Original Research Article

Effect of solvent fractions of crude extract of Liushenqu on gastrointestinal motility in guinea pigs, and the underlying mechanism(s)

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Abstract

Purpose: To study the effect of solvent fractions of the crude extract of liushenqu on gastrointestinal motility in guinea pigs, and the mechanism of action.

Methods: The effects of solvent fractions of crude extract of liushenqu (LSQ) on receptors in guinea pig isolated small intestinal cells were determined by treatment with different receptor blockers, including diphenhydramine (0.067 mg/mL), atropine sulfate (0.064 mg/mL), propranolol hydrochloride (0.033 mg/mL), phentolamine mesylate (0.04 mg/mL) and ondansetron hydrochloride (0.048 mg/mL), to investigate the possible pharmacological mechanism of action.

Results: There was no significant change in the maximum amplitude of muscle tension before and after administration in the control group, petroleum ether fraction group, and dichloromethane fraction group, while muscle tension in the 95% ethanol and n-butanol fractions significantly increased (p < 0.01). The mean changes in tension were significantly different from that of control group (p < 0.01), but ethyl acetate fraction showed significant intestinal muscle inhibition (p < 0.01). Addition of LSQ did not alleviate the inhibition caused by diphenhydramine, but it significantly reversed the inhibition caused by blockers of cholinergic muscarinic receptor, adrenergic alpha- and beta-receptors, and 5-HT receptor (p < 0.01).

Conclusion: These results indicate that n-butanol fraction is the most effective bioactive fraction of LSQ, while ethyl acetate fraction has the opposite effect. In addition, its mechanism of action is related to increase in the amplitude of small intestine smooth muscle contraction and acceleration of small intestine peristalsis.

Keywords: Liushenqu, Intestinal muscle, Gastrointestinal motility, Mechanism of action

INTRODUCTION

Liushenqu (LSQ), also known as Shenqu and Liuqu, is a traditional Chinese medicine for treating indigestion and its related disorders. The main functions of LSQ are related to protection of the spleen and stomach and promotion of digestion, which make it especially suitable for...
formulation of children's food in clinical practice. It is made up of a certain proportion of flour, wheat bran, rice bean powder and bitter apricot seed powder, with water extract of *Artemisia annua* L., *Polygonum barbatum* L. and *Xanthium sibiricum* Patr. Ex Widder, in the ratio of 25:50:1:5:5:5:5. Usually, it is pressed into a small square with a fixed mold, and fermented under constant temperature of 36 °C and a humidity of 75 % [1].

Since LSQ promotes digestion and stimulates appetite, it has a wide range of applications in clinical practice, such as treatment of indigestion, stomach pain or bloating, especially for children indigestion and constipation [2]. At present, the mechanism of action of LSQ in promoting digestion is still unclear, but many scholars believe that the digestive enzymes produced during the fermentation process are the medicinal basis of LSQ, and that they can be used as indicators of changes in the fermentation process [3-5].

The aim of the present study was to identify the most biologically active fraction of LSQ, as well as its active phytochemical composition, and to determine the mechanism through which it promotes digestion by studying its effect on small intestinal smooth muscle receptors in guinea pigs.

**EXPERIMENTAL**

**Materials**

The LSQ used in this study was provided by Sichuan Fuzheng Pharmaceutical Co., Ltd., and was identified by Professor Xian-Ming Lu (College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, China).

**Extract preparation**

LSQ (600 g) was extracted by reflux with 95 % ethanol for 2 h. This was repeated three times, and the filtrates were combined and concentrated to dryness, and re-dissolved in 1000 mL of water. This was subjected to liquid-liquid micro-extraction with different solvents (1000 mL each) in the order: petroleum ether (60 – 90 °C), dichloromethane, ethyl acetate and n-butanol. Each solvent extract was evaporated and taken up in pure water, made into a drug solution of 0.1 g/mL.

**Animal experiments**

A total of 100 male guinea pigs (five months old, and weighing 250 – 300 g) were obtained from the Sichuan Experimental Animal Center (Chengdu, China). All animals received humane care according to the Declaration of Helsinki promulgated in 1964 and amended in 1996 [6]. All experimental protocols were approved by the Animal Care and Use Committee of Chengdu Municipal Hospital of Traditional Chinese Medicine, Chengdu, China (approval no. SCXK (Chuan) 2013-14).

