JOURNAL OF ASIAN NATURAL PRODUCTS RESEARCH
https://doi.org/10.1080/10286020.2022.2134013

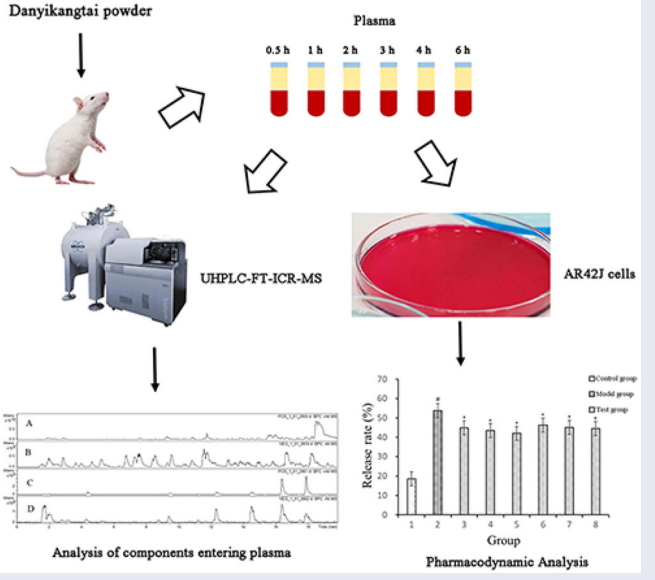
Determination of the components of danyikangtai powder into the plasma and its pharmacodynamic study

Jing-Wei Li[a], Yu-Meng Zhang[a], Chun-Jie Zhao[a], Min Zhao[a] and Yi-He Huang[b]

[a]School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China; [b]School of Public Health, Shenyang Medical College, Shenyang 110034, China

ABSTRACT

Danyikangtai powder has a definite therapeutic effect on pancreatitis. However, the internal mechanism is unclear. The purpose of this experiment is to quickly identify the blood components of danyikangtai powder and evaluate its efficacy. 25 blood components were identified by comparing the components with the same mass spectrometry information from *in vivo* and *in vitro* samples. The AR42J cells of the pancreatitis model were treated with drug-containing plasma, and the drug efficacy was evaluated by investigating the amylase release rate. This study provides a scientific reference for its pharmacological research and rational clinical application.



ARTICLE HISTORY
Received 25 September 2021
Accepted 6 October 2022

KEYWORDS
Danyikangtai powder; UHPLC–FT–ICR–MS; chemical compositions; AR42J; pharmacodynamic effect

CONTACT Yi-He Huang huangyihe@aliyun.com Shenyang Medical College, No. 146 Huanghe North Street, Shenyang 110034, Liaoning, China

Supplemental data for this article can be accessed online at https://doi.org/10.1080/10286020.2022.2134013.

1. Introduction

Pancreatitis is a very serious inflammatory disease. Systemic inflammatory response syndrome (SIRS) is an important form of pancreatitis that predicts pancreatic infection [1]. Many inflammatory mediators in SIRS eventually lead to multiple organ dysfunction syndromes [2]. It is currently believed that the overstimulation of pancreas and obstruction of bile duct will increase the pancreatic duct pressure and reverse the active trypsin. If the intracellular protective mechanism that reduces trypsin activity in the body is weakened, it will lead to pancreatitis [3]. In addition, calcium overload causes acinar and catheter damage, which is also an important factor causing pancreatitis [4]. In view of the complex pathogenesis of pancreatitis, more and more evidences show that traditional Chinese medicine (TCM) is superior in the treatment of pancreatitis, due to its multitarget, multichannel, and comprehensive characteristics. TCM plays an important role in the treatment of pancreatitis by anti-inflammatory, inducing apoptosis, inhibiting pancreatic enzymes, preventing organ damage, and accelerating pancreatic repair and regeneration.

Danyikangtai powder is a TCM formula from Shenyang Military Region, which contains 10 herbal materials, including Scutellariae Radix, Artemisiae Scopariaeporia Herba, Cyperi Rhizoma, Toosendan Fructus, Aurantii Fructus Immaturus, Paeoniae Radix Alba, Pinelliae Rhizoma, Bupleuri Radix, Coptidis Rhizoma, and Taraxaci Herba. It has the functions of clearing away heat and detoxifying, reducing swelling, and relieving pain. Moreover, it has a certain therapeutic effect on acute and chronic cholecystitis and pancreatitis. However, the internal mechanism is still unclear, and there is no report on its blood component and efficacy of the biliary and pancreatic stagnation. Therefore, it is very important to establish a rapid and effective method to identify the chemical components of danyikangtai powder which enter blood and compare them with those which do not enter plasma. This can give a reasonable explanation to the material basis of danyikangtai powder.

For TCMs, the synergy of various chemical components is the material foundation for their pharmacological effects. Liquid chromatography–mass spectrometry with high sensitivity, resolution, and accuracy plays an important role in determining the constituents of TCMs [5]. Ultra-high-performance liquid chromatography coupled with Fourier transform–ion cyclotron–resonance mass spectrometry (UHPLC–FT–ICR–MS) has a great advantage in the quantitative identification of complex composition for its high sensitivity and accuracy. Li *et al.* developed a rapid and reliable method by using UHPLC–FT-ICR-MS to analyze the main components and metabolites of *Rhodiola crenulata* after oral administration [6]. In addition, a rapid and reliable analysis method using UHPLC–FT–ICR–MS was established to study the main components and metabolites of *R. crenulata* after oral administration [6]. In this study, the UHPLC–FT–ICR–MS technique was used for systematical characterization of the blood components of danyikangtai powder by comparing the components with the same mass spectrometry information from the *in vivo* and *in vitro* samples. This constituent characterization and structural elucidation provided significant information for a pharmacological study of danyikangtai powder.

