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Acute and subchronic toxicity studies of seabuckthorn (*Hippophae rhamnoides* L.) oil in rodents

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ABSTRACT

Seabuckthorn (*Hippophae rhamnoides* L.) has been traditionally used as medicine and nutritional supplement for a long period of time. However, information on the systemic toxicity and safety evaluation of seabuckthorn and its extracts is still scarce. The purpose of this study was to evaluate the potential toxicity of seabuckthorn oil by an acute oral toxicity study in mice and a 90-day repeated oral toxicity study in rats. No mortality or signs of toxicity was observed in mice treated with 20 mL/kg body weight seabuckthorn oil in the acute toxicity study. In the subchronic toxicity study, 80 Sprague-Dawley rats (10 animals per sex per treatment group) were administrated with 10, 5, 2.5 and 0 (control) mL/kg body weight of seabuckthorn oil daily for 90 days by gavage. There were no signs of toxicity and treatment-related changes in rats treated with seabuckthorn oil on mortality, body and organ weights, food consumption, blood biochemistry and hematology, gross necropsy and histopathological examinations. Based on the finding of this study, the maximum tolerated dose of seabuckthorn oil was >20 mL/kg for mice for acute toxicity study, and the no-observed-adverse-effect level was 10 mL/kg body weight for both male and female rats for 90-day toxicity study.

1. Introduction

Seabuckthorn is a hardy bush belonging to the genus *Hippophae*, family *Elaegnaceae* (Yildiz et al., 2012). The family includes six species, of which *Hippophae rhamnoides* L. is the major one. The plant naturally grows at a height of 2000–3600 m in higher altitudes and can be resistant to extreme temperatures from −45 to +43 °C (Krejcarová et al., 2015). It is natively widespread throughout the Central Asia and North-Western Europe (Rousi, 1971). Nowadays, cultivated seabuckthorn can also be found in North America (Krejcarová et al., 2015).

Seabuckthorn has been used extensively in traditional Eastern medicine for treatment of different diseases for more than 1000 years. The medicinal value of seabuckthorn was firstly recorded in the 8th century in the Tibetan medicinal classic *rGyud Bzi* (The Four Books of Pharmacopoeia) to treat gastrointestinal diseases, cardiovascular diseases and burn injury (Li et al., 2007; Stobdan et al., 2013). All parts of the plant, including berries, leaves, root and branch are considered to be rich in bioactive compounds and have been traditionally used as medicine and nutritional supplement by inhabitants of Europe, Central and South-Eastern Asia (Krejcarová et al., 2015; Michel et al., 2012). Among all parts of the plant, berries have been in the center of attention due to high contents of biologically active compounds.

Berries of seabuckthorn are oval shaped and 6 mm–9 mm long, with dark yellow, orange or red color when ripe. The fruit consists of a single oval shaped dark brown seed surrounded by a soft and fleshy outer tissue (Michel et al., 2012). Chemical analysis showed that the main constitutes of berries include sugars, amino acids,

Abbreviations: NOAEL, no-observed-adverse-effect level; SD, Sprague-Dawley; SPF, specific pathogen free; EDTA, ethylene-diamine-tetra-acetic acid; MTD, maximum tolerated dose; HPLC, high performance liquid chromatography; HGB, hemoglobin concentration; RBC, red blood cell count; PLT, platelet count; WBC, white blood cell count; LYM, percent of lymphocytes; NEUT, percent of neutrophils; MONO, percent of monocytes; EO, percent of eosinophils; BASO, percent of basophils; AST, aspartate transaminase; ALT, alanine transaminase; BUN, blood urea nitrogen; CR, creatinine; TC, total cholesterol; TG, triglyceride; TP, total protein; ALB, albumin; GLU, glucose.

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fatty acids, phytosterols, flavonoids, carotenoids, vitamins and mineral elements (Guliyev et al., 2004; Stobdan et al., 2013). A number of bioactive compounds have been isolated from berries of seabuckthorn, including quercetin, isorhamnetin, hippophae cerebroside, oleanolic acid, ursolic acid, 19-α-hydroxyursolic acid, dulcioic acid, 5-hydroxymethyl-2-furancarbox-aldehyde, cirsiumaldehyde, octacosanoic acid, palmitic acid and 1-O-hexadecanolenin (Suryakumar and Gupta, 2011; Zheng et al., 2009). Modern pharmacological studies have revealed multiple pharmacological activities of seabuckthorn berries and fruit extracts, for instance juice and oil, among which the hypocholesterolemic, antioxidant, anti-inflammatory, antitumor and skin-protecting effects are the most commonly claimed (Guliyev et al., 2004; Stobdan et al., 2013; Suryakumar and Gupta, 2011; Wani et al., 2016). Due to their excellent medical functions and nourishing effects, berry products of seabuckthorn such as berry juice, oil, drink, etc. are among popular products in many countries including the United States, China, India, Canada, Finland, Germany, and some other European countries (Guliyev et al., 2004; Tulsawani, 2010).

