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Antidiarrheal properties of *Ligusticum chuanxiong* Hort's ethanol extract in mice and its impact on the contraction of rabbits' isolated jejunal smooth muscles

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Abstract: *Ligusticum chuanxiong Hort* (CR) is the dried rhizome of *Ligusticum* belongs to the Umbelliferae family. The present study aimed to assess the antidiarrheal effects of ethanol extracts of CR (CR ext.). The mice were administered castor oil to induce diarrhea, and the antidiarrheal effects of CR ext (250, 500, and 1000 mg/kg) were assessed *in vivo*. The potential effect of CR ext (0.01–10 mg/mL) was examined on isolated rabbit jejunum smooth muscle *in vitro*. CR ext exhibited antidiarrheal effects at a dose ranging from 500 to 1000 mg/kg ($P<0.01$). CR ext (0.01–10 mg/mL) relaxed the smooth muscles in a dose-dependent manner and its median effective concentration (EC_{50}) was 0.55 mg/mL (0.46-0.67, n = 6) ($P<0.05$; $P<0.01$). It alleviated jejunal contraction induced by ACh/K^+ (60 mM) and EC_{50} values were 0.35 mg/mL (0.34–0.37) and 0.11 mg/mL (0.10-0.12), respectively. Similar to the effect of verapamil, CR ext shifted the concentration–response curve of $CaCl_2$ downward to the right. The CR ext exhibits a notable antidiarrheal effect and can inhibit intestinal contraction. This mechanism of action may be based on its ability to inhibit Ca^{2+} channels.

Keywords: Ethanol extract of *Ligusticum chuanxiong* Hort., antidiarrhea, gastrointestinal disorders.

INTRODUCTION

Diarrhea is characterized by increased intestinal movement frequency, abdominal pain, and alterations in stool consistency (Shang *et al*., 2018) and is a major contributor to diseases and deaths in developing countries. Additionally, it is an important clinical issue in developed countries. Synthetic drugs are commonly and effectively used to treat diarrhea; however, owing to their side effects (Li *et al*., 2020) (Wang *et al*., 2022), many scientists have shifted their focus toward using locally available herbal remedies to combat diarrhea. Chinese herbal plants have garnered much attention owing to their advantages such as easy accessibility, efficacy and cost-effectiveness (Yao *et al*., 2020). Moreover, many Chinese herbal plants help treat diarrhea effectively and have been extensively used by local communities and traditional folk medicine practitioners. The powder of Chinese herbal plants *Angelica sinensis* and *Paeonia lactiflora* is a classic Chinese formula that has been effectively used to treat diarrhea in clinical settings. Six herbs in *Angelica sinensis* and *Paeonia lactiflora* powder were screened and the most active compounds were found in chuanxiong (He *et al*., 2021). Additionally, the therapeutic effect of *Angelica sinensis* and *Paeonia lactiflora* powder with or without Sini San was significant against irritable bowel syndrome (Meng *et al*., 2019). The acute toxicity tests of the essential oil extracted from the rhizomes of *Ligusticum chuanxiong* have shown its non-toxic and non-hazardous effects on animals within the range of clinical use (Zhang *et al*., 2012). Thus, the World Health Organization actively promotes and supports the investigation of traditional approaches to treat and prevent diarrhea.

Ligusticum chuanxiong Hort. (CR) is a dried rhizome of *Ligusticum* belongs to Umbelliferae. The extract can promote blood circulation, dispel wind and relieve pain. Moreover, it is one of the most widely used clinical medicinal herbs (Yuan *et al*., 2020). Recently, several studies have shown that CR exerts many useful pharmacological effects, including anti-cerebral ischemia (Yu *et al*., 2021), anti-myocardial ischemia, and anti-myocarditis effects (Chen *et al*., 2018). The major chemical constituents of CR are phthalides (Chen *et al*., 2018), which exert inhibitory effects on oxytocin-induced uterine and gastrointestinal smooth muscle contraction (Almeida *et al*., 2011; Liu *et al*., 2022 and Ni *et al*., 2021) and inhibit smooth muscle cell proliferation (Qi *et al*., 2010). Ferulic acid is one of the most important medicinal components in the rhizome of *Ligusticum chuanxiong* (Guan *et al*., 2017), which possesses antioxidant properties and is useful in preventing thrombosis and coronary heart disease (Li *et al*., 2016 and Tsai *et al*., 2017). Additionally, ferulic acid plays a role in gastrointestinal metabolism (Ge *et al*., 2018). Moreover, *Acanthopanax* root extract improves diarrhea symptoms by increasing intestinal motility under normal conditions (Miyauchi-Wakuda *et al*., 2020). Generally, antidiarrheal