AR42J is a chemically induced rat pancreatic exocrine tumor cell line [7]. AR42J cells have both exocrine and neuroendocrine properties. Moreover, AR42J cells, like

primary pancreatic exocrine cells, have a stable phenotype when cultured *in vitro* [8]. Caerulein, a peptide analog of cholecystokinin, can stimulate the pancreas and induce pancreatic acinar autolysis by promoting the secretion of trypsin. Therefore, it can induce the development of acute pancreatitis at certain dose [9]. Pu et al. studied the effect of baicalein on acinar-to-ductal metaplasia induced by inflammatory cytokines in rat pancreatic acinar cell line AR42J and its possible potential mechanism [8]. In this experiment, an *in vitro* model of pancreatitis induced by caerulein [10] was used to verify the efficacy of blood components of danyikangtai powder.

2. Result

2.1. UHPLC–FT–ICR–MS analysis of danyikangtai powder extract and drug-containing plasma samples

2.1.1. Components absorbed into the blood

The UHPLC–FT–ICR–MS technique was used to detect, identify, and characterize the components in danyikangtai powder extract and drug-containing plasma in positive and negative ion mode. The base peak chromatogram of danyikangtai powder extract and drug-containing plasma is shown in Figure 1. In order to eliminate the interference of the biological matrix and improve the detection sensitivity, the extracted ion chromatograms are shown in Supplementary Figures S1–S3. The accurate molecular weight, retention time, and mass fragmentation information of the compound were obtained by UHPLC–FT–ICR–MS analysis, and the information of the chemical in the danyikangtai powder extract and the drug-containing plasma was compared. A total of 25 compounds were detected in drug-containing plasma, including flavonoids, terpenoids, alkaloids, coumarins, and organic acids. The results are shown in Table 1. Among the 25 compounds, 10 flavonoids prototypes were identified in the drug-containing plasma, including lonicerin, rhoifolin, chrysin-6-C-arabinopyranosyl-8-C-glucopyranoside, hyperoside, narirutin, baicalin, liquiritin, naringenin, isorhamnetin-3-O-glucoside, and hesperidin. Other types of prototype are listed as follows: five terpenes including mudanpioside E, betulonic acid, galloylpaeoniflorin, limonin, and

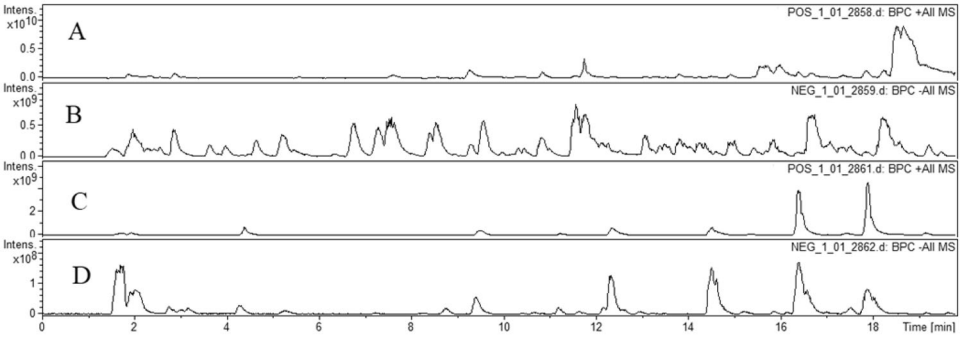


Figure 1. UHPLC–FT–ICR–MS basic peak ion chromatogram (BPC) of danyikangtai powder extract and drug-containing plasma. (A) BPC of danyikangtai powder extract in positive ion mode; (B) BPC of danyikangtai powder extract in the negative ion mode; (C) BPC of drug-containing plasma in the positive ion mode; (D) BPC of drug-containing plasma in the negative ion mode.

Table 1. UHPLC–FT–ICR–MS analysis of components of danyikangtai powder absorbed into blood.

