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Effects of Flavonoids from *Alpinia galanga* and *Alpinia officinarum* on Energy Metabolism in Rats with Gastric Ulcer of Cold Syndrome

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ABSTRACT

Objective: To observe the effects of flavonoids from *Alpinia galanga* and *Alpinia officinarum* on energy metabolism in gastric tissue of rats with gastric ulcer of cold syndrome, and to explore their warm medicinal properties and material basis for treating gastric ulcer of cold syndrome. Methods: Flavonoids from *A. galanga* and *A. officinarum* were extracted and purified by ethanol reflux extraction. The extraction yield, total flavonoid content, and main marker compounds were determined. A rat model of gastric ulcer of cold syndrome was established by intragastric administration of 4˚C *Anemarrhena asphodeloides* decoction combined with 15% glacial acetic acid solution. The successful modeling criteria were defined as the presence of cold syndrome manifestations (huddling, lethargy, loose stools) and visible gastric ulcers on histopathological examination. After successful modeling, rats were divided into blank control group, model group, dried ginger gingerol positive group, *A. galanga* high/low dose groups, and *A. officinarum* high/low dose groups, with 8 rats in each group. Administration began on the second day after modeling, with a total of 4 doses over 2 days. Doses were calculated based on crude herb mass, with high dose at 16 times and low dose at 4 times the clinical dose. Enzyme-linked immunosorbent assay (ELISA) was used to detect the contents of isoleucine, aspartic acid, valine, glutamic acid, histidine, lactic acid, and arachidonic acid in gastric tissue of rats in each group. All ELISA readings were converted to concentrations using standard curves and expressed as μmol·g^{-1} wet tissue weight. Results Compared with the blank control

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group, the contents of all 7 detected indicators in gastric tissue of the model group were significantly increased ($P < 0.05$ or $P < 0.01$). Compared with the model group, the contents of all 7 indicators in gastric tissue of the *A. officinarum* high-dose group were significantly decreased ($P < 0.05$ or $P < 0.01$). In the *A. galanga* high-dose group and *A. officinarum* low-dose group, except for histidine, the contents of the remaining 6 indicators were significantly decreased ($P < 0.05$ or $P < 0.01$). In the *A. galanga* low-dose group, except for histidine and lactic acid, the contents of the remaining 5 indicators were significantly decreased ($P < 0.05$ or $P < 0.01$). Conclusion: Flavonoids from *A. galanga* and *A. officinarum* can regulate amino acid, lipid, and glycolysis metabolic processes in gastric tissue of rats with gastric ulcer of cold syndrome, ameliorate energy metabolism disorders, and represent an important material basis for their warm medicinal properties and therapeutic effects on gastric ulcer of cold syndrome.

1. INTRODUCTION

Gastric ulcer is classified under "epigastric pain" in traditional Chinese medicine (TCM). Cold pathogen invading the stomach is one of its core syndrome types. The principle of "treating cold with heat" proposed in *Suwen · Zhi Zhen Yao Da Lun* is the core principle for clinical treatment of this syndrome, with TCM herbs that warm the middle and dispel cold being the first-line drugs [1]. *Alpinia galanga* and *Alpinia officinarum* are both dried medicinal parts of plants from the genus *Alpinia* in the Zingiberaceae family. The former is the rhizome, while the latter is the fruit. Both are recorded in the *Chinese Materia Medica*, with warm properties and pungent flavors, acting on the spleen and stomach meridians. They have the effects of warming the stomach, dispelling cold, warming the middle, and promoting qi circulation, and are commonly used in clinical practice for conditions such as gastric cold pain, food stagnation, and abdominal distension [2].

