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Acute and subchronic toxicities of the ethanol and hot-water extracts from Chinese sumac (*Rhus chinensis* Mill.) fruits by oral administration in rats

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ABSTRACT

In the present work, acute and subchronic toxicities of the ethanol and hot-water extracts from Rhus chinensis Mill. fruits were performed by oral administration in pathogen-free SD rats. Acute toxicity study was performed at a single dose of 5000 mg/kg for 14 consecutive days. And subchronic toxicity test was conducted by daily oral administration of those two extracts at doses of 312, 625, 1250 and 2500 mg/kg for 30 days. Acute toxicity study showed that the LD50 of the ethanol and hot-water extracts were over 5000 mg /kg. The results of subchronic toxicity showed that no significant adverse effect of those two extracts was observed at 312 and 625 mg/kg. However, the weight gains of rats were significantly inhibited at both 2500 mg/kg groups of the ethanol and hot-water extracts. Moreover, those two extracts exhibited toxicities to heart, liver, spleen and kidney in rats determined by hematological, serum biochemical and/or histological analyses when daily oral administration of 1250 mg/kg and 2500 mg/kg. No significant neurotoxicity was observed in all groups. The results concluded that the low concentration of those two extracts from R. chinensis Mill. fruits can be regarded as safe and used in daily life

1. Introduce

In folk medicine of China and other countries, herbs are extensively used as a main treatment strategy for curing diseases and maintaining health in ancient. Even nowadays, it is reported that approximately 80% of the world's population relies on the folk medicine to treat their diseases, especially in undeveloped countries and regions (Xiang et al., 2015). In recent decades, natural therapies with herbal medicines are getting popular both in developed and developing countries, which may be due to herbal medicines, with thousands of years' usage, are regarded safer than chemical drugs for human beings and/or due to the poor therapeutic effects of chemical drugs for some diseases (Ekor, 2014; Jordan et al., 2010; Traesel et al., 2016). However, most herbs used in folk medicine lack scientific data about their efficacy and safety (Xiang et al., 2015). Those herbs are often used to human beings before being subjected to toxicity testing, which hardly guarantee their safety (Traesel et al., 2016). The globally increasing consumption of herbs medicines results in more concerns about their safety arising. Therefore, substantial efforts have been made in order to test the toxicity or adverse effects of many herbs.

Rhus chinensis, widely distributed in China, Japan and Malaysia, is an individual species of the genus *Rhus* which belongs to the Family *Anacardiaceae* and often called Chinese sumac (Djakpo and Yao, 2010). This plant is a small deciduous tree or shrub with odd pinnate compound leaves that can grow to 2–10 m in height. It bloom in August or September with white flowers and bear fruits in October. The fruits of this plant are red globose and slightly flattened drupes containing one seed at maturity. *R. chinensis* is undemanding to soil conditions and commonly found in uncultivated land (Ren et al., 2008), and also well known as a host of the Chinese aphid, *Schlechtendalia chinensis* (Bell), to produce a famous traditional Chinese medicinal material-*galla chinesis* (Djakpo and Yao, 2010).

In traditional Chinese medicine (TCM), all parts of *R. chinensis* have been used for treating diseases; for example, the leaves are used for treating inflammations and diarrhea; the root is used for treating malaria and jaundice; and the fruits and seeds are often used for treating hepatitis and dysentery (Djakpo and Yao, 2010; Zhang et al., 2018a). Compared with other part of this plant, the fruits are not only used as herbal medicine but also applied as condiments and beverages (Zhang et al., 2018b). Moreover, the mature fruits are often eaten freshly in some parts of China (Zhang et al., 2018b). Therefore, it should pay more attention to the safety of *R. chinensis* fruits. Some studies have

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been performed to identify the phytochemicals in the fruits, mainly containing polyphenols which have been proved to be benefit for human health (Zhang et al., 2018a, 2018b; Giampieri et al., 2017; Tejada et al., 2017; Zhuang et al., 2017). However, to the best of our knowledge, there is no systematic study has been performed on the safety or the potential toxicity of *R. chinensis* fruits. In addition, water-boiling and alcohol-soaking methods are two widely used approaches of herbs consumption for human beings. Thus, the purpose of the present work was to compare the acute and subchronic toxicities of the hot-water (water-boiling method) and ethanol (alcohol-soaking method) extracts from Chinese sumac (*R. chinensis* Mill.) fruits by oral administration in rats.

2. Materials and methods

2.1. Plant material and extract preparation

Mature *R. chinensis* Mill. fruits (collected from Tengchong county, Baoshan City, Yunnan Province, China) were provided by Kunming Plant Classification Biotechnology Co, Ltd. (Kunming, Yunnan, China) in October 2016. The fruits were transported to the laboratory at the low temperature (4–8 °C) and then stored at the −20 °C for further use. Before extraction, the fruits were lyophilized and smashed into powder to pass through a sixty-mesh sieve. Hot-water extraction method was as follows: the fruits powder was boiled for one and half hours in a material-to-solution ratio of 1:20. After being cooled to room temperature, the slurry was centrifuged at 4000 g for 10 min. And then supernatant was collected and concentrated by a rotary evaporator (Hei-VAP, Heidolph, Germany). The condensed sample was finally lyophilized by a lyophilizer (Alpha 1–2 LD plus, Christ, Germany) for toxicity testing. The 80% ethanol extract was prepared according to the previous work (Zhang et al., 2018b). The extraction ratios of the ethanol and hot-water extracts were 10.2% and 13.6%, respectively.