No.	t_R (min)	Identification	Formula	Molecular Weight	Ion mode	MS (*m/z*)	Error (ppm)	MS/MS (*m/z*)
1	2.28	Citric acid	$C_6H_8O_7$	192.123	$[M-H]^-$	191.01987	−0.75	111.0081, 87.0092, 57.0376
2	5.28	Ellagic acid	$C_{14}H_6O_8$	302.194	[M − H]−	301.00012	−3.74	283.9960, 257.0032, 229.0129
3	11.23	Lonicerin	$C_{27}H_{30}O_{15}$	594.5181	$[M+H]^+$	595.16713	−2.33	577.1515, 457.1087, 295.0603
4	11.61	3-O-Feruloylquinic	$C_{17}H_{20}O_{19}$	368.338	[M − H]−	367.10422	−2.08	193.0512
5	11.70	Mudanpioside E	$C_{24}H_{30}O_{13}$	526.1686	[M − H]−	525.16139	−0.06	449.1449, 327.1077, 165.0545
6	12.33	Betulonic acid	$C_{30}H_{46}O_3$	454.695	$[M+H]^+$	455.34992	4.51	409.3527, 203.1782, 111.0838
7	12.46	Rhoifolin	$C_{27}H_{30}O_{14}$	578.523	$[M+H]^+$	579.16935	2.57	271.0598
8	13.80	Chrysin-6-C-arabinopyranosyl -8-C-glucopyranoside	$C_{26}H_{28}O_{13}$	548.497	$[M+H]^+$	549.15959	1.23	255.06519
9	13.86	Scopoletin	$C_{10}H_8O_4$	192.17	$[M+H]^+$	193.0489	−3.3	178.0254, 122.0376
10	14.78	Hyperoside	$C_{21}H_{20}O_{12}$	464.379	$[M+H]^+$	465.10280	−0.11	301.0463, 203.0331, 173.0427
11	14.90	Galloylpaeoniflorin	$C_{30}H_{32}O_{15}$	632.571	[M − H]−	631.16783	−1.57	169.0143, 479.1563
12	14.86	Limonin	$C_{26}H_{30}O_8$	470.518	$[M+H]^+$	471.20226	−1.94	427.2099, 425.1935
13	15.54	Columbamine	$C_{20}H_{20}NO_4+$	338.382	$[M+H]^+$	338.13870	−0.04	322.1069, 294.1120, 265.0730
14	15.67	Berberine	$C_{20}H_{18}NO_4+$	336.366	$[M+H]^+$	336.12283	0.62	321.1025, 320.0934, 306.0786, 292.09844
15	15.76	Coptisine	$C_{19}H_{14}NO_4+$	320.323	$[M+H]^+$	320.09154	0.62	292.0963, 277.0726, 262.0857
16	16.74	Narirutin	$C_{27}H_{32}O_{14}$	580.539	$[M+H]^+$	581.18528	2.06	419.1318, 273.0736, 153.0173
17	17.40	Baicalin	$C_{21}H_{18}O_{11}$	446.364	[M − H]−	445.07832	−1.54	269.0088, 251.0341, 167.0001
18	14.01	Liquiritin	$C_{21}H_{22}O_9$	418.398	$[M-H]^-$	417.11925	−0.34	255.0512, 135.0043, 119.0482
19	16.67	Naringenin	$C_{15}H_{12}O_5$	272.256	$[M+H]^+$	273.07570	0.34	153.0173, 91.0551
20	17.84	Fuziline	$C_{24}H_{39}NO_7$	453.576	$[M+H]^+$	454.28137	−3.17	436.2612, 422.2736
21	18.21	Palmatine	$C_{21}H_{22}NO_4+$	352.409	$[M+H]^+$	352.15407	0.75	337.1337, 336.1346, 308.1082
22	18.65	Isorhamnetin-3-O-glucoside	$C_{22}H_{22}O_{12}$	478.406	$[M+H]^+$	479.1182	0.43	315.045 7
23	18.28	Hesperidin	$C_{16}H_{14}O_6$	302.282	$[M+H]^+$	303.08595	1.21	153.0170, 89.0395
24	19.81	Meranzin	$C_{15}H_{16}O_4$	260.289	$[M+H]^+$	261.1119	0.89	243.1004, 159.0436, 103.0543
25	19.83	Merancin hydrate	$C_{15}H_{18}O_5$	278.304	$[M+H]^+$	279.12256	0.52	261.1128, 243.1004, 189.0543

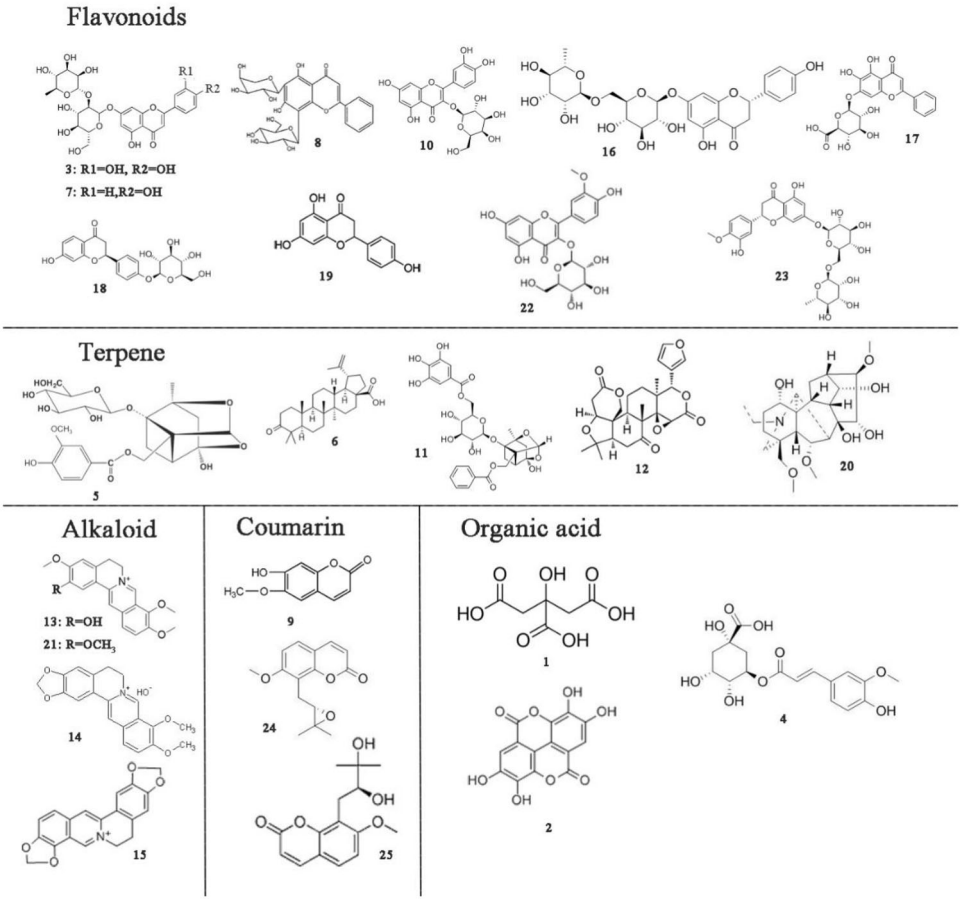


Figure 2. The chemical structures of components of danyikangtai powder absorbed into blood.

fuziline; four alkaloids including columbamine, berberine, coptisine, and palmatine; three coumarins including scopoletin, meranzin, and merancin hydrate; three organic acids including citric acid, ellagic acid, and 3-O-feruloylquinic. The structures of 25 compounds are shown in Figure 2.

