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The dosage-toxicity-efficacy relationship of kansui and licorice in malignant pleural effusion rats based on factor analysis

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ABSTRACT

Ethnopharmacological relevance: The root of *Euphorbia kansui* T.P. Wang (Euphorbiaceae), a well-known traditional Chinese medicine (TCM) with certain toxicity, is known as Gan sui (Chinese: 甘遂) or kansui. It has been used to treat edema, ascites, asthma, and etc. Licorice is the root of *Glycyrrhiza uralensis* Fisch. or *Glycyrrhiza inflate* Bat. or *Glycyrrhiza glabra* L., Leguminosae. It is a widely used herbal medicine native to southern Europe and parts of Asia as an herbal medicine and natural sweetener. Kansui cannot be co-used with licorice, which is recorded in "eighteen incompatible" medicaments in many monographs of TCM.

Aim of the study: The present study was conducted to investigate the dosage-toxicity-efficacy relationship of the co-use of kansui and licorice and to explore its regularity of the toxicity and efficacy change.

Materials and methods: Malignant pleural effusion rats were used and randomly divided into the normal control group, model group, positive control group (furosemide), kansui group, licorice group, and kansui-licorice groups with different ratios (kansui: licorice: 4:1, 2:1, 1:1, 0.5:1, 0.25:1, 0.1:1). Each group was adopted simultaneously to investigate the characteristic of toxicity and effect by measuring the pleural fluid and urine volumes, serum biochemical indexes, and serum TNF-α, IL-2 and IFN-γ levels. The factor analytic approach was used to analyze the dosage-toxicity-efficacy relationship between kansui and licorice.

Results: Two common factors were extracted from 8 indexes concerning toxicity and 5 indexes concerning efficacy. And the total factors related to toxicity (Ft) and efficacy (Fe) were calculated. The curved line of Ft indicated that the toxicity was increased along with the dose increase in licorice. The curved line of Fe indicated that the efficacy was decreased along with the dose increase in licorice. The intersection of these two lines was between the ratios of 2:1 and 1:1, and was deemed the flex point of the dosage-toxicity-efficacy.

Conclusions: Kansui demonstrated a certain efficacy in treating malignant pleural effusion, and the efficacy could be weakened by the co-use of licorice, even causing serious toxicity at the given ratio. The ratio between 2:1 and 1:1 (kansui: licorice) was deemed the flex point of the dosage-toxicity-efficacy of kansui and licorice. The results will be helpful for their better utilization and development.

1. Introduction

Traditional medicine–a system of ancient medical practices that differs in substance, methodology and philosophy from modern medicine–plays an important role in health maintenance for the peoples of Asia and is being increasingly employed in Western countries (Hu et al., 2005; Stephen and Richard, 2004; Xue and Roy, 2003). Formulae are prescription combinations of plant species/minerals used by practitioners of traditional Chinese medicine (TCM) to enhance the therapeutic efficacy and to reduce adverse effects based on clinical experience. TCM uses formulae that contain several herbs to act in unison and restore what TCM practitioners call the patient's 'balance' (Tian, 2011). As its main form, formula has been advocated for more than 2500 years and has undergone much development. With the advances in science and technology, and the increasing health awareness and life

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expectancy of humans, the safety problem of TCM is attracting more and more attention. Drug incompatibility is related to the safety of drug use in the clinic and embodies the essence of interaction between drugs and organisms. It is great scientific problem and focuses and attracted more attention. “Eighteen Incompatible Medicaments”, which represents TCM incompatibility, is experience summarizations based on medications in the clinic and embodies the basic properties of incompatibility theory in TCM (Duan et al., 2012).