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medications are effective in decreasing the secretion of fluids and/or slowing down gastrointestinal muscle movement, thus reducing the symptoms (Tadesse *et al*. 2017). Therefore, based on the above findings from previous studies, we hypothesized that CR extract might exert antidiarrheal effects.

MATERIALS AND METHODS

Medications and chemical compounds

For the experimental work, various research-grade chemicals were used. These included sodium bicarbonate, potassium chloride, magnesium sulfate, glucose, sodium dihydrogen phosphate, sodium chloride, and calcium chloride, all of which were sourced from Chengdu Cologne Chemicals Co. Ltd. (Chengdu, China). Acetylcholine chloride was obtained from Chengdu Huaxia Chemical Testing Co. Ltd. (Chengdu, China), while verapamil was sourced from Med Chem express Co., Ltd. (USA). Neostigmine bromide was available at ApexBio Technology (Shanghai, China). Distilled water was utilized to prepare standard solutions, dilutions and physiological salt solutions, such as Tyrode's solutions.

Equipment

The experimental equipment consisted of various instruments and machines from different manufacturers. These included the BL-420 Biological function experiment system, FT-100 Biological tension sensor, and HW-400E Constant temperature smooth muscle groove from Chengdu Techman Soft Co., Ltd. The HPLC system used was the Agilent-1220 high-performance liquid chromatograph from Agilent in the United States.

The Ultrasonic machine employed was the KQ-300DE from Dongguan Keqiao Ultrasonic Instrument Co., Ltd. in China. The electronic balance utilized was the Discovery PV215CD from OHAUS Co., Ltd. in Switzerland. The pure water manufacturing system was provided by Sichuan ulupure Technology Co., Ltd. The shredding machine was sourced from Tianjin, China. The rotary evaporator used was the RE-52AA from Yarong in Shanghai, China. The vacuum decompression drying oven was from Wuhan, China. Lastly, the constant temperature water bath pot was from Yuhua Instrument Co. Ltd. in Gongyi, China.

Fauna

The Animal Laboratory Center at North Sichuan Medical College in Sichuan, China provided adult male Kun Ming mice (weighing 18-22g) and locally bred rabbits (weighing 2.0-2.5kg) for the study. The animals were kept in standard environmental conditions, with an average temperature of 24 ± 5°C, average humidity of 45 ±5% and a 24-hour light-dark cycle. They had free access to water, but were fasted for 24 hours before the experiments. The research on animals adhered to the guidelines of the Ethical Review Committee of SLAS (Sichuan Association for Laboratory Animal Sciences) and followed animal welfare requirements.

Traditional chinese herb extraction

The CR was obtained from a local pharmacy in Nanchong, Sichuan and verified by Yang Lan, a Pharmacognosy Professor from the School of Pharmacy at North Sichuan Medical College (CBY-2020-0005). The naturally dried CR was crushed into a fine powder using a crusher. 100g of the powdered product was weighed using an electronic analytical balance (Mettle AE 240) and soaked in 800ml of 70% ethanol for 24 hours at room temperature. The extraction process was carried out using a round bottle flask in a water bath, with three rounds of reflux extraction for 30 minutes each at 80°C.

The filtrate was then combined and the solvent was recovered under 60°C using a rotary evaporator (RE-52AA, Yarong, Shanghai, China). The resulting liquid was concentrated to obtain an extract, which was dried using a vacuum decompression drying oven (ZK 6050B, Opson, Wuhan, China). The crude extract of CR had a percentage yield of 17.1%.

Phytochemical analysis

Formulation of the standardized solution

An accurate amount of eleutheroside B and ferulaic acid was weighed precisely using analytical grade methanol as a solvent. These were used to create a reference solution containing 0.244mg/mL of eleutheroside B and 0.100 mg/mL of ferulaic acid. The solution was then filtered through a 0.22mm nylon microporous membrane and stored at 4°C until needed.

