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Antidiarrhoeal and intestinal modulatory activities of Wei-Chang-An-Wan extract

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ABSTRACT

Aim of the study: Wei-Chang-An-Wan (WCAW), a traditional pharmaceutical preparation, has been used for treating various gastrointestinal (GI) diseases for several decades, but it is still poorly understood how it works on those disorders. This study was to investigate the effects of WCAW extract on GI tract.
Materials and methods: The activities of the methanol extract (ME) of WCAW on castor oil-induced diarrhoea, gastrointestinal transit (GIT) in mice, and contractions of isolated rabbit jejunum were investigated. We further assessed the safety of ME in vivo. Additionally, a HPLC fingerprint of ME was appraised to ensure its chemical consistency.
Results: Ten peaks were identified in the HPLC fingerprint of ME. At the doses of 400 and 800 mg/kg, ME significantly protected mice against castor oil-induced diarrhoea as well as the number of faeces and wet faeces. Interestingly, administration of ME significantly accelerated GIT in normal mice and reduced stimulated GIT induced by neostigmine. ME also dose-dependently attenuated spontaneous contractions of the isolated rabbit jejunum, and those induced by acetylcholine (Ach) and neostigmine. Moreover, oral administration of ME up to 5 g/kg did not produce any toxic effects. Taken together, ME is able to inhibit diarrhoea, increase normal GIT, and decrease GIT induced by neostigmine, which indicate that ME might play a bidirectional role in GI tract.
Conclusions: Our study provides a scientific basis for the clinical use of WCAW.

1. Introduction

Diseases of the gastrointestinal (GI) tract are highly common and varied; they include the irritable bowel syndrome and functional dyspepsia, as well as other symptoms (e.g. abdominal pain, nausea, vomiting, diarrhoea, constipation, etc.), which have a substantial effect on quality of life and health-care costs. Given the complexity of the symptoms, most experienced clinicians expect to use a holistic remedy for these diseases (Sanger, 2007), so it is important to open up new ways in which a broader range of symptoms can be attacked.

Among the traditional systems of medicine, traditional Chinese medicine (TCM) is a most extraordinary one with unique theory and practice. TCM is commonly prescribed as complex prescriptions to achieve sufficient treatment in complex conditions through possible mechanisms known as multiple components to multiple pathophysiological targets, yet proof of systematic scientific efficacy and safety is generally lacking when compared with synthesized chemical medicines (Peter, 2002).

Wei-Chang-An-Wan (WCAW), a standard Chinese herbal preparation, consists of ten Chinese medicinal herbs including *Aucklandia lappa* Decne. (family Compositae), *Aquilaria sinensis* (Lour.) Gilg (family Thymelaeaceae), *Citrus aurantium* L. (family Rutaceae), *Magnolia officinalis* Rehd. et Wils. (family Magnoliaceae), *Santalum album* L. (family Santalaceae), *Rheum officinale* Baill. (family Polygonaceae), *Croton tiglium* L. (family Euphorbiaceae), *Moschus* (family Cervidae), *Ligusticum chuanxiong* Hort. (family Umbelliferae), and *Ziziphus jujuba* Mill. (family Rhamnaceae). These herbs are milled into fine powder, mixed and made into water pills, which possess the properties of eliminating damp pathogen, regulating vital energy to alleviate pain, and removing food in the stomach and intestine due to indigestion (Committee of National Pharmacopoeia, 2005). This product has been marketed in China for more than 20 years for the management of GI diseases such as diarrhoea, enteritis, dysentery, irritable bowel syndrome, nausea, vomiting, indigestion, abdominal pain and distension (Ye et al., 2004; Ling et al., 2005). Despite the popular medicinal use of WCAW, no data are available with respect to its usefulness in GI

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disorders. Therefore, the aim of this study was to examine the effects of the methanol extract (ME) of WCAW on GI tract so as to assess some of the possible mechanisms involved in the clinical treatment.

2. Materials and methods

2.1. Plant materials and preparation of ME

WCAW (Lot No. 20070507) was prepared with the following ten herbs: 25% the dried root of *Aucklandia lappa* Decne., 10% the resinous wood of *Aquilaria sinensis* (Lour.) Gilg, 15% the immature fruit of *Citrus aurantium* L., 15% the dried bark of *Magnolia officinalis* Rehd. et Wils., 10% the duramen of *Santalum album* L., 7% the dried rhizoma of *Rheum officinale* Baill., 5% the mature fruit of *Croton tiglium* L., 0.5% *Moschus*, 5% the dried rhizoma of *Ligusticum chuanxiong* Hort., and 7.5% the fruit of *Ziziphus jujuba* Mill in Tianjin Lerentang Pharmaceutical Factory (Committee of National Pharmacopoeia, 2005; Ling et al., 2005). These herbs were purchased from Medicinal Materials Company (Hebei Province) and identified by Professor Wen-Yuan Gao from School of Pharmaceutical Science and Technology, Tianjin University, China. All the voucher specimens (Voucher No. WCAW-060601–060610) were available in the herbarium of Research Center of Tianjin Zhongxin Pharmaceuticals. The quality of WCAW was controlled by HPLC analysis as described previously (Committee of National Pharmacopoeia, 2005; Ling et al., 2005).

