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Evaluation of the sub-chronic toxicity of a standardized flavonoid extract of safflower in rats

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ABSTRACT

Carthamus tinctorius L., or safflower, is an annual herbaceous crop belonging to the family Asteraceae, which is cultivated throughout China and used as a traditional Chinese medicine. Our previous study revealed anti-Parkinson's disease effects of an isolated standardized safflower flavonoid extract (SAFE). The purpose of this study is to evaluate the potential sub-chronic toxicity of SAFE. Male and female Sprague Dawley rats received three doses of SAFE (100, 300, and 500 mg/kg) q.d. by gavage for four weeks. Body weights were measured during the experiment, and blood samples were collected once per week for hematological and serum biochemical parameters. Major organs were examined after execution and histopathological analyses were performed. Body weight gain in the administration groups showed no decline compared to the control group. However, there were changes in values of aspartate transaminase ($p < 0.05$), alanine transaminase ($p < 0.05$), and blood glucose ($p < 0.05$) between treatments. SAFE influenced parameters related to platelets in rats receiving SAFE for both sexes under different dosages ($p < 0.05$). No histopathological changes were observed. SAFE might have influence on conglomeration of platelets, transaminases, and blood glucose. SAFE caused no significant toxicity and further studies may be needed to ensure safety of SAFE.

1. Introduction

In the history of humankind, herbal medicinal products have been used to prevent and treat diseases since ancient times. China is one of the countries where products extracted from medicinal plants are most frequently utilized.

Carthamus tinctorius L., commonly known as safflower, is an annual herbaceous crop belonging to the family Asteraceae. *Carthamus* species are known to have been cultivated in China, India, and Iran almost from prehistoric times (Asgarpanah and Kazemivash, 2013), with the flower being used as a coloring and added to food as a flavoring agent. Safflower is a famous traditional Chinese medicine that promotes blood circulation by inhibiting platelet aggregation, so the safflower florets are utilized for the treatment of cardiovascular, cerebrovascular, and gynecological diseases. The water extract from safflower and its active components have been developed into injection agents (such as safflower yellow injection) to treat cardio-cerebrovascular ailments in China (Yue et al., 2016).

More than 200 compounds have been isolated from safflower including flavonoids, phenylethanoid glycosides, coumarins, fatty acids, steroids, and safflower polysaccharides (Fan et al., 2009). Flavonoids are commonly considered the main active components of the safflower having medicinal properties.

Carthamins yellow, which is isolated from safflower, can significantly decrease whole blood viscosity, plasma viscosity, and erythrocyte aggregation index (Li et al., 2009). Hydroxysafflor yellow A attenuates pulmonary fibrosis induced by bleomycin in mice, decreasing the lung consolidation area and collagen deposition in mice having pulmonary fibrosis (Jin et al., 2016). Safflor yellow B might act as a potential neuroprotective agent against the cerebral ischemia-induced injury in rat brains by reducing lipid peroxides, scavenging free radicals, and improving energy metabolism (Wang et al., 2007). The phenolic content and composition of safflower

Abbreviations: SAFE, standardized safflower flavonoid extract; AHYB, anhydrosafflor yellow B; 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease.

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seed extracts have antioxidant and anti-adipogenic activities (Yu et al., 2013). Besides, extracts of safflower also exhibit cardioprotective effects in myocardial ischemia, operating partially by reducing oxidative stress-induced damage and apoptosis (Han et al., 2009), and has been demonstrated to have potential as a novel therapeutic drug for heart failure (Jia et al., 2015).

In a previous study, our group isolated a standardized safflower flavonoid extract (SAFE) from safflower, which mainly contained flavonoids with two marker compounds, i.e., kaempferol 3-O-rutinoside and anhydrosafflor yellow B (AHYB). The SAFE exhibited neuroprotective effects, improving the behavioral performances in the 6-hydroxydopamine (6-OHDA)-induced rat model of Parkinson's disease (PD) and inhibiting the changes of extracellular space diffusion parameters induced by 6-OHDA (Ren et al., 2016). The neuroprotective effects of SAFE were further confirmed in studies on rotenone-induced Parkinson rats (Ablat et al., 2016).

Herbal toxicity possesses serious threats to human health and represents an important issue to be managed (Mirhoseini et al., 2012). It is necessary for health researchers to illustrate any herbal toxicity and patients to understand the potential toxicological effects of particular herbal products. The present study was designed to investigate the toxicological effects of SAFE, whose neuroprotective effects against PD have been demonstrated previously by our group (Ren et al., 2016; Ablat et al., 2016). The toxicological effects of the components of SAFE with marker compounds of kaempferol 3-O-rutinoside and AHYB were studied for the first time, and provided support for continued study of the components to develop a potential new clinical use for this extract.