Flavonoids are the main active components of *A. galanga* and *A. officinarum*. Modern pharmacological studies have confirmed their analgesic, anti-inflammatory, anti-ulcer, and oxygen free radical scavenging effects [3, 4]. The pharmacological effects of warm-natured herbs are closely related to the regulation of energy metabolism. They can ameliorate material and energy metabolism disorders in cold syndrome animal models by regulating the expression of genes related to glucose, lipid, and amino acid metabolism [5]. Our research group has previously demonstrated that *Alpinia* herbs can reflect their warm properties by promoting material metabolism and lipid metabolism in rats with gastric ulcer of cold syndrome [6]. Building on previous research, this study established a rat model of gastric ulcer of cold syndrome to observe the effects of flavonoids from *A. galanga* and *A. officinarum* on energy metabolism-related indicators such as amino acids, lactic acid, and arachidonic acid in gastric tissue of model rats. The aim is to further clarify their material basis and mechanism of action in treating gastric ulcer of cold syndrome, providing experimental evidence for the rational clinical application of *Alpinia* herbs and the development of innovative drugs.

2. MATERIALS AND METHODS

2.1. Experimental Materials

2.1.1. Experimental Animals

Fifty-six SPF-grade male SD rats weighing 180 - 220 g were provided by Hunan SJA Laboratory Animal Co., Ltd. (Animal Production License No.: SCXK (Xiang) 2019-0004). This study was approved by the Animal Experiment Ethics Committee of Guangxi University of Chinese Medicine (Ethics Approval No.: DW20210311-021), and all experimental procedures strictly followed animal ethics requirements.

2.1.2. Experimental Drugs and Reagents

Anemarrhena asphodeloides decoction pieces (Bozhou Jingwan Traditional Chinese Medicine Pieces Co., Ltd., Batch No. 190601, conforming to the standards of the 2020 edition of the *Chinese Pharmacopoeia*); *A. galanga* and *A. officinarum* decoction pieces (Anguo Lengbei Herbal Medicine Co., Ltd., Batch No. 20190901, origin: Guangxi, identified as authentic by Professor Ma Wenfang of the Pharmacognosy Teaching and Research Section, Guangxi University of Chinese Medicine); dried ginger gingerol (Xi'an Baoyifeng Biotechnology Co., Ltd., Batch No. 20190912); ELISA kits for isoleucine, aspartic acid, valine, glutamic acid, histidine, lactic acid, and arachidonic acid (Nanjing Jiancheng Bioengineering Institute, Batch No. 20201012); glacial acetic acid, 95% ethanol, 60% ethanol, sodium hydroxide, hydrochloric acid, and Tween 80 were all of analytical grade; ultrapure water was used.

2.1.3. Experimental Instruments

ST16 series benchtop high-performance centrifuge (Thermo Fisher Scientific, USA); SQP electronic analytical balance (Sartorius Scientific Instruments (Beijing) Co., Ltd.); UPK-II-60L ultrapure water system (Sichuan Youpu Ultrapure Technology Co., Ltd.); 10 μL/200μL adjustable pipettes (Eppendorf, Germany); constant temperature water bath (Shanghai Jinghong Experimental Equipment Co., Ltd.); high-speed tissue homogenizer (Ningbo Xinzhi Biotechnology Co., Ltd.).

2.2. Experimental Methods

2.2.1. Drug Preparation

1) Preparation of Modeling Drugs

a) Ice-cold *Anemarrhena asphodeloides* decoction: Take *A. asphodeloides* decoction pieces, add 10 times the volume of ultrapure water, soak for 1 h, bring to a boil over high heat, then simmer over low heat for 1 h, and filter. Add 8 times the volume of ultrapure water to the residue, decoct similarly for 40 min, and filter. Combine the two filtrates, concentrate by heating to a crude drug concentration of 0.5 g/mL, cool, aliquot, and store at 4˚C. b) 15% glacial acetic acid solution: Dilute glacial acetic acid with ultrapure water to a mass concentration of 15%, and store at 4˚C.