Three hundred grams of WCAW was powdered and extracted with 3 l of methanol for 2 h in a reflux condenser. The filtrate was collected and the residue was re-extracted with 3 l of methanol. The process was repeated four times for complete extraction. Then the solvent was removed under reduced pressure in a rotary evaporator (Buchi B-480), with a yield of 21.7% (w/w). Then the methanol extract (ME) was made up to a final concentration of 100 mg/ml in 0.5% carboxymethyl cellulose (CMC) suspension in distilled water prior to each experiment. The vehicle, 0.5% CMC suspension, in a volume equivalent to that of the ME solution (200 μl), did not produce changes in the test system.

2.2. Animals

Adult male and female ICR mice (18–22 g) and New Zealand White rabbits (2.0–2.5 kg) were obtained from Laboratory Animal Center of Health Science, Peking University, Beijing, China. All animals were housed at the Animal Breeding Laboratory of Tianjin Institute of Pharmaceutical Research and kept under standard environmental conditions. Animals had free access to water, but food was withdrawn 24 h before experiments. This animal study was approved by the Institutional Animal Care and Use Committee of China, and institutional guidelines for animal welfare and experimental conduct were followed.

2.3. Drugs and reagents

Acetylcholine (Ach) and isosorbide dinitrate (ISDN) were purchased from Sigma (St. Louis, MO, USA) and neostigmine methylsulfate was obtained from Xinyi Co., Ltd. (Shanghai, China). Loperamide hydrochloride was supplied by Xian-Janssen Pharmaceutical Co., Ltd. (Beijing, China). Mosapride citrate was provided by Lunan Pharmaceutical Co., Ltd. (Shandong, China). Other chemicals were of the highest grade available.

2.4. HPLC fingerprint of ME

ME was made up to a concentration of 5 mg/ml in methanol and filtered through a 0.45 μm membrane before being used for the HPLC analysis. Waters-1525 Alliance HPLC instrument (Waters Corporation, USA) equipped with an on-line degasser, and a 2998 photodiode array detector were used. A HiQ sil C18 column (4.6 mm × 250 mm, 5 μm, No. 00W00354 KYA TECH Corporation, Japan) was used. The mobile phase consisted of (A) CH_3CN and (B) (CH_3COOH:H_2O = 0.5:100) using a linear gradient of 20–35% of (A) in 20 min, 35–85% of (A) in 10 min, 85–90% of (A) in 30 min. The injection volume was 20 μl and flow rate was 1 ml/min with UV absorbance detection at 280 nm. The column temperature was set at 30 °C. All assigned peaks were identified with standard samples compared with UV spectral data. Fig. 1 shows the HPLC fingerprint of ME.

2.5. Castor oil-induced diarrhoea

Fifty animals were randomly divided into five groups. Vehicle control group received 0.5% CMC suspension (10 ml/kg, p.o.), positive control group received loperamide (10 mg/kg, p.o.), ME-treated groups were administered orally at the doses of 200, 400,

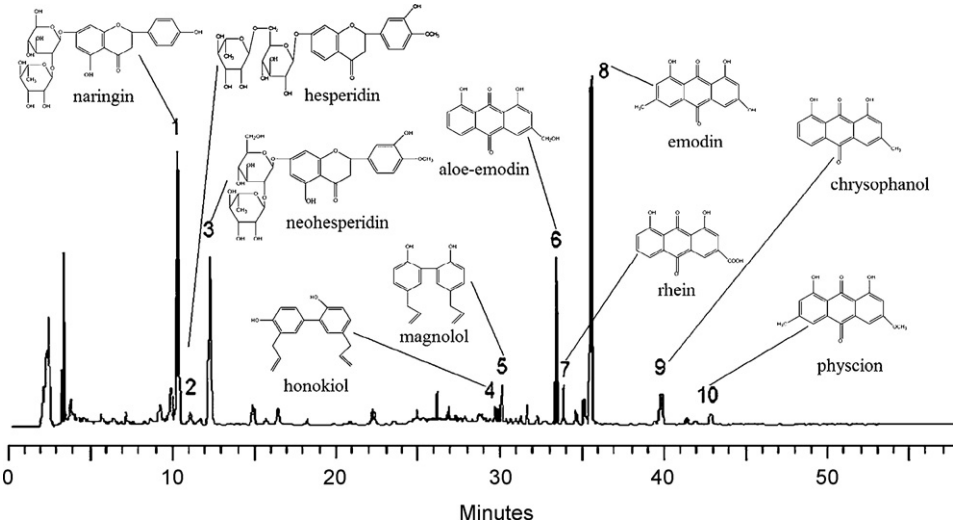

Fig. 1. HPLC fingerprint of ME at absorbance 280 nm. Ten peaks corresponding to naringin, neohesperidin, hesperidin, honokiol, magnolol, aloe-emodin, rhein, emodin, chrysophanol, physcion were identified. The mobile phase consisted of (A) CH_3CN and (B) (CH_3COOH:H_2O = 0.5:100) using a linear gradient of 20–35% of (A) in 20 min, 35–85% of (A) in 10 min, 85–90% of (A) in 30 min. The injection volume was 20 μL and flow rate was 1 ml/min with UV absorbance detection at 280 nm. The column temperature was set at 30 °C.