Although this plant and its extracts have been used as medicine and nutritional supplement in traditional Eastern medicine for centuries, data on the systemic toxicity and safety evaluation of seabuckthorn is still insufficient. Efforts have been spent to explore the pharmacological activities while only a few studies have been performed on the safety evaluation of the plant extracts (Wani et al., 2016). Given the potential utility of developing an extract from seabuckthorn berries that would be a rich source of bioactive compounds, and noting the relative absence of safety evaluation on seabuckthorn berries or its extract *per se*, it was considered prudent to conduct a comprehensive toxicological assessment to demonstrate the safety of such a product for possible use in food. This study aimed to examine the acute and subchronic toxicity of seabuckthorn oil by an acute oral toxicity study in mice and a 90-day repeated oral toxicity study in rats, so as to provide useful information for assessment the safe use of seabuckthorn oil in food or as a dietary supplement.

2. Methods and materials

2.1. Plant material and oil extraction

Well-ripened seabuckthorn berries were collected from Altay Prefecture region in Xinjiang province, China and stored at −20 °C before used. The whole berries were vacuum-dried and ground to powder to pass 0.6 mm mesh. A supercritical carbon dioxide method was used to extract oil from the powder following method developed by Xu et al. (Xu et al., 2008). Briefly, 200 g of berry powder was placed into the extraction vessel of a supercritical fluid extraction system (HA220-50-06, Nantong Hua'an Co. Ltd, Jiangsu, China). Liquefied carbon dioxide was pumped into the extraction vessel to maintain an extraction pressure of 27.6 MPa with a flow rate of 17 L/h. The extraction temperature was set to 34.5 °C and the extraction time lasted for 82 min. One gram of dried seabuckthorn berry powder produced 0.19 g of seabuckthorn oil and the oil had a density of 0.936 g/mL.

2.2. Standardization of seabuckthorn oil

Fatty acid composition of seabuckthorn oil was determined according to the standardized protocol set by China Food and Drug Administration (China Food and Drug Administration, 2016). Seabuckthorn oil sample was evaporated to dryness under nitrogen. The sample was then methylated in 2% NaOH in methanol at 80 °C. When cooled, the resulting methyl esters were extracted into *n*-heptane and transferred to vials containing anhydrous Na_2SO_4 as the dehydrating agent. Fatty acid methyl esters were separated and quantified by using a Shimadzu GC-2010 gas chromatograph (Kyoto, Japan) equipped with an Agilent HP-88 capillary column (100 m × 0.25 mm × 0.2 μm, Agilent Technologies, USA). The injection volume was 1 μL and the carrier gas was helium. The injector temperature was set at 270 °C and the detector (flame ionization) temperature at 280 °C. The oven temperature was 130 °C initially, heated to 220 °C at 4 °C/min, held for 20 min, heated to 240 °C at 10 °C/min, and held for 10 min. Fatty acid methyl esters were identified based on the retention time to authentic lipid standards obtained from Nu-Chek-Prep, Inc. (Elysian, MN, USA).

Isorhamnetin content of the seabuckthorn oil was determined by high performance liquid chromatography (HPLC) according to the procedure described by Liu and Yang et al. (Liu and Yang, 2010) with slight modifications. HPCL was performed on an Agilent 1100 HPLC system equipped with a diode array detector. Separation of isorhamnetin was done with an Eclipse XDB-C18 column (250 mm × 4.0 mm × 5 μm). The mobile phase was a 58:42 (v/v) mixture of methanol and 0.4% phosphoric acid solution with a flow rate of 1.0 mL/min. The peak was detected at 370 nm.

2.3. Experimental animals

Specific pathogen free Kunming mice and Sprague-Dawley (SD) rats were used for the toxicological tests. Male and female mice (3-week-old) and rats (3-week-old) were provided by the Animal Experimental Center at Guangdong Academy of Medical Science (Guangzhou, China). Animals were housed in the animal house with controlled temperature of 23 ± 1 °C, relative humidity of 60 ± 5%, and a 12-hr light/dark cycle. Male and female animals were separated and kept in polycarbonated cages with 5 mice per cage and 2 or 3 rats per cage. Conventional diets (Animal Experimental Center at Guangdong Academy of Medical Science, Guangzhou, China) and sterilized tap water were given *ad libitum*. Animals were allowed to acclimatize for at least 3 days and only healthy animals were used for the experiments. Animal studies were performed under license from Department of Science and Technology of Guangxi and endorsed by the Animal Experimentation Ethics Committee at Guangxi Center for Disease Prevention and Control (Nanning, China).

2.4. Acute oral toxicity study in mice

Ten healthy Kunming mice of each sex at weight 18–22 g were randomly selected and used for the study. Mice were fasted overnight and administered seabuckthorn oil at a single dose of 20 mL/kg body weight through oral gavage. After the treatment, mice were provided with conventional diets and water immediately and monitored for any signs of toxic symptoms or mortality for 24 h and daily for the next 14 days. The body weights of mice were measured before dosing (Day 0) and on Day 7 and 14. At the end of the study, mice were sacrificed and general gross necropsy was performed on all animals.

2.5. 90-day repeated oral toxicity study in rats

The 90-day repeated oral toxicity study was conducted following standardized protocol set by the Ministry of Health of the PR China (Ministry of Health of the PR China, 2003; Qin et al., 2017). A total of 80 SD rats, weighted 74.0 ± 4.0 g, were randomly divided into four groups (three treatment groups and one control group), with 10 males and 10 females in each group. Male and female rats were housed separately in polycarbonated cages with a maximum of 3 rats per cage. Rats in the treatment groups were given 10, 5,