Formulation of the sample solution

To prepare the sample solution, a precise amount of *Chuanxiong rhizoma* extract (3.03g/g DW) was weighed and mixed with 70% methanol (analytical grade) to achieve a concentration of 0.212g/mL. The solution was then filtered through a 0.22μm nylon micro porous membrane and subsequently used for HPLC analysis.

Chromatographic parameters

The Aglient-ZORBAX SB-C18 column (250 mm × 4.6 mm, 5μm) was used in this study. Mobile phase A consisted of 0.1% formic acid in purified water, while mobile phase B was chromatographic acetonitrile. The mobile phase was then filtered through a 0.45μm filter membrane. The compounds loaded onto the column were eluted using a gradient mobile phase of 0.1% formic acid and acetonitrile (see table 1). The detection wavelength was changed from 260 nm to 321 nm at 15 minutes, with a flow rate of 0.6mL/min. The column temperature was maintained at 28°C and a 5 L sample of the solution was directly injected.

In vivo

Acute toxicity screening

The lethal dose 50 (LD_{50}) for CR was investigated using an *in vivo* model of Kunming mice. The mice were randomized into six groups (n = 6). The test groups were orally administered with CR at doses of 16000, 8000, 4000, 2000, 1000 and 5000mg/kg body weight (equivalent to 4.19g crude drug/g) and were closely monitored for 2 weeks for any signs of mortality or toxicity. Throughout the study, all animals had unrestricted access to water and food (Li *et al*., 2019).

Effect of CR ext on diarrhea induced by castor oil in mice

The mice used in this experiment were randomized into five groups (n = 10). The experimental groups were orally administered with CR ext at doses of 1000, 500 and 250 mg/kg, whereas the positive control group was administered with 50mg/kg verapamil. The negative control group was given 20mL/kg normal saline. After 30 min, 0.4mL castor oil was orally administered to each mouse using a gavage needle.

Then, each mouse was put back in its own cage and an ink-absorbing piece of paper slightly larger than the cage was placed 3 cm below the cage. The initial time of semi-solid feces appearance and the total amount of solid, semi-solid, and liquid feces were recorded for the next 4 h. The severity of diarrhea was assessed using the evacuation index (EI) method (Tadesse *et al*., 2014). The severity of diarrhea, as measured by the EI, was calculated by multiplying the number of liquid feces by 3, the number of semi-solid feces by 2 and adding the number of solid feces.

In vitro

Tissue processing

We selected healthy rabbits as the experimental animals for this study. These rabbits were allowed to drink and eat as usual but were required to fast for 24h before the experiment. The rabbits were sacrificed through air embolism. After separating the jejunum (a part of the small intestine) and removing attached feces, it was immersed in a Tyrode solution at 4°C for later use. It was subsequently cut into 2 cm pieces using scissors and suspended vertically in a 20mL organ bath filled with 37 ±0.5°C Tyrode's solution. The bath was aerated with a mixture of 5% CO_2 and 95% O_2.

After preloading the tissue with 1g, the tissue was stabilized in Tyrode's solution for approximately 30 min. Intestinal smooth muscle activity was measured and recorded by a force transducer and the BL-420F System. The spontaneous contraction of the jejunal tissue in rabbits, under the specified experimental conditions, enabled the testing of the antispasmodic effect of the test material without the need for an agonist.

Effect of CR ext on the intrinsic movement of rabbit jejunum

Following a 30-min equilibration period in Tyrode's solution, we introduced various concentrations of CR ext (0.01-0mg·mL^{-1}) into the organ bath to evaluate the diverse mechanisms underlying its spasmolytic activity. We used spasmo genic agents, including K+ (60mM) and ACh (1μM). The inhibitory responses, dependent on the concentration of the test compound, were observed via cumulative addition. Before administration, the tension was recorded to be 100%. Changes in spontaneous contraction of jejunal smooth muscle were recorded after administration. The jejunum was subjected to treatment with verapamil (0.01, 0.03, 0.1, 0.3, 1, 3, 10μM) and the data were recorded.

