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Toxicological evaluation of Yulangsan polysaccharide in Wistar rats: A 26-week oral gavage study

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ABSTRACT

Although numerous studies have proven the medicinal values of Yulangsan polysaccharide (YLSP), the toxicity of this active ingredient is unknown. In the acute toxicity study, a single oral administration of 24 g/kg YLSP caused neither toxicological symptoms nor mortality, and the LD_{50} was estimated >24 g/kg. In the chronic toxicity study, we administered doses of 0, 0.6, 1.2 and 2.4 g/kg YLSP in rats by oral gavage for 26 weeks followed by a 3-week recovery period. There was no mortality or remarkable clinical signs observed during this 26-week study. Additionally, there were no toxic differences in the following parameters: body weight, food consumption, hematology, clinical biochemistry, organ weight, and macroscopic findings. There were no adverse effects on histopathology observed in males or female rats treated with YLSP. Based on the results, the no-observed-adverse-effect-level of YLSP in rats is greater than 2.4 g/kg when administered orally for 26 consecutive weeks.

1. Introduction

Traditional oriental herbal therapies have become increasingly popular and are widely used for the treatment and prevention of various diseases (Liang et al., 2014; Cai et al., 2011; Lara-Diaz et al., 2009). It is believed that herbal medicines are harmless and are free from adverse effects. Herbal medicines are also available at a relatively low cost (Li et al., 2013; Lee et al., 2012). However, concerns have been raised over the lack of scientific evidence supporting the effectiveness and safety of herbal medicines.

Yulangsan is also called Longyanshen and is the root of the Millettia pulchra Kurz var. laxior (Dunn) Z. Wei plant. It is widely cultivated in Guangxi province (FDA, 2008) and is a commonly used traditional Chinese medicine for the remedy of anemia, dizziness, hyposomnia, infantile malnutrition, stroke hemiplegia and other conditions. Yulangsan polysaccharide (YLSP) is the major effective ingredient YLS extract (Lin et al., 2014). Previous studies have demonstrated that YLSP has the following broad spectrum therapeutic properties: anti-aging (Lin et al., 2013; Huang et al., 2008a; Doan et al., 2015), anti-inflammatory (Huang et al., 2008b), antiviral (Tao et al., 2014), anti-alcoholism (Liang et al., 2011), anti-liver fibrosis (Lv et al., 2011) and enhanced immunity (Cai et al., 2011). YLSP is effective in attenuating ischemia-reperfusion injury (Chen and Huang, 2009), isoniazid or rifampicin + isoniazid-induced hepatic injury (Dong et al., 2014), and D-galactose-induced cognitive impairment (Lin et al., 2014). Interestingly, YLSP also attenuates withdrawal symptoms in morphine-dependent rats (Chen et al., 2014). Furthermore, YLSP inhibits depression by modulating the neurotransmitters norepinephrine and 5-hydroxytryptamine (Liang et al., 2012).

Despite the evidence supporting the biological effects of YLS, its toxicity is still unknown. Therefore, it is extremely important to evaluate its toxicity using both acute and chronic oral administration in Wistar rats.

2. Materials and methods

2.1. Preparation of YLSP extract

Millettia pulchra Kurz var. laxior (Dunn) Z. Wei was purchased from Nanning Qianjinzi Chinese Pharmaceutical Co. Ltd (Nanning, China). A voucher specimen (YLS20110312619) was identified by Professor Quanfang Huang in the Department of Pharmacognosy, The First Affiliated Hospital of Guangxi University of Chinese

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Medicine and deposited in the herbarium of Department of Pharmacology of Guangxi Medical University.

YLSP was prepared by the method described previously (Lin et al., 2014). The root of Yulangsan was dried, powdered, and extracted three times with boiling water. The polysaccharide in the filtrate was precipitated fractionally with alcohol. The protein in the product was removed using the Savage method and further purified using DEAE fast flow Sepharose anion exchange chromatography. The components of the saccharide were finally purified by Sephadex G-75 gel permeation chromatography. Trifluoroacetic acid hydrolysis method and high-performance capillary electrophoresis analysis showed that YLSP consists of D-glucose and D-arabinose in a molar ratio of 90.79% and 9.21%, with an average molecular weight of 14,301 Da.

2.2. Animals

In this study, 10 male and 10 female mice weighing 18–22 g and 40 male and 40 female Wistar rats weighing 80–120 g were obtained from the Experimental Animal Center of Guangxi Medical University [SYXK 2009-0002]. The animals were allowed to acclimate in quarantine for one week prior to experimentation. The research was conducted according to protocols approved by our institutional ethical committee. The animals were housed five per cage in a temperature-controlled room at 22 ± 2 °C with a 12 h light and dark cycle (lights turned on from 8:00 AM to 8:00 PM). The animals had access to food and water ad libitum. The animal protocol was approved by the institutional ethical committee of Guangxi Medical University (approval No. 20110501202), and this study was conducted in accordance with the US guidelines (NIH publication No. 85-23, revised in 1985) for laboratory animal use and care.