2.1.2. Components not absorbed into blood

With UHPLC–FT–ICR–MS, the exact molecular weight, retention time, and mass fragment information of the compounds obtained from danyikangtai powder extract and drug-containing plasma were analyzed and compared. Compounds that were detectable in the extractive solution but not in the drug-containing plasma were considered likely to be chemical components that could not be absorbed into the blood, that will be verified in further experiments. There were 44 kinds of components undetected in drug-containing plasma, and the results are shown in Supplementary Table S1.

2.2. Characterization of chemical constituents of danyikangtai powder absorbed into blood

2.2.1. Characterization of flavonoids

Flavonoids are the main compounds of danyikangtai powder absorbed into the blood. The identification process is illustrated by taking liquiritin as an example. The peak of no. 18 showed an *m/z* 417.11925 $[M-H]^-$ in the negative ion mode. The molecular formula is presumed to be $C_{21}H_{22}O_9$ by mass spectrometry software. In its secondary mass spectrum (Figure 3(a)), the fragment at *m/z* 255.0512 is presumed to be a fragment of liquiritin that is depleted of one molecule of glucose. It is speculated that *m/z* 135.0043 and *m/z* 119.0482 are fragment ions with broken C1–C2 and C3–C4 bonds in the core B ring after partial glucose removal. Therefore, the peak of no. 18 is identified as liquiritin. The cracking pattern is shown in Figure 4(a). The peaks of 3, 7, 8, 10, 16, 17, 19, 22, and 23 are identified as lonicerin, rhoifolin, chrysin-6-C-arabinopyranosyl-8-C-glucopyranoside, hyperoside, narirutin, baicalin, naringenin, isorhamnetin-3-O-glucoside, and hesperidin, respectively.

2.2.2. Characterization of terpenoids

The terpenoids use limonin as an example to illustrate their identification process. The peak of no. 12 in positive ion mode showed an *m/z* 471.20226 $[M+H]^+$. The molecular formula is presumed to be $C_{26}H_{30}O_8$ by the mass spectrometry software. In its secondary mass spectrum (Figure 3(b)), the fragment at *m/z* 427.2099 is presumed to be excimer ion ($[M+H]^+$) stripped of CO_2 (44 Da). Excimer ion ($[M+H]^+$) detached H_2O (18 Da) and CO (28 Da) to obtain the fragment at *m/z* 425.1935. Therefore, the peak of no. 12 is identified as limonin. The spectrum of mass spectrometry is shown in Figure 4(b). The peaks of 5, 6, 11, and 20 are identified as mudanpioside E, betulonic acid, galloylpaeoniflorin, and fuziline, respectively.

2.2.3. Characterization of alkaloids

The alkaloids use palmatine as an example to illustrate their identification process. The *m/z* of the 21st peak in positive ion mode is 352.15407 ($[M+H]^+$). The molecular formula is presumed to be $C_{21}H_{22}NO_4^+$ by mass spectrometry software. In its secondary mass spectrum (Figure 3(c)), the fragment ion at *m/z* 337.1337 is presumed to be a fragment ion after removal of a methyl group (15 Da). For the fragment of *m/z* 337.1337, the methoxy group is cyclized to remove a fragment of H (1 Da) to provide *m/z* 336.1346, followed by removal of CO (28 Da) to form the fragment ion at *m/z* 308.1082. Therefore, the peak of no. 21 is identified as palmatine, and the spectrum of the mass spectrometry is shown in Figure 4(c). The peaks 13, 14, and 15 are identified as columbamine, berberine, and coptisine, respectively.

2.2.4. Characterization of coumarins

The coumarins are described by taking scopoletin as an example to illustrate their identification process. The *m/z* of the 9th peak in the positive ion mode is 193.0489 ($[M+H]^+$). The molecular formula is presumed to be $C_{10}H_8O_4$ by mass spectrometry software. In its secondary mass spectrum (Figure 3(d)), the fragment at *m/z* 178.0254 is a fragment of the excimer ion ($[M+H]^+$) after removal of one methyl group