Effect of CR ext on $CaCl_2$-induced cumulative contractions

To remove calcium ions from the jejunal fragments, the tissue was incubated in a Ca^{2+}-free high K^+ (60mM) solution containing ethylenediaminetetraacetic acid (0.1 mM) for 30 min. Following that, tissue fragments were placed in a Ca^{2+}-free high-K^+ (60mM) solution. Dose-effect curves were created for $CaCl_2$ after 15 min (3×10^{-5}- $\times 10^{-2}$ M) with and without verapamil (0.3, 1 μM) or CR ext (0.3, 1 g·L^{-1}). Verapamil was used as a positive control drug. Without CR ext and verapamil, 3×10^{-2} M $CaCl_2$-induced contraction was recorded as 100%.

STATISTICAL ANALYSIS

All data were presented as the mean ± standard error and processed through SPSS software followed by Dunnett's test of a one-way analysis of variance. A p-value ≤ 0.05 was considered significant statistically.

RESULTS

The identified chemical composition in CR ext by HPLC analysis

Fig. 1 displays the liquid chromatography results demonstrating the notable separation achieved between eleutheroside B and ferulaic acid under optimal conditions. The figures presented include the blank solution (fig. 1A), the reference solution (fig. 1B) and the sample solution (fig. 1C). Particularly, both the reference solution (fig. 1B) and the sample solutions (fig. 1C) exhibited chromatographic peaks at the same time.

Animal model studies

Acute toxicity screening

During the LD_{50} test, no instances of mortality or toxicity were observed within the designated observation period after orally administering CR ext at the specified dose level. Similarly, no changes in mouse death or behavior were recorded during the single maximum dose test period. These results reveal that the LD_{50} for CR ext was considered to be more than 16,000 mg/kg.

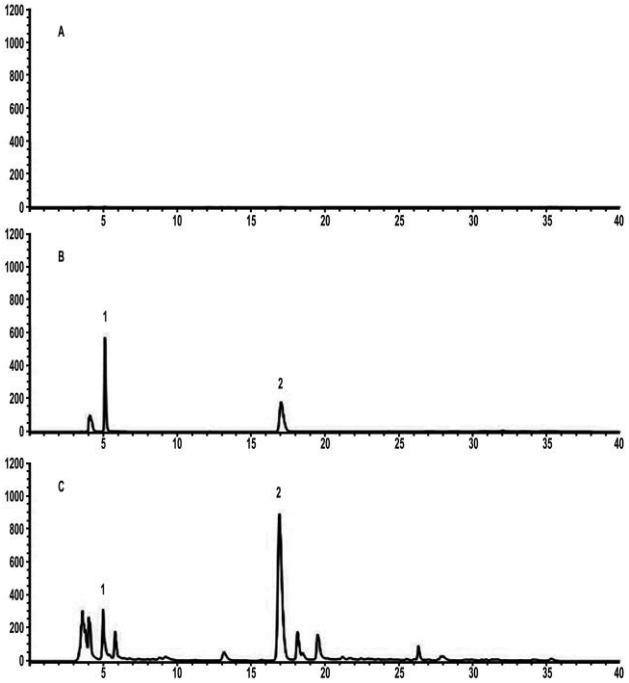

Fig. 1: HPLC chromatograms of blank (A), reference substances (B) and the CR ext (C) (1 eleutheroside B, 2 ferulaic acid)

Effect on diarrhea induced by castor oil

Negative control mice given saline developed acute diarrhea symptoms after castor oil. However, CR ext administration (500mg/kg and 1000mg/kg) significantly suppressed diarrhea symptoms. This was evidenced by a significantly delayed onset time (OT) of semi-solid feces (60.4±4.20 and 65.5±3.96min) and a significant reduction in the diarrhea severity (EI of 8.2±0.91 and 6.2±0.57) compared to 20mL/kg normal saline group (40.14±2.90 min; 13.93±1.45) (p<0.001). Furthermore, verapamil administration also led to a significant effect on diarrhea symptoms, with an OT of 70.2±4.3 min and an EI of 6.8± 1.02 (p<0.001) (fig. 2).

