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A comparative study on the tissue distributions of rhubarb anthraquinones in normal and CCl_4-injured rats orally administered rhubarb extract

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ABSTRACT

Aim of the study: The present study comparatively investigated the tissue distributions of rhubarb anthraquinone derivatives (AQs) to examine whether they undergo different uptakes in normal or CCl_4-induced liver-damaged rats, to explore possible reasons for the different toxicities of AQs in pathological model rats and normal rats at the tissue distribution level.
Materials and methods: The total rhubarb extract (14.49 g kg^{-1} of body weight per day based on the quantity of crude material) was administrated orally to normal and model rats for 12 weeks. The concentrations of free AQs in tissues were quantitated by liquid chromatography–tandem mass spectrometry (LC–MS). After drug withdrawal for 4 weeks, tissue distributions were again determined.
Results: The five free AQs—aloe-emodin, rhein, emodin, chrysophanol and physcion—were detected in the liver, kidney and spleen, while only rhein, aloe-emodin and emodin reached the quantitative limit. The tissue distributions of rhein ($p < 0.001$), aloe-emodin ($p < 0.001$) and emodin ($p < 0.05$) in normal rats were higher than those in model rats with rhein > aloe-emodin > emodin in kidney and spleen tissues and aloe-emodin > rhein > emodin in liver tissues. Free AQs were not detected in the tissues after drug withdrawal for 4 weeks.
Conclusions: These results suggest that the tissue toxicity of AQs in normal animals is higher than that in pathological model animals with little accumulative toxicity of rhubarb. The results are concordant with the traditional Chinese theory of *You Gu Wu Yun* recorded first in *Su Wen*, a classical Chinese medical treatise.

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1. Introduction

Rhubarb (*Radix et rhizoma rhei*, Dahuang in Chinese) has been used in traditional Chinese medicine (TCM) for more than 2000 years and was first documented in "The Shen Nong Ben Cao Jing", the earliest book on materia medica in the world. Rhubarb was named general with beneficial effects on constipation (Jong et al., 2010), hepatitis (Cui et al., 2010), cholecystitis (Jiao and Liu, 1982), diabetic nephropathy (DN) (Gu et al., 2003) and chronic renal failure (CRF) (Xiao et al., 2002). Rhubarb contains five free anthraquinone derivatives (AQs), which account for the main medicinal properties of rhubarb and have similar chemical structures, as shown in Fig. 1. These AQs have been documented to have numerous therapeutic benefits, including inducing purgation (Sun, 1992), inhibiting bacterial growth (Wang et al., 2010), protecting the liver (Huang et al., 1997; Zhao et al., 2009) and treating CRF (Wang et al., 2009a) and jaundice (Ho, 1996). However, recent studies have reported that AQs are cytotoxic to the liver and kidney (Wojcikowski et al., 2006) and have mutagenic and carcinogenic effects in vitro. A NIH 2001 panel conducted a two-year experimental study on the toxic effects of emodin in animals (National Toxicology Program (NTP), 2001). They showed that emodin has no carcinogenic effects and leads to obvious pathological changes of the renal tubule. Recent risk assessments of rhubarb in animals also concluded that AQs cause injury to the liver and kidney (Wang et al., 2007a, 2009a, 2011; Xing et al., 2011). The discrepancies among these studies have raised doubts concerning the medicinal use of rhubarb. Therefore, the protective or destructive effects of rhubarb should be assessed scientifically; the results of such studies will be of great significance to clinical diagnosis and treatment.

We previously determined that rhubarb-induced injury to the liver or kidney is more pronounced in normal rats than in

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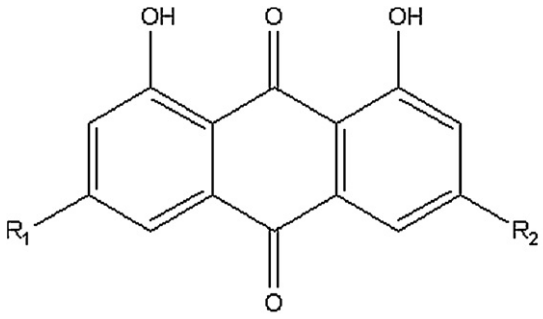

	R_1	R_2
Aloe-emodin	CH_2OH	H
Rhein	COOH	H
Emodin	CH_3	OH
Chrysophanol	CH_3	H
Physcion	CH_3	OCH_3

Fig. 1. Chemical structures of aloe-emodin, rhein, emodin, chrysophanol and physcion.

pathological rat models. This finding suggests that the medicinal effects of rhubarb are dependent on the pathological status (Wang et al., 2009a). In general, the protective or toxic effects of a drug on target organs are related to the concentration of the drug in the respective tissues. Therefore, the differing effects of rhubarb are possibly due to accumulation of the drug in the organs leading to different pathological conditions. To date, there have not been any studies of the tissue distribution of AQs in healthy rats compared to pathological rat models. In the present study, we determined the concentrations of free AQs in different tissues and evaluated their toxic effects on the liver or kidney in normal rats and in rats with CCl_4-induced liver damage. Possible explanations for the differences in free AQ tissue distribution are explored preliminarily.