2.2. Animals

In the present study, one hundred and five Sprague-Dawley (SD) rats (specific pathogen free, SPF) at 4–8 week old and 200 g ± 5% of weight from both sexes (60 female and 45 male) were used. The experimental animals were purchased from Liaoning Changsheng Biotechnology Co., Ltd. (certificate number: SCKK (Liao) 2015–0001). The basic animal feed was provided by Kunming Medical University (60% carbohydrate, 20% protein, 5% fat, 5% fiber). Rats were placed under standard conditions (23 °C ± 2 °C, 40%–75% humidity, and 12 h light dark cycle from 08:00 to 20:00). Male and female mice were housed separately in sterile polypropylene cages, and fed with basic feed and tap water. All rats must adapt to the new environment for 7 days before the study. All animal procedures were strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Ethical Committee for Animal Experimentation of Kunming University of Science and Technology. The schematic illustration of experimental design is presented in Fig. 1.

2.3. Acute toxicity

The acute toxicity test was conducted according to the Organization for Economic Co-operation and Development (OECD) Guidebook 425 edited by the Asia-Pacific Financial Organization. The “Up and Down procedure” was applied in the present study to determine the median lethal dose (LD_{50}) (Rispin et al., 2002; OECD, 2008b). The formal acute toxicity experiment was performed using a single oral concentration of 5000 mg/kg, because no obvious abnormity of rat was observed in the preliminary experiments with the 2000 mg/kg concentration (OECD 407, 2008a; OECD 425, 2008b). After adjusting to the environment, experimental animals were divided into three groups with 5 female rats in each group. The first group is the control group, and the second group is the ethanol extract group, and the third group is the hot-water extract group. The rats in experimental groups were administered with samples (dissolved in distilled water), and the control group was treated with distilled water. The gavage was performed with a volume of 5 ml/kg. All animals were fasted for 12 h before experimentation. Each rat was marked before the experiment and the initial body weight (fasting for 12 h) was recorded. Observations were made at 30 min, 1 h, 2 h, and 4 h after the sample administration. And then, feed with known weight was offered. Water and dietary intake were recorded daily during the 14 days experiment. Meanwhile, mortality (if any) and abnormity of rat was also recorded (Xiang et al., 2015). The pole tests (Xiang et al., 2015) and organ coefficients were also performed. At the end of 14 days, the LD_{50} was calculated using the Karn's method.

2.4. 30-Day subchronic toxicity

2.4.1. Treatments

The 30-day subchronic toxicity experiment was performed according to the OECD Guideline 407 programme (OECD, 2008a). Ninety rats were divided into 9 groups, 5 males and 5 females in each group. Male and female rats in each group were housed separately in different cages. There were four groups for each kind of extract (hot-water extract or ethanol extract). Grouping was as follows: Group 1 - control (G1), Group 2–312 mg/kg (G2), Group 3–625 mg/kg (G3), Group 4–1250 mg/kg (G4), Group 5–2500 mg/kg (G5), Group 6–312 mg/kg (G6), Group 7–625 mg/kg (G7), Group 8–1250 mg/kg (G8), Group 9–2500 mg/kg (G9), in which G2 -G5 were administrated with ethanol extract and G6-G9 were fed with hot-water extract. Subchronic toxicity test was conducted based on acute toxicity.

2.4.2. Body weight, dietary and water intake

The behaviors and conditions of all rats were checked before and after each gavage, no less than twice a day. The rat's fur, skin, mucous membrane, moving posture, central nervous system, and mortality (if any) were observed and recorded. The weight was recorded every seven days after the initial record. The food and water consumptions were record daily. At the end of the experiment, rats were anesthetized with chloral hydrate and then the liver, lung, heart, spleen, kidney, testis and ovary were collected and the ratio of these organs to body weight (organ coefficient) was calculated. When the rats were finally euthanized, the blood and tissues were properly handled as soon as possible in order to prevent self-concentration between tissues (Czéh et al., 2016).

2.4.3. Hematological analysis

A vacuum blood collection tube containing heparin lithium was used to collect 1 ml of rat venous blood. The hematological parameters included: leukocyte (WBC), lymphocyte (LYM), percentage of lymphocytes (LYM%), monocytes (MON), percentage of granulocytes (GRA%), red blood cell (RBC), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), red blood cell volume (HCT), platelet (PLT), platelet pressure (PCT), and mean platelet volume (MPV).

2.4.4. Serum biochemistry

A suitable amount of blood from each rat was taken in a negative pressure general blood collection tube. The solidified blood samples were centrifuged at 1800 g in 4 °C to obtain serum. The serum biochemistry parameters were analyzed by kits purchased from Nanjing Jiancheng Biological Co., Ltd., including total protein (TP), albumin (ALB), triglyceride (TG), total cholesterol (T-CHO), alanine aminotransferase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), sodium (Na), potassium (K), and chlorine (Cl).