In vitro

Effect of CR ext on the intrinsic movement of rabbit jejunum

CR ext (0.01-0mg·mL^{-1}) produces concentration-dependent relaxation of spontaneous contraction with an EC_{50} of 0.43mg·mL^{-1} (0.37-0.49 mg·mL^{-1}, n = 6, fig. 3A (a)), similar to verapamil (0.01-0 μM) with an EC_{50} value of 1.35μM (1.18-.54, n = 6, fig. 3B (b)). In addition, CR ext demonstrated inhibitory effects against acetylcholine (ACh, 10^{-5} M) and potassium (K^+, 60 mM)-induced contractions in a concentration-dependent manner. The EC50 values for CR ext against ACh and K^+ were 0.32 mg·mL^{-1} (0.27-0.35, n = 6) and 0.12 mg·mL^{-1} (0.10-0.14, n=6), respectively. Similarly, verapamil exhibited inhibition against ACh and K^+ at concentrations of 0.001-3μM and 0.041μM, respectively, with the corresponding EC50 values of 0.33 μM (0.30-0.35, n = 6) and 0.04 μM (0.03-0.05, 95% CI, n = 6, fig. 4).

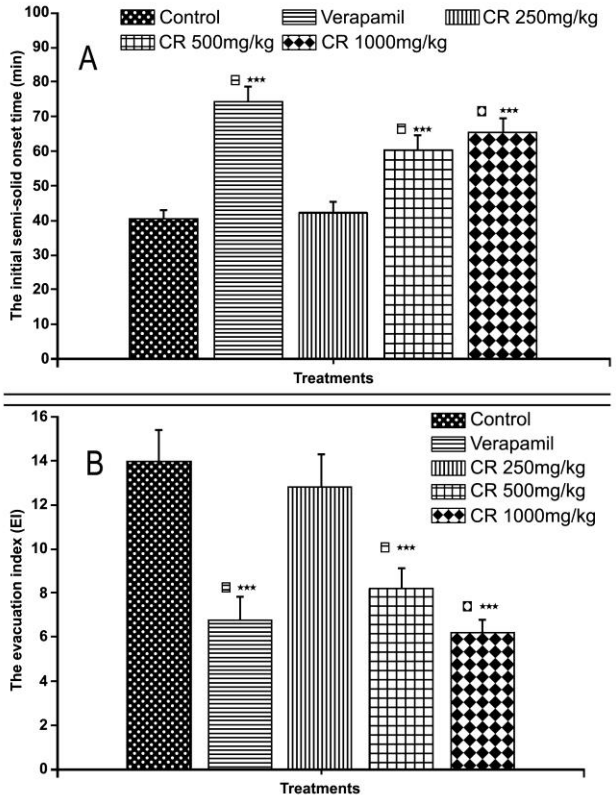

Fig. 2: Effect of CR ext on castor oil-induced diarrhea in mice. A. The initial semisolid onset time (min), B. The Evacuation Index (EI). **p<0.01, ***p<0.001, compared with the negative control group; Volumes presented as the mean ± SEM, n = 10.

Impact of CR ext on cumulative contraction induced by $CaCl_2$

The concentration-dependent effect of CR ext (0.3, 1 g·L^{-1}) on $CaCl_2$-induced cumulative contraction was observed through the reduction of maximum concentration-response curves. Further evaluation revealed that as the dose of CR ext increased, the $CaCl_2$ curve shifted downward and to the right, similar to the verapamil curve, a calcium channel antagonist. Both CR ext (0.3, 1 g·L^{-1}) and verapamil (0.3, 1μM) significantly reduced the maximum contraction induced by 3×10^{-2} M $CaCl_2$ in comparison to the control group, as shown in fig.5.

DISCUSSION

Through HPLC analysis, it is evident that eleutheroside B and ferulic acid exhibit excellent separation when subjected to optimal liquid chromatography conditions. Literature reviews indicate that ferulic acid exhibits antidiarrheal effects (Zhang *et al.,* 1991). Concurrently, studies have demonstrated that eleuthero root extract has a

Table 1: Liquid Chromatography Conditions. The detective wavelength was adjusted to 310 nm, replacing the previous setting of 260 nm at 20 minutes.