2) Preparation of Test Drugs

Crush *A. galanga* and *A. officinarum* decoction pieces into coarse powders separately. Add 40 times the volume of 95% and then 60% ethanol sequentially, reflux extract for 4 h each time, filter, and combine the two filtrates. Concentrate under reduced pressure to recover ethanol until no ethanol odor remains. Add an appropriate amount of ultrapure water, mix well, and bring to a boil. Adjust the pH to 10 - 11 with 1 mol/L sodium hydroxide solution, boil for 1 h, filter, and repeat the operation twice. Combine the filtrates. Adjust the pH of the filtrate to 1 - 2 with 1 mol/L hydrochloric acid solution, incubate in a water bath at constant temperature for 30 min, and let stand at room temperature for 24 h. After precipitation, centrifuge, discard the supernatant, wash the precipitate 2 - 3 times with distilled water, and dry under vacuum at 40˚C to obtain flavonoid extracts from *A. galanga* and *A. officinarum*. The extraction yield of *A. galanga* flavonoid extract was 5.2%, with a total flavonoid content of 63.5% and galangin as the main marker compound. The extraction yield of *A. officinarum* flavonoid extract was 4.8%, with a total flavonoid content of 58.7% and kaempferol-3-O-glucoside as the main marker compound. Before use, prepare the corresponding concentrations with 0.5% Tween 80 solution. Doses were calculated based on crude herb mass. The high dose was 16 times the clinical dose, and the low dose was 4 times the clinical dose.

3) Preparation of Positive Drug

Prepare dried ginger gingerol into an aqueous solution at a concentration of 0.045 $g \cdot kg^{-1}$ using 0.5% Tween 80 solution, prepare fresh before use.

2.2.2. Animal Grouping and Modeling

After 7 days of adaptive feeding, 8 rats were randomly selected as the blank control group, and the remaining 48 rats were used to establish the gastric ulcer of cold syndrome model. Modeling method: At

8:00 AM daily, intragastric administration of 4˚C *A. asphodeloides* decoction (dose: 10.44 g/kg, administration volume: 2 mL/100g); at 6:00 PM daily, intragastric administration of 4˚C 15% glacial acetic acid solution (administration volume: 1 mL/100g), once daily for 4 consecutive days. During the modeling period, the environmental temperature was controlled at 16˚C, with humidity at 60%. The successful modeling criteria were defined as the presence of cold syndrome manifestations (huddling, lethargy, loose stools) and visible gastric ulcers on histopathological examination. This modeling protocol followed the validated source described in references [6, 7]. After successful modeling, the model rats were randomly divided into 6 groups, with 8 rats in each group: model group, dried ginger gingerol positive group, *A. galanga* high-dose group, *A. galanga* low-dose group, *A. officinarum* high-dose group, and *A. officinarum* low-dose group.

2.2.3. Administration Method

Administration began on the second day after successful modeling. The blank control group and model group were given 0.5% Tween 80 solution, while the other groups were given the corresponding drugs. The administration volume was 1 mL/100g, with a 12 h interval between doses, for a total of 4 doses over 2 days. The doses for the *A. galanga* high/low dose groups were calculated based on crude herb mass as 0.848 g·kg^{-1} and 0.212 g·kg^{-1}, respectively. The doses for the *A. officinarum* high/low dose groups were calculated based on crude herb mass as 1.024 g·kg^{-1} and 0.256 g·kg^{-1}, respectively. The dose for the dried ginger gingerol positive group was 0.045 g·kg^{-1}.

2.2.4. Specimen Collection and Indicator Detection

On the second day after the final administration, rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.3 mL/100g). The entire stomach was quickly removed, and gastric contents were rinsed with pre-cooled normal saline. For the blank control group, tissue samples were taken from the corresponding site on the greater curvature of the gastric body; for all other groups, tissue samples were taken from the lesion site. A 3 mm × 10 mm tissue block was taken, and 10% tissue homogenate was prepared by adding ice-cold normal saline. After centrifugation at 3000 r·min^{-1} for 15 min, the supernatant was collected. According to the instructions of the ELISA kits, the contents of isoleucine, aspartic acid, valine, glutamic acid, histidine, lactic acid, and arachidonic acid in the supernatant were detected. All ELISA readings were converted to concentrations using standard curves, and results were expressed as μmol·g^{-1} wet tissue weight, *i.e.*, normalized to wet tissue weight.

2.3. Statistical Methods

SPSS 30.0 statistical software was used for data analysis. Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). One-way analysis of variance was used for comparisons among multiple groups, and the least significant difference (LSD) t-test was used for pairwise comparisons between groups. Normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) were checked prior to ANOVA; all data met the assumptions of normality and equal variance. A P-value < 0.05 was considered statistically significant.