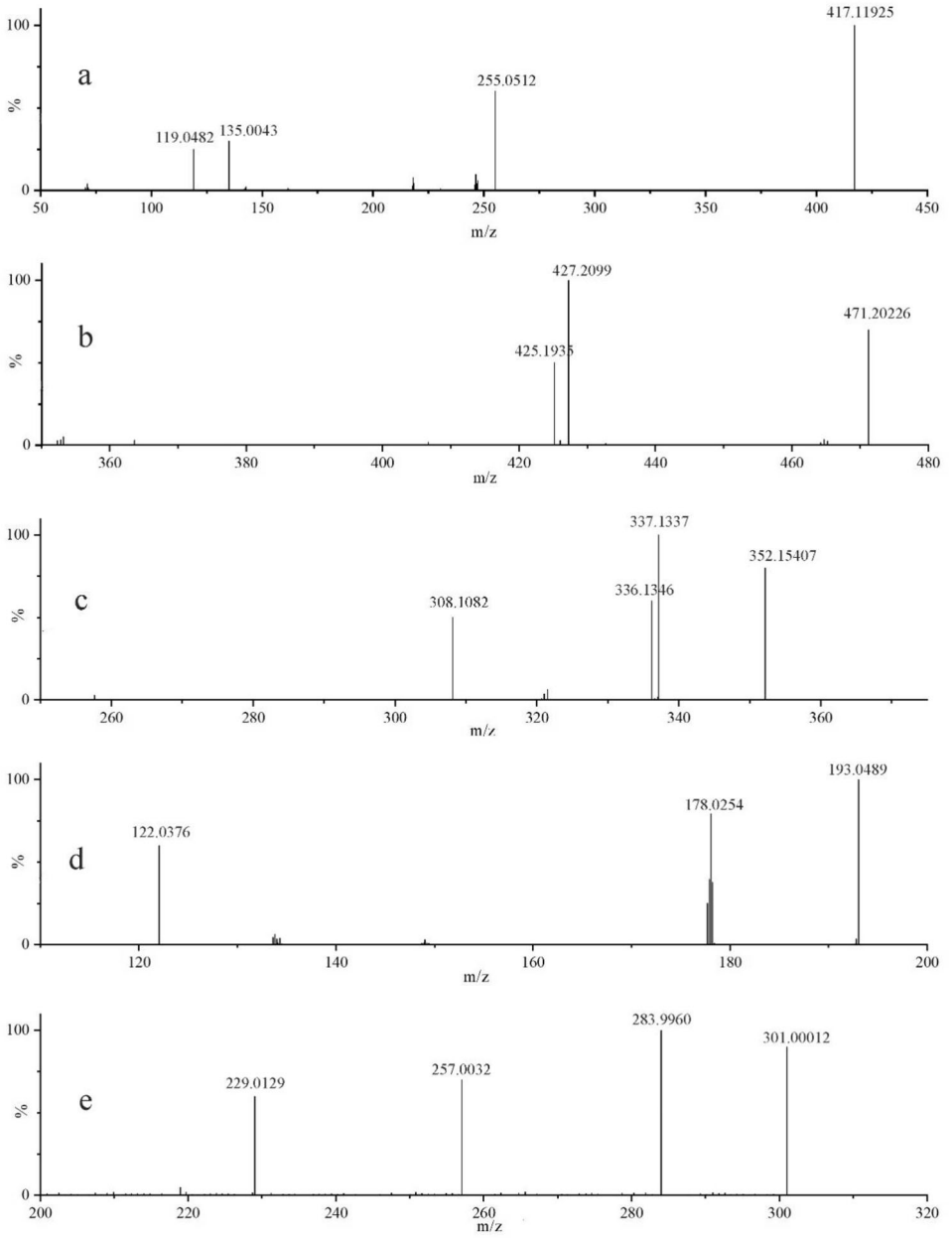


Figure 3. The MS/MS spectra of liquiritin (a), limonin (b), palmatine (c), scopoletin (d), and ellagic acid (e).

(15 Da). *m/z* 122.0376 is a fragment produced by the breakup of the core matrix of the excimer ion ($[M+H]^+$). Therefore, the no. 9 peak is identified as scutellarin, and the spectrum of the mass spectrometry is shown in Figure 4(d). The peaks 24 and 25 are identified as meranzin and merancin hydrate, respectively.