Duration(min)	Phase A composition (%)	Phase B composition (%)
0~10	80	20
10~30	80→40	20→60
30~35	40→80	60→20
35~40	80	20

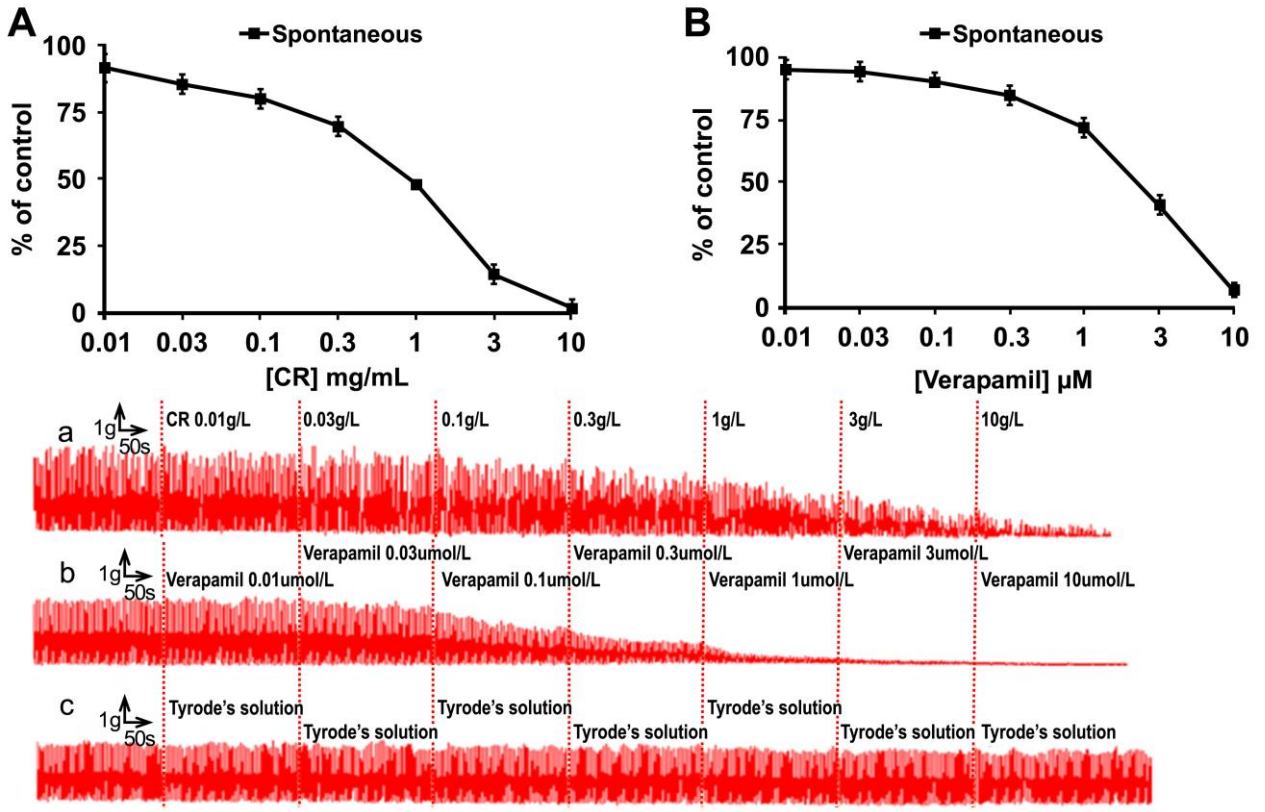

Fig. 3: Concentration-dependent inhibitory effect of (A(a)) crude extract of CR and (B(b)) verapamil, on spontaneously contraction of the isolated jejunum smooth muscle. Tracing showing (c) spontaneous contraction of the isolated jejunum smooth muscle. Results are expressed as mean ± SEM, n = 6.

dual effect on the ileal function of mice, causing muscarinic (M)-receptor contraction and direct relaxation. The relaxation effect can be employed to alleviate diarrhea symptoms, consequently increasing parasympathetic nerves (Miyauchi-Wakuda *et al.*, 2020). Therefore, eleutheroside B and ferulic acid were initially selected as markers. However, it was noted in subsequent phases of this experiment that their antidiarrheal effect was not prominent, and as a result, further discussion on this matter was omitted.

