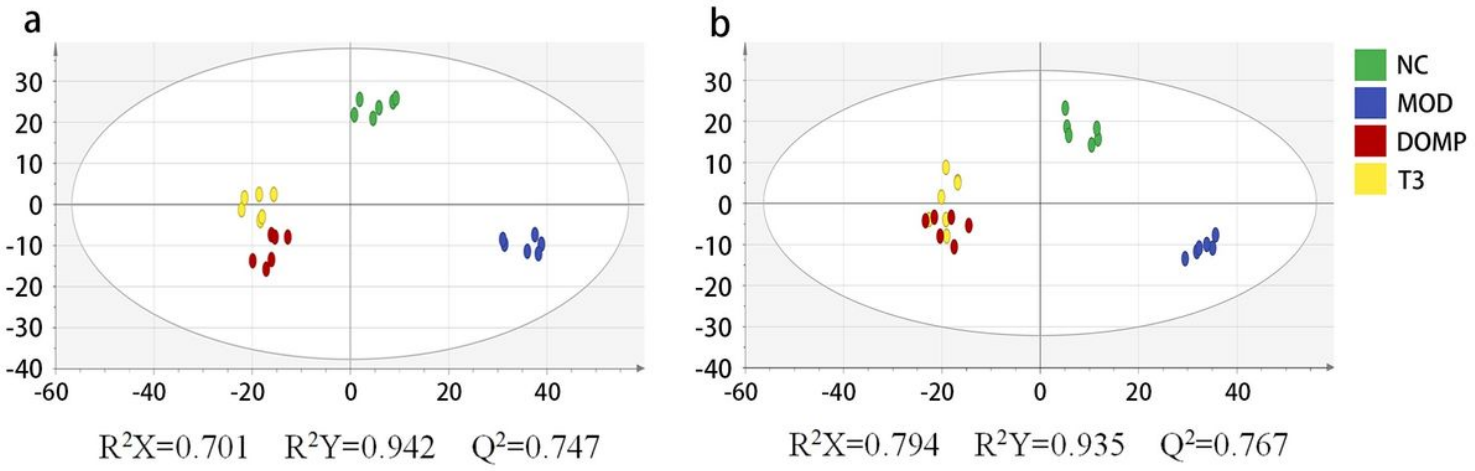


Figure 4

Score plot of OPLS-DA of different groups; (a) positive ion mode (b) negative ion mode

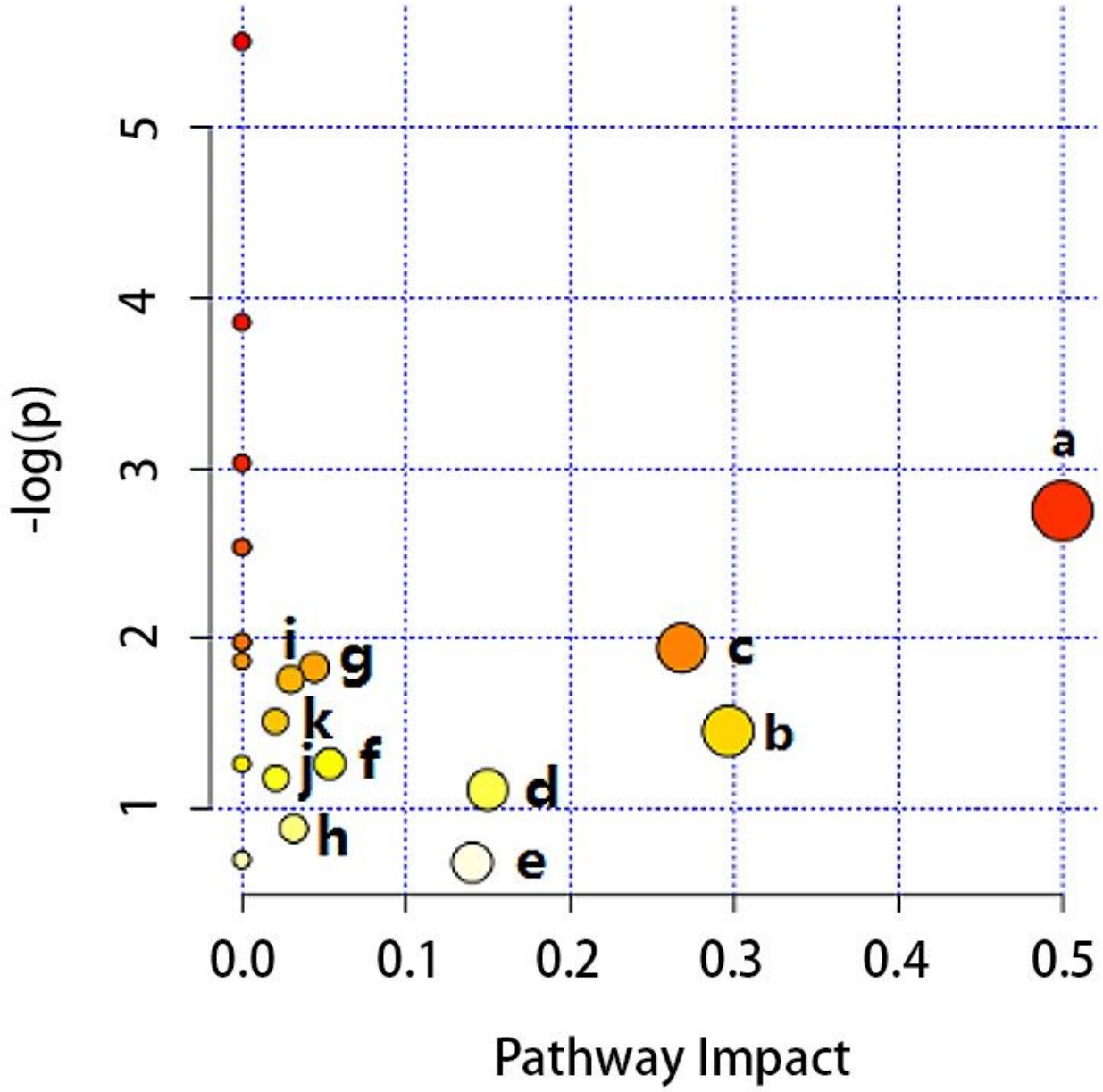


Figure 5

The pathway impact