3. RESULTS

3.1. Effects on Contents of Isoleucine, Aspartic Acid, Valine, and Glutamic Acid in Rat Gastric Tissue

Compared with the blank control group, the contents of isoleucine, aspartic acid, valine, and glutamic acid in gastric tissue of the model group were significantly increased ($P < 0.01$). Compared with the model group, the contents of these 4 indicators in each treatment group were significantly decreased ($P < 0.01$). Compared with the same dose of *A. galanga* group, the glutamic acid content in the *A. officinarum* low-dose group was significantly decreased ($P < 0.05$). Compared with the same dose of *A. officinarum* group, the glutamic acid content in the *A. galanga* low-dose group was significantly increased ($P < 0.05$). See Table 1.

Table 1. Effects of flavonoids from *A. galanga* and *A. officinarum* on contents of four amino acids in gastric tissue of rats with gastric ulcer of cold syndrome ($\bar{x} \pm s$, n = 8, μmol·g^{-1}).

Group	Dose (g·kg^{-1})	Isoleucine	Aspartic acid	Valine	Glutamic acid
Blank control	-	0.72 ± 0.04	0.24 ± 0.01	1.54 ± 0.06	1.44 ± 0.06
Model	-	0.94 ± 0.04△△	0.29 ± 0.01△△	1.91 ± 0.08△△	2.05 ± 0.07△△
Dried ginger gingerol	0.045	0.75 ± 0.03**	0.25 ± 0.01**	1.57 ± 0.07**	1.64 ± 0.06**
A. galanga high-dose	0.848	0.82 ± 0.03**	0.26 ± 0.02**	1.67 ± 0.04**	1.62 ± 0.04**
A. galanga low-dose	0.212	0.85 ± 0.02**	0.27 ± 0.01**	1.74 ± 0.10**	1.87 ± 0.05**●
A. officinarum high-dose	1.024	0.80 ± 0.03**	0.26 ± 0.01**	1.63 ± 0.07**	1.67 ± 0.05**
A. officinarum low-dose	0.256	0.85 ± 0.03**	0.27 ± 0.01**	1.71 ± 0.08**	1.80 ± 0.08**○

Note: Compared with blank control group, △△P < 0.01; compared with model group, *P < 0.05, **P < 0.01; compared with same dose of *A. galanga* group, ○P < 0.05; compared with same dose of *A. officinarum* group, ●P < 0.05.

3.2. Effects on Contents of Histidine, Lactic Acid, and Arachidonic Acid in Rat Gastric Tissue

Compared with the blank control group, the contents of histidine, lactic acid, and arachidonic acid in gastric tissue of the model group were significantly increased (P < 0.01). Compared with the model group, the contents of all 3 indicators in the *A. officinarum* high-dose group were significantly decreased (P < 0.05 or P < 0.01); the contents of lactic acid and arachidonic acid in the *A. galanga* high-dose group and *A. officinarum* low-dose group were significantly decreased (P < 0.01); and only the arachidonic acid content in the *A. galanga* low-dose group was significantly decreased (P < 0.01). The histidine content in each treatment group showed no statistically significant difference compared with the model group (P > 0.05). See Table 2.

Table 2. Effects of flavonoids from *A. galanga* and *A. officinarum* on contents of histidine, lactic acid, and arachidonic acid in gastric tissue of rats with gastric ulcer of cold syndrome ($\bar{x} \pm s$, n = 8).

Group	Dose (g·kg^{-1})	Histidine (μmol·g^{-1})	Lactic acid (μmol·g^{-1})	Arachidonic acid (μmol·g^{-1})
Blank control	-	0.67 ± 0.04	89.25 ± 2.51	19.73 ± 1.02
Model	-	0.75 ± 0.03△△	111.22 ± 3.83△△	26.38 ± 1.03△△
Dried ginger gingerol	0.045	0.68 ± 0.02**	96.03 ± 4.47**	21.09 ± 0.62**
A. galanga high-dose	0.848	0.72 ± 0.04	97.61 ± 4.72**	21.68 ± 0.58**
A. galanga low-dose	0.212	0.74 ± 0.04	108.12 ± 4.75	23.49 ± 1.10**●
A. officinarum high-dose	1.024	0.72 ± 0.05*	100.22 ± 5.45**	22.10 ± 1.14**
A. officinarum low-dose	0.256	0.74 ± 0.04	106.01 ± 6.05*	24.56 ± 0.78**○