**Preparation and isolation of guinea pig intestinal muscle**

After 7 days of adaptive feeding, the guinea pigs were fasted for 24 h prior to experimental procedure. The animals were humanely sacrificed via cervical spine dislocation, then laparotomy was quickly carried out. The ileum was cut 10 cm long, gently rinsed with Krebs solution, and placed in the nutrient solution. The smooth muscle was separated from unwanted tissue and cut into 1.0 cm long sections for later use [7]. One end of the muscle strip was hung on the bottom hook of a bath containing 30 mL of Krebs solution, and the other end was tied with an inelastic cotton thread on a muscle tension transducer hook. A load of about 1.0 g was then applied. All changes in isometric tension were recorded through a force transducer coupled with a bridge amplifier data acquisition system, and the experiment was started after balance was attained [8].

**Evaluation of the effect of LSQ extract on isolated intestinal muscles**

The experiment was divided into five groups: control group (CG), ethanol extract group (EEG), petroleum ether group (PEG), dichloromethane group (DIG), ethyl acetate group (EAG) and n-butanol group (n-BG). The bio-signal acquisition system first traced a normal intestinal smooth muscle standard curve. After the contraction was stabilized, 1.5 mL of 0.1g/mL solution of each solvent fraction was added to the bath separately. For the control group, 1.5 mL of pure water was used. The intestinal muscle contraction curve was recorded after addition of drug solution. At the end of the experiment, the log file was saved, and the muscle tension was measured within 1 min before the administration (AA), and at the first 1 min after the administration (AB) using the interval measurement method provided by the bio-signal software. The average tension change value (AV) was calculated as in Eq 1.

\[
AV = (AA - AB) \quad \text{(1)}
\]
Assessment of the effect of LSQ extract on isolated intestinal muscle receptors

The experiment was divided into five groups: diphenhydramine group (DG, 25 mg/mL), atropine sulfate group (ASG, 0.024 mg/mL), propranolol hydrochloride group (PG, 1 mg/mL), phentolamine mesylate group (PMG, 0.8 mg/mL) and ondansetron hydrochloride group (OHG, 4.8 mg/mL). The biosignal acquisition system first traced a normal intestinal smooth muscle standard curve. After stabilization of the contraction, the corresponding receptor blocker was added to the bath, and the muscle tension was measured within 1 min before the administration, and at the 1 min after the administration using the interval measurement method provided by the bio-signal software. Then, 2 mL 0.1g/mL ethanol extract of LSQ was added, and the change in intestinal muscle tension was recorded within 1 min. At the end of the experiment, the log file was saved, and the muscle tension was measured 1 min before the receptor blocker administration (BA), 1 min after the receptor blocker administration (AR), and the first minute after the administration of the LSQ (BL), using the interval measurement method provided by the bio-signal software. The average tension change value (AC) was calculated as indicated in Equation 2.

\[ AC = (BL - AR) \]

\(............... (2)\)

Statistical analysis

Data from animal experiments are expressed as mean ± standard deviation (SD), and were analyzed using SPSS 21.0 software with single factor analysis of variance between groups. Values of \( p < 0.05 \) were considered significant.

RESULTS

Effect of different solvent extracts of LSQ on isolated intestinal muscles

As seen from Table 1, the maximum and amplitude of muscle tension were significantly increased in the ethanol extract (EE, 5mg/mL) and n-butanol groups (n-BG, 5mg/mL), relative to control group (\( p < 0.01 \)). The average tension changes were significantly different from that of the control group (\( p < 0.01 \)). It can be seen from the results that intestinal muscle tension was significantly increased in EE and n-BG groups (\( p < 0.01 \)). Moreover, the n-butanol fraction produced the most significant changes. There was no significant change in the maximum and amplitude of muscle tension before and after administration in petroleum ether group (5mg/mL) and dichloromethane group (5mg/mL), and the average change value was not significantly different from that in the control group, indicating that the components of the petroleum ether and dichloromethane had no effect on smooth muscle contraction. The maximum muscle tension before and after administration of ethyl acetate (5 mg/mL) was significantly reduced (\( p < 0.01 \)), and the amplitude after administration was close to zero, showing a significant inhibitory effect. Thus, there may be substances inhibiting smooth muscle in this fraction. The representative intestinal muscle movement curve of each group is shown in Figure 1.

