

Data S1: Acute toxicity test of ethanol extract of *Rubia yunnanensis* by oral administration in mice (maximum dose)

## Materials and methods

**Animals.** 40 C57BL/6J mice, SPF grade, half male and half female, weight 18-22 g, were obtained from Spefford (Beijing) Biotechnology Co., LTD. [certificate no. SCXK (Beijing) 2019-0010].

**Reagents.** The clinical adult daily dose is 15 g (60 kg/person, 0.25 g/kg body weight) (1).

**Animal experiments.** A total of 40 C57BL/6J mice were divided into administration group and blank control group according to body weight (n=20/group). Before the test, water was not allowed for 12 h. The administration group was given a dose of 30 g/kg body weight *Rubia yunnanensis* and the administration volume was 0.4 ml/10 g body weight; blank control group was given an equal volume of normal saline. The appearance, behavior and organ toxicity of animals in each group were observed every day for 14 days after administration and the weight of animals was recorded (Table SI). Animal food intake was recorded (Table SII). On the 14th day

of the experiment, the mice were killed and dissected and the appearance of their major organs was observed by naked eye.

## Results

There was a decrease in autonomous activity, but no death, within 4 h of administration. Within 14 days of administration, the animals did not die and were generally in good condition, with normal diet, bowel movement, appearance, fur, behavior and respiration, no abnormal secretion from the nose, eyes and mouth, and no other obvious abnormal reactions. The change in body was similar between the administration and the blank control group and the food intake was stable after administration. No notable abnormalities were observed in the major organs by gross anatomy. The maximum dose of alcohol extract of *R. yunnanensis* was 30 g crude drug/kg. Calculated according to the clinical dosage of 15 g for adults (60 kg weight), the maximum dosage of ethanol extract of *R. yunnanensis* in mice was 120 times the clinical dosage for adults.

## Reference

1. Kong C, Chen Q and Chen P: Ethnic drug of *Rubia yunnanensis* on the prevention and involvement of cardiovascular disease research *Medicine & Pharmacy of Chinese Minorities* 16: 41-43, 2010.

Figure S1. Hematoxylin and eosin staining. Carotid artery vessels in (A) control, (B) model and ethanol extract of (C) high-, (D) medium- and (E) low-dose *Rubia yunnanensis* group.