2. Materials and methods

2.1. Materials and instruments

Rhubarb is the peeled and dried root of *Rheum palmatum* L., *Rheum tanguticum Maxim. ex* Balf. or *Rheum officinale* Baill. (Polygonaceae family) described in the Chinese Pharmacopoeia (PPRC, 2010). Rhubarb is also officially listed in the European and Japanese Pharmacopoeia (European Pharmacopoeia, 2001; Japanese Pharmacopoeia, 2006). *Rheum palmatum* was specifically examined in these studies because it is documented in all three pharmacopoeias mentioned above and is more commonly used than other species. The dried root and rhizoma of *Rheum palmatum* L. of the Polygonaceae family were collected in Lixian County, Gansu province of China, and were classified by Professor Xiaohe Xiao, a taxonomist at the PLA Institute of Traditional Chinese Material Medica.

Standard chemicals (aloe-emodin, emodin, rhein, chrysophanol, physcion and 1,8-dihydroxy anthraquinone) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol was HPLC grade (Fisher, USA). Formic acid was of analytical grade purity and was purchased from Beijing Chemical Regents Inc., PRC (Beijing, China). High-purity water was produced by the Milli-Q water purification system (Millipore, Bedford, MA, USA). Carbon tetrachloride (20060525, Beijing Beihua Fine Chemicals Co. Ltd.), and olive oil (F20080219, Sinopharm Chemical Reagent Beijing Co. Ltd.) were of analytical grade.UPLC analyses of free AQs in rhubarb total extract were performed using a Waters Acquity System equipped with a binary solvent delivery pump, an auto sampler and a photodiode array detector. The UPLC–ESI-MS/MS analyses for tissue distribution of AQs were performed using an Acquity UPLC–MS/MS System (Waters Corp., Milford, MA, USA) with a Waters tandem quadrupole mass spectrometer (Milford, MA, USA) equipped with an electrospray source. An Acquity BEH UPLC C_{18} column (100 mm × 2.1 mm, 1.7 μm) was used for the separation. The signal acquisition, peak integration and concentration determination were performed using ChemStation software (MassLynx 4.1) supplied by Waters Technologies. Other instruments used included a solid-phase extraction column with an OASIS MAX cartridge 3 cc (60 mg) (Waters, USA), a Waters extraction manifold system (Waters, USA), an ALl04 electronic balance, a low-speed centrifuge, and a manually adjustable pipette gun.

2.2. Animals

Male and female Sprague Dawley (SD) rats 6–8 weeks of age and weighing 180 ± 30 g were obtained from the Laboratory Animal Center of the Academy of Military Medical Sciences (License No. SYXK 2007-004). The animals were separated by gender and were given unlimited access to food and water in an environmentally controlled breeding room (temperature 22 ± 2 °C, humidity 60–80%). The breeding room was illuminated by artificial light with a 12-h light/12-h dark cycle every day; the room was disinfected regularly.

2.3. Preparation of extracts

A total of 100 kg of rhubarb was added to 600 L of 90% ethanol, heated and extracted 3 times for 1 h each. The residual rhubarb after extraction was added to 1000 L of water and heated and extracted for 1 h (Wang et al., 2011). The filtrate was merged and spray-dried, and the final extract yield was 29.3%. The samples were stored at 4 °C for later experiments.

2.4. Animal experiments and sample collection

In this study, a CCl_4-induced liver injury model was used because it has been well researched and demonstrates good reproducibility and high reliability. The mechanism by which CCl_4 induces liver injury involves the formation of free radicals and the subsequent peroxidation chain reaction, which leads to a significant and chronic injury of hepatic cells (Jian et al., 2008).

The experiment encompassed a total of 16 weeks. First, 54 rats were randomized into three groups of 18 animals each (9 males and 9 females). One group (the model group) was injected intraperitoneally with CCl_4 oil (containing 1 portion CCl_4 and 9 portions olive oil, 5 mL/kg) two times per week for a total of 12 weeks to induce chronic liver injury. The others received physiologic levels of saline. The medication group was administered the RE intragastrically once every day from the 4th week after modeling to the end of the 16th week. The control and model groups were administered physiologic levels of saline intragastrically. The dosage of total RE was 14.69 g herbs kg^{-1} of body weight per day and is equivalent to 4.9 times (converted with body surface area) (Wang et al., 2011) the upper dosage limit for humans as described in the Chinese Pharmacopoeia (0.5 g kg^{-1}) (PPRC, 2010). Food and water were available to the animals ad libitum.

All experiments using rodents were performed in accordance with the applicable guidelines and regulations. All rats received humane care in compliance with the institutional animal care

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