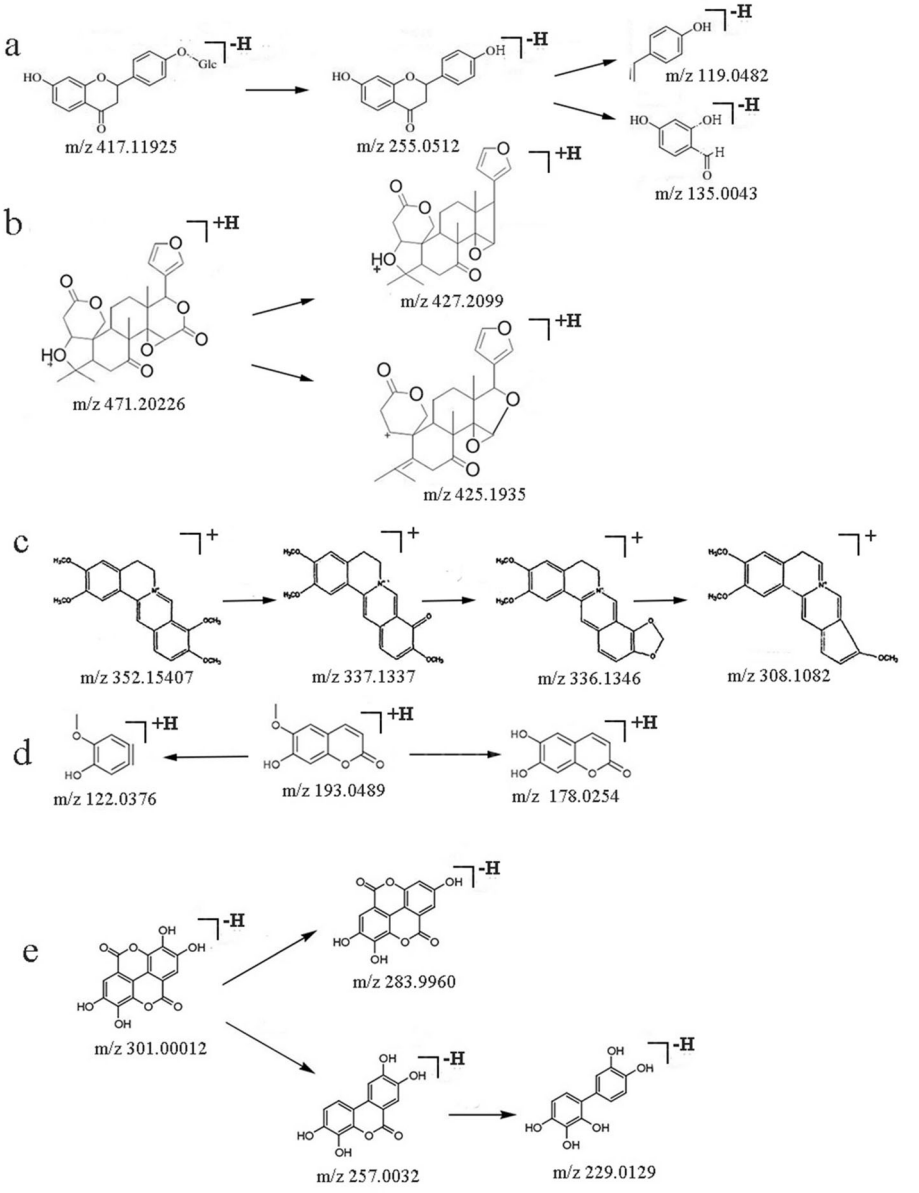


Figure 4. The fragmentation pathways of liquiritin (a), limonin (b), palmatine (c), scopoletin (d), and ellagic acid (e).

2.2.5. Characterization of organic acids

The organic acid is described by ellagic acid as an example. The *m/z* of the no. 2 peak in the negative ion mode is 301.00012 ($[M-H]^-$), and the molecular formula is presumed to be $C_{14}H_6O_8$ by mass spectrometry software. In its secondary mass spectrum (Figure 3(e)), the fragment at *m/z* 283.9960 is a fragment of the excimer ion ($[M-H]^-$) after the removal of a –OH (17 Da). *m/z* 257.0032 is a fragment of the

excimer ion ($[M-H]^-$) after the removal of one molecule of CO_2 (44 Da). The *m/z* 257.0032 fragment is stripped of one molecule of CO (28 Da) to obtain the fragment at *m/z* 229.0129. Therefore, the peak of no. 2 is identified as ellagic acid, and the spectrum of the mass spectrometry is shown in Figure 4(e). Peaks 1 and 4 are identified as citric acid and 3-O-feruloylquinic, respectively.

2.3. Effects of the ingredients in the blood on the release of amylase from AR42J cells induced by cerulean

After adding plasma samples, the release rate of amylase in AR42J cells at different time points is shown in Figure 5. Compared with the control group, the release rate of amylase in the model group was significantly increased ($p < .01$), indicating that the pancreatitis model was successfully established. There was significant difference between each test group and the model group, the release rate of amylase in each test group was significantly lower than that in the model group ($p < .01$), indicating that the drug-containing plasma had a therapeutic effect on pancreatitis. Among them, the release rate of amylase in the fifth group (the drug-containing plasma test group 2 h after administration) was the lowest, and it was speculated that danyikangtai powder had the most obvious effect after administration for 2 h.

3. Discussion

3.1. Absorbed components and their effects in the treatment of pancreatitis

Pancreatitis is caused by the premature activation of digestive enzymes produced by pancreatic acinar cells, which leads to the self-digestion of acinar cells and further leads to the inflammation of pancreas [11]. Recent studies have shown that oxidative stress and activation of pro-inflammatory signaling play an important role in the development of pancreatitis [12]. In this study, 25 kinds of chemical components absorbed into blood were identified, most of which were flavonoids. Flavonoids have abundant pharmacological activities, among which anti-inflammatory and analgesic effects have been paid more and more attention. Studies have shown that flavonoids play an anti-inflammatory and analgesic role by regulating oxidative stress, pro-inflammatory cytokines, signal pathways, and leukocytes [13]. It has been reported that naringenin can inhibit oxidative stress and inflammatory reaction through NLRP3 and Nrf2/HO-1 pathway. Naringenin can treat pancreatitis by reducing the activity of a series of inflammatory cytokines and myeloperoxidase (MPO) and reducing the production of anaerobic free radicals in pancreas [14]. What's more, naringenin can also reduce the activity of N-acetyl-β-D-glucosaminidase, reduce the recruitment of neutrophils and monocytes, and inhibit the activation of NF-κB [15]. All these are beneficial to exert its anti-inflammatory effect to treat pancreatitis. In addition, baicalin can also reduce pancreatic acinar cell necrosis and inhibit the expression of TLR4/NLRP3-related mRNA [16]. Similarly, lonicerin, rhoifolin, hyperoside, narirutin, and liquiritin also have been reported about their anti-inflammatory activities [17]. Therefore, it is speculated that flavonoids in danyikangtai