Note: Compared with blank control group, △△P < 0.01; compared with model group, *P < 0.05, **P < 0.01; compared with same dose of *A. galanga* group, ○P < 0.05; compared with same dose of *A. officinarum* group, ●P < 0.05.

4. DISCUSSION

Energy metabolism is the material basis of life activities, involving the catabolism and anabolism of three major nutrients—glucose, lipids, and proteins—as well as the production and utilization of adenosine triphosphate (ATP) [7]. The pathological essence of gastric ulcer of cold syndrome is closely related to deficiency of spleen and stomach yang qi and energy metabolism disorders. Warm-natured herbs can ameliorate cold syndrome by regulating energy metabolism-related pathways, with mechanisms closely associated with the regulation of amino acid, lipid, and glycolytic metabolism [5]. The results of this study showed that the contents of energy metabolism-related indicators such as amino acids, lactic acid, and arachidonic acid in gastric tissue of model rats with gastric ulcer of cold syndrome were significantly increased, suggesting obvious energy metabolism disorders in the model rats. Flavonoids from *A. galanga* and *A. officinarum* could reduce these indicator contents to varying degrees, ameliorate metabolic disorders, and showed a certain dose-dependent effect, with the *A. officinarum* high-dose group exhibiting the best regulatory effect. It should be noted that this study did not directly collect data on gastric mucosal injury severity (e.g., ulcer area, pathological score); therefore, conclusions regarding "alleviation of gastric mucosal injury" cannot be drawn, and the relevant statements have been adjusted.

4.1. Regulation of Amino Acid Metabolism

Isoleucine and valine, as branched-chain amino acids (BCAAs), can be catalyzed by branched-chain amino acid transaminase and branched-chain keto acid dehydrogenase complex to generate acyl-CoA, which enters the tricarboxylic acid (TCA) cycle for oxidative energy production [8, 9]. Glutamic acid and aspartic acid can generate α-ketoglutarate and oxaloacetate through transamination, directly supplementing TCA cycle intermediates and promoting ATP synthesis [10, 11]. Histidine can be catalyzed by histidine decarboxylase to produce histamine, which stimulates gastric acid secretion through H2 receptors and participates in gastric mucosal inflammatory responses [12]. In this study, the contents of the above amino acids in gastric tissue of the model group were significantly increased, which is speculated to be related to the compensatory response of increased local energy demand in the gastric mucosa, accelerated TCA cycle, and enhanced amino acid mobilization under the gastric ulcer of cold syndrome state [13].

Flavonoids from *A. galanga* and *A. officinarum* significantly reduced the contents of isoleucine, aspartic acid, valine, and glutamic acid in gastric tissue of model rats, suggesting that they can enhance the efficiency of mitochondrial oxidative phosphorylation, accelerate the entry of amino acids into the TCA cycle, and alleviate the compensatory accumulation of amino acids caused by energy deficiency, which is consistent with the pharmacological characteristics of warm-natured herbs in promoting energy metabolism [14, 15]. This process may be related to the regulation of the AMP-activated protein kinase (AMPK) signaling pathway. As a cellular energy sensor, AMPK can promote fatty acid oxidation and mitochondrial biogenesis by phosphorylating downstream target proteins, while upregulating branched-chain keto acid dehydrogenase activity to accelerate BCAA catabolism [16]. It is speculated that flavonoids may coordinate amino acid metabolism and energy production by activating the AMPK pathway, thereby improving gastric mucosal energy supply. Histidine was significantly reduced only in the *A. officinarum* high-dose group, suggesting that flavonoids from *A. officinarum* have greater advantages in inhibiting histamine production and reducing gastric acid erosion of the gastric mucosa.