Effect of LSQ on isolated intestinal muscle receptors

As shown in Table 2, after the addition of diphenhydramine (D, 0.067mg/mL), atropine sulfate (AS, 0.064mg/mL), propranolol hydrochloride (PG, 0.033mg/mL), phentolamine mesylate (PM, 0.04mg/mL) and ondansetron hydrochloride (OH, 0.048mg/mL), maximal muscle tension decreased significantly such that the amplitude was close to 0, and the receptor blocker significantly blocked the smooth muscle contractility (\( p < 0.01 \)). After addition of ethanol extract of LSQ (6.67mg/mL), there was no significant change in the indices of diphenhydramine (0.067 mg/mL) group; the changes in muscle tension in other groups were significant (\( p < 0.01 \)). The changes in intestinal muscle contraction curve before and after of administration for each group are shown in Figure 2.
DISCUSSION

Traditional Chinese Medicines (TCMs) are considered useful agents for treating various human diseases [9]. In fermented TCM, changes in various conditions during fermentation affect the quality of the finished product [10]. Although the chemical composition and mechanism of action of Liushenqu is still not clear, its significant pharmacological activity means that it will continue to be of use in Chinese medicine. Thus, it is necessary to provide scientific data to serve as a basis for its use in Chinese medicine. The purpose of this study was to investigate the components of LSQ, and to explore its mechanism of action in promoting digestion.

Separation of the components of LSQ with different solvents was carried out according to solvent polarities. The pharmacological activity of lower polarity component was substantially lower, and the components having a significant influence on the gastrointestinal contraction were found in the n-butanol fraction. The n-butanol fraction contained presumably saccharide, glycoside or protein, which is consistent with opinion in the literature that digestive enzymes are the main medicinal substances of LSQ [11]. Some scholars have discovered that the effect of LSQ on GIT pharmacology can be improved by high temperature treatment [12]. However, enzyme components are destroyed at high temperatures. This contradiction suggests that there must be a significant class of high temperature-resistant components in LSQ which have significant pharmacological activities. The results of this study suggest that the active principle of LSQ may be present in the n-butanol fraction, which lays the foundation for further research on this fraction. It was also observed that the ethyl acetate extract had significant intestinal muscle tone inhibition, which is being reported for the first time.

In addition, the ethyl acetate fraction showed a significant inhibition of intestinal peristalsis, which means that there are chemical components in the LSQ that are antagonistic to digestion. In addition, there may be other pharmacological effects of LSQ, such as anti diarrheal effects.

Receptors are important factors for regulating gastrointestinal motility. Together with the nervous system and gastrointestinal hormones, they participate in the regulation of gastrointestinal motility [13]. At present, it is believed that there are mainly cholinergic muscarinic receptors, adrenergic alpha and beta receptors, 5-HT receptors, histamine receptors and various gastrointestinal hormone receptors in the gastrointestinal tract [14]. In this study, the contraction of the intestinal muscle was blocked using several receptor blockers, and the effects

Table 1: Effect of different fractions of LSQ on intestinal muscle tension

| Fraction | Concentration (mg/mL) | Before administration | After administration | Mean tension change |
|----------|------------------------|-----------------------|----------------------|-------------------|
|          |                        | Maximum | Amplitude | Maximum | Amplitude |          |
| Control  | 0                      | 1.62±0.13 | 0.22±0.09 | 1.64±0.15 | 0.23±0.09 | 0.04±0.03 |
| EE       | 5                      | 1.88±0.40 | 0.47±0.19 | 7.67±1.54 | 4.57±1.49 | 2.97±0.92 |
| PE       | 5                      | 3.03±0.28 | 0.55±0.22 | 3.43±0.66 | 1.23±0.64 | 0.26±0.16 |
| DI       | 5                      | 2.76±0.22 | 0.59±0.21 | 3.62±1.55 | 1.19±0.64 | 1.08±0.83 |
| EA       | 5                      | 0.29±0.26 | 0.83±0.36 | -0.86±0.4 | 0.07±0.33 | 0.79±0.35 |
| n-B      | 5                      | 0.31±0.21 | 0.89±0.33 | 6.63±1.42 | 6.24±1.09 | 4.78±0.88 |

EE: ethanol extract group; PE: petroleum ether group; DI: dichloromethane group; EA: ethyl acetate group; n-B: n-butanol group. Data are expressed as mean ± SD (n = 10); *P < 0.05, **P < 0.01, compared with control group.