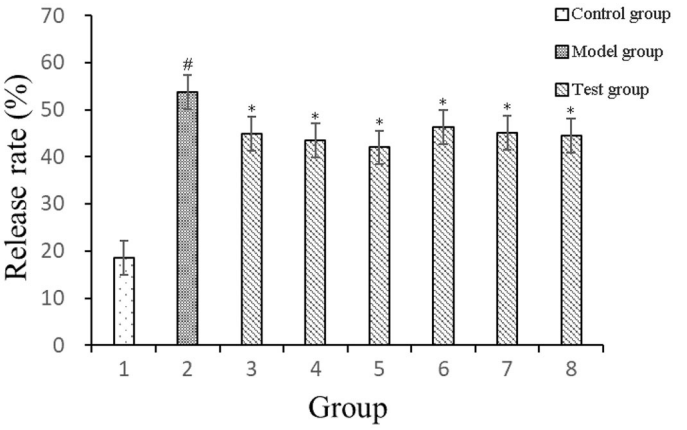


Figure 5. The time-dependent relationship of danyikangtai powder components in plasma on the release of amylase from AR42J cells at different times after intragastric administration of Sprague–Dawley rats. (1 – control group; 2 – model group; 3–9 – test group: 0.5, 1, 2, 3, 4, 6 h after administration). Data are expressed as mean ± SD. #$p < .01$, the model group was compared with the control group; *$p < .01$, the test group was compared with the model group.

powder absorbed into blood can play an anti-inflammatory effect and reduce oxidative stress in the treatment of pancreatitis.

For terpenoids, studies have shown that betulonic acid can inhibit pancreatic cancer cells and induce apoptosis of pancreatic cancer cells. Further analysis proves that betulonic acid specifically induces apoptosis by targeting mTOR signals [18]. Similarly, it has been reported that galloylpaeoniflorin and limonin can inhibit the proliferation and metastasis of cancer cells through AMPK signaling pathway and Wnt/β-catenin pathway, respectively [19]. In addition, fuziline can improve cell vitality and reduce isoproterenol-induced apoptosis, reduce reactive oxygen species production and restore mitochondrial function [20]. For organic acids, studies have found that ellagic acid can inhibit key cell functions and pancreatic stellate cells (PSC) activation by inhibiting IL-1β- and TNF-α- induced activation of activator protein-1 and mitogen-activated protein kinases. As we know, PSC play a pivotal role in the pathogenesis of pancreatic fibrosis and inflammation [21]. Therefore, it is speculated that the terpenoids and organic acids in danyikangtai powder mainly restore the health of pancreas through the regulation of cells. At the same time, berberine and coptisine in the alkaloids, and scopoletin in coumarins absorbed into the blood in danyikangtai powder also have the effects of anti-inflammatory, inhibiting oxidative stress, and inhibiting the proliferation of cancer cells [22].

In summary, the flavonoids, terpenoids, alkaloids, coumarins, and organic acids absorbed into the blood in danyikangtai powder mainly play a role in treating pancreatitis by reducing oxidative stress and pro-inflammatory cytokines, regulating signal pathways, and pancreatic cells. At the same time, the therapeutic effect of the components of danyikangtai powder entering the blood has been further verified in cell experiments. The experimental results of AR42J cells (through the release rate of cellular amylase) show that danyikangtai powder has a certain therapeutic effect on caerulein-induced pancreatitis. The drug-containing plasma two hours after

administration has the best therapeutic effect, which will provide a scientific reference for the design of drug administration routes in the future.

3.2. Undetected chemical components in drug-containing plasma

A total of 44 compounds were not detected in the plasma by comparing the extract of danyikangtai powder with the plasma containing drugs. Among them, there are 21 kinds of flavonoids (nos. 17, 20, 21, 23–29, 32–37, 39–43 in Supplementary Table S1), 4 kinds of alkaloids (nos. 3, 11, 22, 30 in Supplementary Table S1), 12 kinds of organic acids (nos. 1, 2, 4–10, 16, 18, 19 in Supplementary Table S1), 5 kinds of terpenoids (nos. 13–15, 38, 44 in Supplementary Table S1), 1 coumarin (no. 12 in Supplementary Table S1), and 1 lignan (no. 31 in Supplementary Table S1). It can be seen that there are still a lot of flavonoids which are not absorbed into the blood. According to the above analysis, it can be known that flavonoids are the main components of danyikangtai powder, which have anti-inflammatory effects and play a key role in its efficacy. Taking hesperidin which has not entered the blood as an example, hesperidin can reduce the activities of amylase and lipase in plasma, reduce the level of inflammatory factors, increase the level of insulin and resist oxidation, and play a role in treating pancreatitis by alleviating oxidative stress and inflammation [23]. Therefore, in order to give full play to its pharmacological effects, so that as many active ingredients as possible can be absorbed to exert their efficacy, it is necessary to make further research on its administration mode.

The established UHPLC–FT–ICR–MS method can be used to identify the blood components of danyikangtai powder. A total of 25 compounds were identified in the drug-containing plasma, including flavonoids, terpenoids, alkaloids, coumarins, and organic acids. Among them, flavonoids are the main components of danyikangtai powder entering the blood. It is speculated that danyikangtai powder can treat pancreatitis mainly by reducing oxidative stress and pro-inflammatory cytokines, regulating signal pathways and pancreatic cells. The therapeutic effect of the components of danyikangtai powder entering the blood has been further verified by investigating the amylase release rate of AR42J cells. The preliminary pharmacodynamic experiments proved that danyikangtai powder has a certain therapeutic effect on caerulein-induced pancreatitis, and the drug effect is the most obvious 2 h after administration. This study provides a basis for the pharmacological research and reasonable clinical application of danyikangtai powder.

4. Experimental

4.1. Materials

Methanol and acetonitrile of HPLC grade were purchased from Fisher Inc. (Town, USA). Formic acid of LC grade was purchased from Kemiou Chemical Co., Ltd. (Tianjin, China). The purified water was purchased from Wahaha Co., Ltd. (Hangzhou, China). Caerulein was purchased from Dalian Meilun Biotechnology Co., Ltd. (Dalian, China). Fetal bovine serum (FBS), penicillin, streptomycin, and Dulbecco's Modified Eagle Medium (DMEM [H]) were purchased from HyClone Inc.