2.3. Acute toxicity study in mice

The 20 mice were randomly divided into either the control group or the YLSP group. Each group consisted of 10 mice of each sex. YLSP was administered orally as a single dose (24 g/kg) by using a ball-tipped incubation steel needle placed on a graded disposable syringe. This dose is the maximum technically feasible dose in mice and is the maximum concentration and volume that can be given intragastrically (i.g.) (Takano et al., 2013). The control mice received an intragastric administration of distilled water (10 ml/kg). All animals were observed for mortality, signs of gross toxicity, behavioral changes, and body weight change during the first several hours after treatment and once daily for 14 d after dosing (Irwin, 1968). Necropsies were performed on all animals at sacrifice.

2.4. Chronic toxicity study in rats

2.4.1. Experiment design

After confirming normal health status at the end of the acclimation period, 40 rats of each sex were selected and randomly assigned to the YLSP 0.6, 1.2, and 2.4 g/kg groups and a control group. Each group consisted of 10 rats of each sex. The daily application volume (10 ml/kg body weight) of YLSP was calculated in advance based on the most recently recorded body weight of each individual animal. The YLSP was administered orally to rats for 26 weeks. Distilled water was given to the animals in the vehicle control group. The rats were observed twice daily during the experimentation period.

2.4.2. Clinical observation, body weight, food and water consumption

After the acclimation period, the common symptoms, activities, appetite, urine and stool were observed. Any animal mortality was also recorded. The animal food and water consumption were recorded once daily. The amounts of food and water given and their remnants on the next day were measured to calculate the difference, which was regarded as daily consumption. The body weight was measured once a week, and the body weight change was calculated.

2.4.3. Hematology and serum biochemistry

At the end of the 26-week experimentation period, 48 rats ($n = 12$) were sacrificed by cervical dislocation after collecting blood samples via the supra-orbital vein. The remaining 32 animals ($n = 8$) were sacrificed after 3 weeks without treatment, and the same parameters were measured.

Blood samples for hematological examination were collected in an Ethylene Diamine Tetraacetic Acid (EDTA)-2K (Sewon Medical, Republic of Korea) blood collection tube. The hematological parameters were analyzed using an automatic analyzer (Sysmex KX-21NV; Sysmex Co., Hyogo, Japan) to determine the following characteristics: the red blood cell count (RBC), hemoglobin concentration (HGB), mean corpuscular cell hemoglobin (MCH), mean corpuscular cell hemoglobin concentration (MCHC), hematocrit (HCT), mean corpuscular cell volume (MCV), white blood cell count (WBC), granulocyte ratio (NEUTR), lymphocyte ratio (LYR), platelet count (PLT), mean platelet volume (MPV), and platelet crit (PCT). The blood clotting time (CT) was also determined in blood samples using a glass plate (Coagrez-100s, Japan).

To analyze the serum biochemistry, the blood samples were first centrifuged at 3000 rpm for 10 min. The serum was analyzed using a 200FR NEO (Toshiba, Japan). The serum biochemistry parameters examined included the following: total protein (TP), albumin (ALB), globulin (GLB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactic dehydrogenase (LDH), alkaline phosphatase (ALP), bilirubin (T-Bil), triglyceride (TG), total cholesterol (TCHO), blood urea nitrogen (BUN), creatinine (CREA), glucose (GLU), potassium (K), sodium (Na), and chloride (Cl).

2.4.4. Macroscopic examination and organ weights

A complete necropsy was conducted on all animals after the terminal blood collection. The necropsies included the external surfaces of the body, all orifices and the cranial, thoracic and abdominal cavities and their contents.

The organ weights of the heart, liver, lung, brain, thymus, spleen, kidneys, adrenal gland, bladder, testis, prostate plus seminal vesicle, epididymides, uterus, and ovaries were measured. The organ index (absolute organ weight/body weight) was then calculated. Paired organs were weighed together.

2.4.5. Histopathological examination

The following organs were collected from all of the groups: the brain, hypophysis, heart, liver, lung, cerebellum, thymus, spleen, kidneys, adrenal gland, esophagus, stomach, duodenum, rectum, bladder, testis, prostate + seminal vesicle, epididymides, uterus, ovaries, bone marrow, spinal cord, ischiadic nerve, aorta, bronchus, thyroid gland, parathyroid gland, lymph node, pancreas, and salivary gland. All organs were fixed in 10% neutral buffered formalin and stained with hematoxylin and eosin (HE). A microscopic examination was performed for specimens from all of the rats.

2.5. Statistical analysis

The values are expressed as the mean ± SD. The data were tested by Levene's test and one-way analysis of variance (ANOVA) using SPSS 13.0 statistical software. The LSD t-test was used for post hoc analysis. Additionally, the levels of female ALT and GLU in YLSP