In the acute oral toxicity study, graded doses of CR ext were orally administered and no signs of toxicity or mortality were observed within the observation period, even at the highest dosage of 16,000 mg·kg^{-1}. Presently, no standard guidelines or references are reported for LD_{50} values in Chuanxiong. The results showed that the LD_{50} of chuanxiong volatile oil soft gel capsule core gavage in mice was 1594.92mg/kg and its 95% confidence limit was 1443.80-1765.51mg/kg. The LD_{50} of chuanxiong volatile oil soft gel gavage in rats was 2115.24mg/kg and its 95% confidence limit was 1849.86-2438.34mg/kg (Zhang *et al.*, 2015). The findings of relevant studies showed no distinct acute toxicity in cranial pain chuanxiong capsules. The LD_{50} was not detected and the maximum daily dose of mice was 16.0 g·kg^{-}1·d^{-}1, which is equivalent to 256 times the daily dose of adults (Liu *et al.* 2013). It is generally believed that a substance that does not cause any side effects at a concentration of 5 g·kg^{-1} is relatively safe (Li *et al*., 2019). The results indicated that CR ext exhibits a relatively good safety range in these preclinical experiments. This showed the safety of the CR ext and establishes a basis for further pharmacological investigations.

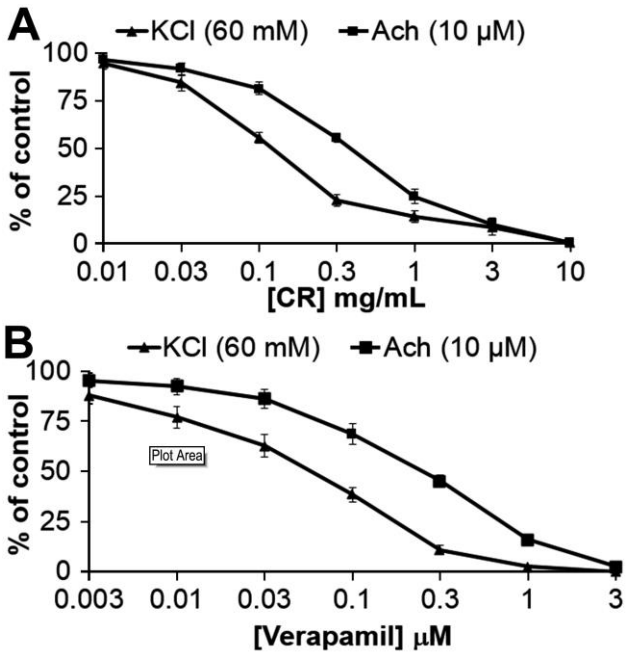

Fig. 4: Concentration-dependent inhibitory effect of (A) crude extract of CR and (B) verapamil on high K^+ (60 mM) and ACh (10^{-5} M) induced contraction of the isolated jejunum smooth muscle. Results are expressed as mean ± SEM, n = 6.

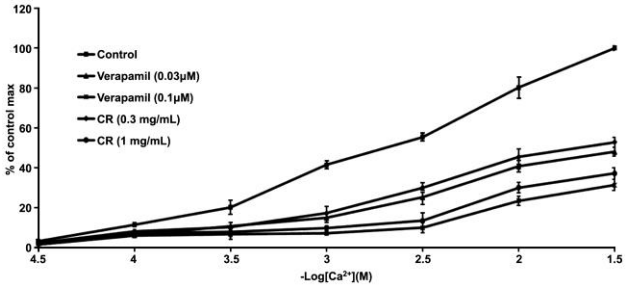

Fig. 5: Concentration-response curves of $CaCl_2$ on isolated rabbit jejunum smooth muscle in the absence (■) and in the presence of CR (◆ 0.3mg/mL; • 1mg/mL) and verapamil (▲ 0.03µM; × 0.1µM). Results are mean ± SEM, n = 6.

We established an *in vivo* diarrhea model in mice using castor oil to induce accelerated intestinal motility. Castor oil used in this study was obtained from the seeds of the Ricinus communis plant belonging to the *Euphorbiaceae* family (Xu *et al*., 2021). Yoshida *et al*. (2020) and Li *et al*. (2019) reported that ricinoleic acid, a hydrolytic metabolite of castor oil, changes ion transport and water movement in the intestines. Furthermore, it can cause the release of several mediators, such as tachykinin, cAMP, platelet activation factor, nitric oxide and prostaglandin, by stimulating the intestinal mucosa and causing inflammatory effects (Chen *et al*., 2022). Ching *et al*. (2013) reported that the evaluation of a potential antidiarrheal agent primarily lies in its ability to inhibit and decrease fecal output. The study revealed that CR ext significantly inhibited diarrhea by increasing the time to the onset of semi-solid feces and reducing the fecal EI.