(Utah, USA). Dimethyl sulfoxide (DMSO) was purchased from Damao Chemical Reagent Factory (Tianjin, China). Rat amylase (AMS) ELISA Kit was purchased from Qianxi Biological Co., Ltd. (Shanghai, China). Danyikangtai powder (batch number: 20181219) was provided by Shenyang Military Region (Shenyang, China)

4.2. Preparation of danyikangtai powder extract

About 1250 mL of purified water (1:10 w/v) was added to 125 g of danyikangtai powder, followed by soaking for 40 min and decocting for 30 min. It was then filtered through four layers of gauze. Five hundred milliliters of purified water (1:4 w/v) was added to the dregs and decocted for 30 min. After filtration through four layers of gauze, the two filtrates were combined and concentrated to 75 mL.

4.3. Animals and treatment

Six male Sprague–Dawley rats (10 weeks, 200–220 g) were purchased from the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The breeding was approved by the Medical Ethics Committee of Shenyang Pharmaceutical University and in accordance with the guidelines of the National Institutes of Health on Animal Care (2004). In addition, all operations were executed under standard conditions.

Six rats were housed for 7 days under the conditions of a temperature range of 22–24 °C and a relative humidity of 50–60%. They were fasted for 12 h before administration with free access to water. Danyikangtai powder was administered by intragastric administration at a dose of 16.7 g/kg (powder/body weight), which was converted based on the human dose. After administration, about 0.5 mL of blood was taken from the orbital vein of the rat with a heparin-coated EP tube at 0.5, 1, 2, 3, 4, and 6 h. After centrifugation at 4000 rpm for 10 min, the supernatant was placed in another EP tube and stored in a −80 °C freezer.

4.4. Sample processing

4.4.1. Pretreatment of medicinal extracts

The extract was diluted in a ratio of 1:20 and subjected to UHPLC–FT–ICR–MS analysis after passing through a 0.22 μm micropore filter.

4.4.2. Pretreatment of plasma samples

Plasma samples were mixed at various time points after administration. Four hundred microliters of acetonitrile was added to the 200 μL mixed sample. The mixture was vortexed and centrifuged at 13,000 rpm for 10 min. The supernatant was dried by blowing under a nitrogen stream at 35 °C. The residue was reconstituted with 200 μL of 0.1% formic acid in water–acetonitrile (90:10, v/v), and subjected to UHPLC–FT–ICR–MS analysis after passing through a 0.22 μm micropore filter.

4.5. Analysis condition

4.5.1. Chromatographic conditions

All analyses were performed on a Universal XB C_{18} column (150 mm × 2.1 mm, 1.8 μm; Kromat, USA) coupled to a Bruker Solarix 7.0 T FT-ICR MS system (Bruker, Germany) equipped with an electrospray ionization source (ESI). The column temperature was 35 °C, the flow rate was 0.20 mL/min, and the injection volume was 5 μL. The mobile phase consisted of 0.1% formic acid water (A) and acetonitrile (B). The gradient elution procedure was as follows: 10–20% (B) in 0–5 min, 20–28% (B) in 5–14 min, 28–50% (B) in 14–16 min, 50–70% (B) in 16–20 min.

4.5.2. Mass spectrometry condition

The positive ion and negative ion modes of ESI were used; the capillary voltage was 4.5 kV; the desolvation gas temperature was 200 °C, and the desolvation gas flow rate was 8 L/min. The atomizing gas was high purity nitrogen, the atomizing gas pressure was 4 bar; the collision gas was high purity argon gas. With full scan mode, the full scan quality range was 100–1000 Da. The collision energy of the secondary mass spectrum ranges from 10 to 30 eV.

4.6. Anti-acute pancreatitis cell experiment

There were eight groups of control, model and test (0.5, 1, 2, 3, 4, 6 h after administration), and each group had six duplicate wells. AR42J cells were seeded at 1×10^5 cells/well in six-well plates, and cultured in DMEM [H] medium containing 10% FBS and 1% penicillin and streptomycin at 37 °C and 5% CO_2. The experiment was carried out after 24 h of incubation. In the control group and the model group, 2 mL of 10% blank plasma culture solution was added, and the test group was added with 2 mL of 10% drug-containing plasma culture solution. The model of *in vitro* pancreatitis was established according to the literature and reagent instructions [7]. Briefly, after 30 min of culture, the model group and the test group were added with 6 μL of the molding solution (the cerulean was dissolved in DMSO, and the final concentration was 10^{-7} mol/L), and the control group was added with 6 μL of DMSO, and further incubated for 24 h. The cells and the supernatant were collected for the amylase content by the kit method, and the ratio of the amylase in the cytoplasm of the damaged cells to the supernatant was obtained to evaluate the cell death rate, thereby evaluating the anti-acute pancreatitis activity of the enrolled component. One milliliter of PBS was added to the collected cells and disrupted twice under ultrasound. The disrupted cells and cell supernatant were centrifuged at 3000 rpm for 10 min, respectively. Standard well and sample well were set according to the kit method, and the absorbance (A) value of each well was measured at the wavelength of 450 nm after the corresponding solution was added. The linear regression curve of reference standard was drawn, and the concentration of each sample was calculated according to the curve equation. The amylase release rate was calculated according to the formula: amylase release rate (%) = 100% × total extracellular amylase activity/total amylase activity.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported there is no funding associated with the work featured in this article.

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