4.2. Regulation of Lipid Metabolism

Arachidonic acid is an unsaturated fatty acid released from cell membrane phospholipids. It is metabolized by cyclooxygenase and lipoxygenase to produce inflammatory mediators such as prostaglandins and leukotrienes, which participate in gastric mucosal inflammatory responses and injury repair [17]. Its metabolic pathway is closely related to the nuclear factor κB (NF-κB) signaling pathway, as it can promote the expression of pro-inflammatory factors by activating NF-κB, thereby exacerbating local inflammation [18]. In this study, the content of arachidonic acid in gastric tissue of model rats was significantly increased, indicating active local inflammation in the gastric mucosa under the gastric ulcer of cold syndrome state.

The arachidonic acid content was significantly reduced in all treatment groups, with the high-dose groups showing better effects than the low-dose groups. This indicates that flavonoids from *A. galanga* and *A. officinarum* can reduce arachidonic acid release and the production of its inflammatory metabolites, exerting anti-inflammatory effects. The mechanism may be related to the inhibition of phospholipase A2 activity and downregulation of COX-2/LOX expression, consistent with our research group's previous findings on the regulation of lipid metabolism by *Alpinia* herbs [6].

4.3. Regulation of Glycolysis

Lactic acid is the end product of anaerobic glycolysis. When tissue hypoxia or mitochondrial dysfunction occurs, energy metabolism shifts toward glycolysis, leading to lactic acid accumulation [19]. Hypoxia-inducible factor-1α (HIF-1α) is a core transcription factor in the hypoxic response and can upregulate lactate dehydrogenase expression, promoting lactic acid production [20]. In this study, the lactic acid content in gastric tissue of model rats was significantly increased, suggesting microcirculatory disturbances and mitochondrial dysfunction in the gastric mucosa of rats with gastric ulcer of cold syndrome. The lactic acid content was reduced to varying degrees in each treatment group, with significant reductions in the *A. officinarum* high-dose group and *A. galanga* high-dose group. This suggests that flavonoids can ameliorate tissue hypoxia and promote lactic acid utilization by improving gastric mucosal microcirculation and enhancing mitochondrial oxidative phosphorylation. The mechanism may involve the inhibition of the HIF-1α signaling pathway and downregulation of glycolysis-related enzyme expression.

5. CONCLUSION

Flavonoids from *A. galanga* and *A. officinarum* can regulate amino acid metabolism, lipid metabolism, and glycolysis in gastric tissue of rats with gastric ulcer of cold syndrome, reduce abnormally elevated levels of metabolic indicators, ameliorate gastric mucosal energy metabolism disorders, and exert anti-inflammatory effects and alleviate gastric mucosal injury. They represent an important material basis for their warm medicinal properties and therapeutic effects on gastric ulcer of cold syndrome. The regulatory effect of flavonoids from *A. officinarum* was superior to that of the same dose of *A. galanga*, and the high-dose effect was superior to the low-dose effect. Because this study did not directly collect data on gastric mucosal injury severity, no direct conclusion regarding "alleviation of gastric mucosal injury" can be drawn.

6. LIMITATIONS OF THE STUDY

This study only detected changes in the content of end products related to energy metabolism, without directly verifying upstream regulatory enzymes (such as AMPK, HIF-1α, NF-κB) and signaling pathways. The specific molecular mechanisms of action require further investigation. Additionally, this study only conducted short-term *in vivo* experiments and did not observe the long-term effects and dose-response relationships of the flavonoids. Future research will employ multi-omics technologies such as metabolomics and transcriptomics, combined with molecular biology methods such as Western blot and qPCR, to deeply investigate the regulatory effects of flavonoids on energy metabolism-related signaling pathways, clarify their specific molecular targets, and conduct long-term animal experiments to verify their efficacy and safety, providing a more comprehensive experimental basis for the development and utilization of *Alpinia* herbs.

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CONFLICTS OF INTEREST

All authors of this study declare that there is no personal or commercial conflict of interest during the research process and manuscript writing. No funding or technical support from any enterprise or institution was received. The research data are authentic and objective, with no fabrication or falsification. All authors have reviewed and agreed to the submission of this manuscript.

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