2. Materials and methods

2.1. Preparation of SAFE

The safflower used in the present study was purchased from Sanhe Pharmacy Industry Ltd. (Beijing) and identified by Professor Min Ye, Peking University, Beijing, China. Based on the previous study of neuroprotective effects (Ren et al., 2016), the dried flower petals were twice extracted with 50% EtOH at a ratio of 1:8 (i.e., 1 kg plant material to 8 L 50% EtOH). The collective extract was concentrated in a rotary evaporator and chromatographed on an AB-8 macropore resin column using gradient elution with 15%, 30%, 50%, 70%, and 95% EtOH. To detect the main components, high performance liquid chromatography was employed and the main ingredients of AHYB and kaempferol 3-O-rutinoside were guaranteed in the collection from the 30% EtOH. The 30% EtOH fraction was collected, concentrated, and freeze-dried. Qualitative analysis of components in the extracts was conducted using liquid chromatography/mass spectrometry (LC/MS). The extract was dissolved in normal saline before administration to experimental animals.

2.2. Experimental animals

The Sprague Dawley rats used in this study, including male rats (6 weeks old; 160–170 g) and female rats (6 weeks old; 120–130 g) were obtained from Charles River Laboratories, Inc. (Beijing, China) with the confirmation number SCXK (Jing) 2012–0001.

The animals were maintained in adequately ventilated hygienic compartments, sustained under standard environmental conditions (23 ± 1 °C, 45% ± 5% humidity, and 12 h/12 h light/dark cycle), and fed standard rodent diet (Keaoxieli Company) and water ad libitum. Different cages were divided between the sexes, with cage beddings and water bottles cleaned daily. The animals were allowed five days of acclimatization before the commencement of experimental procedures.

The investigational procedures adopted in this study were in accordance with the requirements of the Experimentation Ethics Committee on Animal Use of the College of Medicine, Peking University, Beijing, China and the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals (National Research Council [US] Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011).

2.3. Sub-chronic oral toxicity study

Forty 6-week-old Sprague Dawley rats were randomly divided into 4 groups containing 10 rats each (5 males and 5 females). SAFE dissolved in normal saline was administered to groups of rats at the concentrations of 100, 300, and 500 mg/kg by gavage of 1 mL/100 g for four weeks. The control group received saline solvent only. The general physical condition of each animal was observed during the experimental period and individual body weight was recorded daily.

2.4. Blood sample collection

Blood samples were collected from the ophthalmic venous plexus at the beginning of each week (four times in total) and at the end of the experiment, after 16-h fasting of feed but drinking water allowed. The blood samples for complete blood counts were collected into EDTA-treated sample tubes. The blood samples for serum biochemicals were collected into Eppendorf tubes, allowed 2 h to clot, and centrifuged at 3500 rpm for 10 min to obtain the serum.

2.5. Hematological assays

Complete blood counts were determined by the Department of Laboratory Animal Science, Peking University Health Science Center, using a blood cell counter (MEK-6318K, Japan) and an automatic biochemical analyzer (AUTOLAB-18, Italy). Complete blood count included: white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), red blood cell specific volume (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell volume distribution width (RDW), platelet (PLT), platelet cubic measure distribution width (PCT), mean platelet volume (MPV), platelet distribution width (PDW), lymphocyte (LYM), intermediate cell (MID), and granulocyte (GRN).

2.6. Determination of serum biochemical parameters

Serum biochemistry assays were performed by the Laboratory Medicine Department, Peking University Third Hospital, using an automatic biochemistry analyzer (AU2700, Japan). Serum biochemistry assays included alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (Alp), total protein (Tp), albumin (Alb), albumin/globulin (A/G), total bilirubin (T-bil), urea nitrogen (UREA), creatinine (Cre), blood glucose (Glu), creatine kinase (CK), cholesterol (CHOL), triglyceride (TG), and electrolytes [sodium (Na), potassium (K), and chlorinum (Cl)].

2.7. Measurement of organ weights

Necropsies were performed on all animals at the termination of the study. Selected organs were identified, cleared of adherent tissues, and harvested. The organs were then rinsed with normal saline and blotted with filter paper. The organ weights of the heart, spleen, kidneys, liver, lung, brain, and testes were measured. The ratio of each organ to terminal body weight (relative organ weight) was calculated as [organ weight (g)/body weight of rat (g)] × 100.