Kansui is the root of *Euphorbia kansui* T.N. Liou ex T.P. Wang. As a well-known traditional Chinese medicine (TCM), it was recorded in Sheng Nungs Herbal as a toxic herb. It has been widely utilized for centuries in China as a remedy for edema, ascites, and asthma (Committee for the Pharmacopoeia of PR China, 2015). Licorice is the root of *Glycyrrhiza uralensis* Fisch. or *Glycyrrhiza glabra* L. (Leguminosae). It is a widely used herbal medicine native to southern Europe and parts of Asia as an herbal medicine and natural sweetener. Kansui cannot be co-used with licorice, which is recorded in “eighteen incompatible” medicaments in many monographs of TCM (Guo et al., 2014). The toxicity of kansui might be enhanced by licorice; and on the other side the effect of kansui might be decreased by licorice. Huang Wen-quan et al. (Huang et al., 2004) found that the combined application of licorice and kansui has a significant impact on cardiac and hepatic function, and slightly affected the histomorphology of the heart, liver and kidney in rats. Ding Ai-hua et al. (Ding et al., 2012) found that the contraction-promoting effect of kansui was markedly weakened when combined with licorice. The mechanisms of the incompatible co-use of kansui and licorice were also studied. Our previous study (Shen et al., 2013) found that licorice could clearly promote the dissolution of most diterpenes and triterpenes from kansui. Jing Xin-yue et al. (Jing et al., 2015) studied the combined effect of kansui and licorice on the metabolism of kansuinine A (KA) and kansuinine B (KB), two compounds with certain toxicities from kansui. The results showed that KA and KB might be the substrates of CYP2C19, and the co-use of kansui and licorice could inhibit the activity of CYP2C19, resulting in the slowing down and accumulation of KA and KB metabolism. It may be one of the mechanisms of kansui-licorice incompatibility. Although, many studies and clinical Chinese medical practitioners at all times have considered kansui and licorice as incompatibility, the tradition to utilize them for remedying human disease has been passed down from one generation to another (Tang et al., 2010). Wang Xi et al. (Wang et al., 2013) found that Gansui Banxia Tang had therapeutic effect on malignant ascites rats; it could decrease the ascite fluid volume and inhibit cytokines. Additionally, the mechanisms of Gansui Banxia Tang on reversing the imbalanced network of hepatocellular carcinoma was studied by Zhang Yan-qiong et al. (Zhang et al., 2014) by a systems-level investigation combined with network analysis and experimental validation. Liu Jia et al. (Liu et al., 2013) research the literature of clinical articles using formulas comprising the combination of kansui and licorice. The results indicated that kansui and licorice can be co-used at a suitable dosage and ratio. Thus, the co-use of kansui and licorice leads to toxicity or efficacy depending on some terms, such as the dosage and ratio. The relationship of dosage-toxicity-efficacy of kansui and licorice should be urgently studied clearly.

In this study, we investigate the dosage-toxicity-efficacy relationship of the co-use of kansui and licorice and explore its regularity of the toxicity and efficacy change.

2. Materials and methods

2.1. Reagents and equipment

The roots of *Euphorbia kansui* T.N. Liou ex T.P. Wang (kansui) were collected from Honghegu, Shanxi Province, China, and the dried roots and rhizomes of *Glycyrrhiza uralensis* Fisch. (licorice) were collected from Lingwu City, Ningxia Province, China. The two herbs were identified by Professor Chungen Wang (Department of Pharmacognosy, College of Pharmacy, Nanjing University of Chinese Medicine, Nanjing, China). In our previous study (Shen et al., 2013), the chemical analyses of kansui and licorice were performed by UHPLC-PDA-TQ-MS, and the major peaks of each herb found in the total ion chromatograms were identified and quantified. The voucher specimens (No. NJUTCM-20100520 for *Euphorbia kansui* and No. NJUTCM-20101215 for *Glycyrrhiza uralensis*) have been deposited in the Herbarium of Nanjing University of Chinese Medicine. The AU 680 automatic biochemistry analyzer and blood biochemistry [alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB), total protein (TP), urea nitrogen (BUN), creatinine (CREA), total bilirubin (TBIL), glucose (GLU), triglycerides (TG), cholesterol (CHOL), lactate dehydrogenase (LDH), α-hydroxybutyrate dehydrogenase (HBDH), and creatine kinase (CK)] reagents were purchased from Beckman Coulter, Inc., USA. TNF-α, IFN-γ and IL-2 enzyme-linked immunosorbent assay (ELISA) kits were purchased from eBiosciences, Inc., USA.

2.2. Preparation of drug solution

The dried kansui was ground into powder and then sieved through a 100 mesh stainless steel sieve. Licorice was accurately weighed into a round bottom flask, and refluxed for 2 h with 10 fold water each time for twice. The kansui powder was added to the licorice decoction to make the different ratios of kansui-licorice suspension before it was given to the rats.

2.3. Animals and treatment

SPF male Wistar rats were purchased from Vital River Laboratory Animal Technology Corp., Beijing, China (license number: SCXK (Beijing) 2012-0001). All of the rats were kept in Drug Safety Evaluation Center of Nanjing University of Chinese Medicine, Nanjing, China. All of the studies on animals were in accordance with the guidelines of the Animal Ethics Committee of Nanjing University of Chinese Medicine.

After a week of adaptive feeding, eighty-eight rats weighing 250～280 g were divided into eleven groups: control group, model group, positive control group (furosemide), kansui group, licorice group, and kansui-licorice groups at different ratios of (kansui: licorice: 4:1, 2:1, 1:1, 0.5:1, 0.25:1, 0.1:1). The models were developed by once intrapleural injection with Walker 256 cells (1×10^7 /mL, 0.3 mL per rat). After intrapleural injection, the rats of the different groups were given different drugs by gavage for 7 days. The control group and model group were given 0.9% saline, and the positive control group were given 4 mg/kg of furosemide. The kansui-licorice groups were given the corresponding kansui-licorice suspensions, and the ratios and doses are listed in Table 1.

2.4. Sample collection and preparation

Twenty-four hours urine samples from rats housed in metabolism cages were collected and measured on the 6th day after administration. At the end of the experiments, all of the rats were fasted for 12 h, and blood samples were collected from the abdominal aorta, allowed to clot on ice and subsequently subjected