Table 2: Effect of LSQ on isolated intestinal muscle receptors

| Fraction | Dosage (mg/mL) | Before the receptor blocker | After the receptor blocker | Mean tension change |
|----------|----------------|----------------------------|---------------------------|--------------------|
|          |                | Maximum | Amplitude | Maximum | Amplitude |          |
| DP       | 0.067          | 2.89±0.40 | 0.85±0.33 | 2.07±0.31 | 0.16±0.05 | 0.12±0.11 | 0.05±0.04 |
| PM       | 0.04           | 0.26±0.24 | 0.70±0.19 | -0.43±0.12 | 0.15±0.07 | 1.42±0.79 | 1.71±0.59 | 0.89±0.48 |
| AS       | 0.064          | 2.14±0.15 | 0.57±0.17 | 1.57±0.17 | 0.11±0.02 | 3.87±1.69 | 2.21±1.41 | 1.04±0.96 |
| OH       | 0.048          | 0.07±0.21 | 0.73±0.42 | -0.86±0.36 | 0.10±0.06 | 1.09±0.82 | 1.81±0.75 | 1.20±0.62 |
| PL       | 0.033          | 2.96±0.42 | 0.75±0.56 | 2.16±0.32 | 0.14±0.07 | 3.10±0.52 | 0.92±0.47 | 0.50±0.25 |

DP: diphenhydramine group; PM: phentolamine mesylate group; AS: atropine sulfate group; OH: ondansetron hydrochloride group; PL: propranolol hydrochloride group. Data are expressed as mean ± SD (n = 10); *P < 0.05, **P < 0.01, comparison before and after addition of receptor blocker in each group.
of the extract of LSQ was monitored to verify which receptor LSQ acted on.

Histamine receptors are active substances widely distributed in the human body. Diphenhydramine has an anti-histamine H1 receptor; it has a strong inhibitory effect on the central nervous system, and acts as a H1 receptor blocker. The experimental results showed that Liushenqu could not reverse the inhibition caused by diphenhydramine, indicating that it does not act on histamine receptors. The parasympathetic nerves that innervate the gastrointestinal tract are mainly from the vagus nerve. The stimulatory effect of the vagus nerve on the gastrointestinal nerve is achieved through choline fibers. Some of the post-ganglionic nerve endings release Ach which causes excitatory effects through the M receptor. In addition, phentolamine mesylate is a short-acting, non-selective α-receptor (a1, a2) blocker, and ondansetron hydrochloride is a selective serotonin 3 (5-HT3). The antagonist propranolol hydrochloride, is a non-selective β-blocker that has antagonistic effects on both β1 and β2 receptors. The experimental results show that LSQ reversed the inhibition of these receptors, indicating that it acts on the four receptors.

Thus, LSQ acts on the cholinergic M receptor, the adrenergic α and β receptors, and the 5-HT3 receptor. That is to say, LSQ acts on the cholinergic M receptor, enhances the sympathetic nerve excitation, thereby inducing contraction of the gastrointestinal smooth muscle and increasing the secretion of the digestive gland. It also acts on the adrenal α-receptor and β2 receptor, stimulates the small intestine smooth muscle, enhances relaxation of the small intestine, and acts on the 5-HT3 receptor to mediate gastrointestinal contraction and hormone secretion. The total effect is reflected in an increase in the contraction amplitude of the intestinal muscles and an increase in intestinal peristalsis.

**CONCLUSION**

The results of this study show that LSQ significantly enhances smooth muscle contraction in the small intestine of guinea pig. Its active principle is concentrated in the n-butanol fraction, while the ethyl acetate fraction has components that inhibit gastrointestinal motility. The mechanism involved in its enhancement of digestion is related to increase in the contraction amplitude of small intestinal smooth muscle and the acceleration of intestinal peristalsis.

**DECLARATIONS**

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**Conflict of interest**

No conflict of interest is associated with this work.

**Contribution of authors**

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. All authors have read and approved the manuscript for publication.

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