Inflammation causes abdominal pain and diarrhea. Astragaloside IV (ASI) is a single compound extracted from the traditional Chinese herb Ligusticum chuanxiong with anti-inflammatory properties. *In vitro* and *in* vivo studies have shown that ASI effectively improved experimental ulcerative colitis (UC) by inhibiting inflammatory molecules and suppressing NF-κB signaling (Wu *et al*., 2019). Therefore, we can reasonably assume that CR ext may improve the symptoms of diarrhea by inhibiting inflammatory factors and related pathways. However, in this study, we aimed to inhibit gastrointestinal motility to improve diarrhea; therefore, inflammatory factors and related pathways were not thoroughly studied, which can be verified and analyzed in future experiments.

Antidiarrheal effects can be achieved by decreasing gastrointestinal motility (Fang *et al*., 2022). The onset time for semi-solid stool after administering the CR ext at doses of 500 and 1000 mg/kg was 60.4±4.20 and 65.5± 3.96 min, respectively. On the other hand, the onset time for semi-solid stool was 70.2±4.3min ($p<0.001$) in the verapamil group and 40.14±2.90min in the negative control group. These findings suggest that the CR ext exerts antidiarrheal effects. Because the antidiarrheal effect of CR ext (1000mg/kg) is closer to that of verapamil, we can hypothesize that the antidiarrheal effect of CR ext is concentration-dependent. This observed antidiarrheal effect of the CR ext can be attributed to its ability to inhibit intestinal contraction in a concentration-dependent manner, as revealed in the *in vitro* study.

ACh is a major neurotransmitter that regulates intestinal peristalsis (Russell *et al*., 2019) (Hayakawa *et al*., 2017) (Takahashi *et al*., 2021), resulting in smooth muscle contraction by agitating the M receptor. In a study, researchers demonstrated that the CR ext can diastole the ACh-induced tightening of isolated smooth muscle in the intestine, suggesting cholinergic neurotransmitter release and M receptor action in this process (Ghayur *et al*., 2005). High K^+ at a concentration of 60 mM can induce the depolarization of voltage-gated Ca^{2+} channels, whereas high K^+ at a concentration of 430 mM can lead to smooth muscle contraction by opening voltage-gated L-type calcium channels (Tanahashi *et al*., 2020) (Soder *et al*., 2011) (Türck *et al*., 2010). Therefore, it allows extracellular Ca^{2+} to flow into the cells and induces contractile effects (Aleem *et al*., 2018) (Li *et al*., 2019). In addition, calcium is vital for regulating the cellular process involved in smooth muscle contraction. Smooth muscle contraction relies on both extracellular and intracellular calcium levels (PLOS *et al*., 2019). Spontaneous smooth muscle contraction is primarily

controlled by rhythmic depolarization and repolarization cycles. Depolarization is triggered by calcium release from the internal stores and rapid Ca^{2+} influx into the cytoplasm via voltage-gated calcium channels. Contractile responses and normal muscle tone maintenance highly depend on these processes. On the other hand, relaxation occurs when cytosolic calcium levels decrease (Li *et al*., 2019). Together with the abovementioned two points, we explored the relationship between calcium flow and gastrointestinal movement. We used a KCl solution (60 mM) to induce intestinal contraction and verapamil, a type of L-type calcium channel blocker, as the positive control group (Awe *et al*., 2011) and observed that the CR ext can inhibit KCl (60mM)-induced rabbit jejunum contraction. The inhibition was enhanced with an increase in the concentration of the CR ext; this demonstrates that the CR ext inhibits smooth muscle contraction in the jejunum and that this inhibition may be related to the inhibition of L-type calcium channels. Furthermore, as the dosage of the CR ext increased, a noticeable shift was observed in the $CaCl_2$ effect curve toward the lower right. This indicates that the CR ext impedes the entry of extracellular calcium, similar to the curve observed for verapamil.

CONCLUSION

The CR ext exhibits a notable antidiarrheal effect in mice with castor oil-induced diarrhea. Moreover, it can relax the spontaneous contraction of the isolated rabbit jejunum smooth muscle as well as the contractions induced by ACh (10^{-5} M) and K^+ (60 mM). This mechanism of action may be attributed to its ability to inhibit Ca^{2+} channels. Our study findings provide a solid pharmacological foundation for using the CR ext in gastrointestinal disorders.

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