Food and Chemical Toxicology 107 (2017) 1–9

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Nonclinical safety of astilbin: A 4-week oral toxicity study in rats with genotoxicity, chromosomal aberration, and mammalian micronucleus tests

Yonglin Gao [a, 1], Chunmei Li [b, 1], Yunzhi Wang [a], Yunguo Liu [a], Guisheng Li [b, *], Xiaochen Fan [a], Yanshen Li [a], Jingwei Tian [b, **], Albert W. Lee [c]

[a] School of Life Sciences, Yantai University, Yantai, 264005, PR China
[b] School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai, 264005, PR China
[c] NutraSource, Inc., Clarksville, MD, 21029, USA

ARTICLE INFO

Article history:
Received 21 March 2017
Received in revised form
5 June 2017
Accepted 13 June 2017
Available online 15 June 2017

Keywords:
Rhizoma Smilacis Glabrae
Flavonoid
Salmonella typhimurium
Chinese hamster ovary cells
Mice

ABSTRACT

Astilbin is an active flavonoid compound isolated from *Rhizoma Smilacis Glabrae*. It has been widely used as an anti-hepatic, anti-arthritic, and anti-renal injury agent. However, its safety has not yet been established. The objective of this study was to evaluate 4-week repeated oral toxicity and genotoxicity of astilbin. We examined oral toxicity in Sprague-Dawley rats after daily oral administration of astilbin at 50, 150, and 500 mg/kg for 4 weeks. Negative control animals received the same volume of the solvent. Astilbin administration did not lead to death, body weight gain, food consumption, or adverse events. There were no significant differences in toxicity between the astilbin and control group; we observed no toxic effects on hematological or urinalysis parameters, biochemical values, organ weight, or histopathological findings. We assessed the genotoxicity of astilbin with the Ames test (TA97a, TA98, TA100, TA102, and TA1535), chromosomal aberration assay (using Chinese hamster ovary cells), and mammalian micronucleus test (in mice). We found no genotoxicity in any tested strains. The no-observed-adverse-effect level (NOAEL) for astilbin in the 4-week repeated oral toxicity study in rats was greater than 500 mg/kg body weight/day, regardless of gender. Results also suggested that astilbin does not have genotoxicity potential.

1. Introduction

Rhizoma Smilacis Glabrae belongs to the Smilacaceae family. Traditional Chinese medical literature, including the *Compendium of Materia Medica* and the *State Pharmacopoeia of the People's Republic of China,* describe it as effective for detoxification and easing joint movement. *Rhizoma Smilacis Glabrae* exists widely in plants, is rich in flavonoids, and possesses potent antioxidant activity *in vitro* and *in vivo* (Chen et al., 2007; Wang et al., 2016). In addition to its role as an antioxidant (Zheng et al., 2013; Li et al., 2015; Lou et al., 2014; Wang et al., 2012), it also has anti-inflammatory (Jiang et al., 1997) and immunomodulatory (Meng et al., 2016) properties (Diao et al., 2014; Xu et al., 2013; Tang et al., 2013). Astilbin (Fig. 1) is an active flavonoid compound isolated from *Rhizoma Smilacis Glabrae*. It has been used to treat arthritis (Kong et al., 2016), and as an agent to prevent hepatic (Wang et al., 2004; Xu et al., 1999) and renal injuries (Chen et al., 2011). Although astilbin and *Rhizoma Smilacis Glabrae* have a long history of use in China and many other countries, its natural origin and widespread use do not guarantee its safety.

Short-term toxicity studies with rodents are generally conducted for 28 days (one month). Results of this study will provide safety information include: (1) can help predict appropriate doses of the test substance for future subchronic or chronic toxicity studies, (2) can be used to determine the no-observed-adverse-effect level (NOAEL) for some toxicology endpoints, and (3) allow future studies in rodents to be designed with special emphasis on identified target organs. At the same time, genotoxicity tests are

* Corresponding author.
** Corresponding author.
E-mail addresses: liguishsheng@luye.cn (G. Li), tianjingwei@luye.cn (J. Tian).
[1] These authors contributed equally to this work and are the co-first authors.

http://dx.doi.org/10.1016/j.fct.2017.06.024
0278-6915/

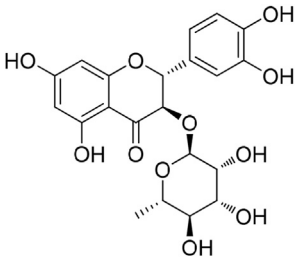

Fig. 1. Chemical structure of astilbin.

in vitro and *in vivo* tests designed to detect compounds that induce genetic damage, which is associated with adverse human health effects include gene mutations, chromosomal rearrangements or deletions, and loss or gain of whole chromosomes (aneuploidy) or chromosomal segments.

This preclinical 4-week repeated oral toxicity study will provide safety information that can help estimate appropriate doses of astilbin for future subchronic or chronic toxicity studies, and determine the NOAEL for some toxicology endpoints. Outcomes will also enable future rodent studies to focus on identified target organs. Moreover, we used the standard battery of tests (i.e., Ames assay, chromosomal aberrations assay using Chinese hamster ovary cells, and *in vivo* mammalian micronucleus test) to investigate genotoxicity of astilbin. This study was performed in compliance with the testing guidelines of the China Food and Drug Administration (CFDA) and OECD guidelines.

2. Materials and methods

2.1. Materials

We obtained astilbin with purity >98.5% from Shandong Engineering Research Center for Natural Drugs (Yantai, PR China). Sprague-Dawley rats and Kunming mice (SPF grade) came from the Experimental Animal Center of Shandong Luye Pharmaceutical Co., Ltd. (Yantai, PR China). Standard animal feed was purchased from Beijing Keao Xieli Co., Ltd. (Beijing, China). Molecular Toxicology, Inc. (Moltox®, Boone, NC) provided five strains of *Salmonella typhimurium* (TA97a, TA98, TA100, TA102, and TA1535). Reagents and control chemicals included Dexon (Laboratory of Dr. Ehrenstorfer-Schäfers), dimethylsulfoxide (DMSO, Sinopharm Chemical Reagent Co., Ltd.), sodium azide (SA, Sigma), 2-Aminoanthracene (2-AN, Sigma), 2-Aminofluorene (2-AF, Energy Chemical), cyclophosphamide (CP, Jiangsu Hengrui Medicine Co., Ltd.), Mitomycin C (MMC, Roche), and S9 (Moltox). The Stem Cell Bank at the Chinese Academy of Sciences provided CHO-K1 cells.

2.2. A 4-week repeated oral toxicity study

2.2.1. Study design

The study was performed in accordance with OECD Guideline 407 for Repeated Dose 28-day Oral Toxicity Study in Rodents (OECD, 1995) and Good Laboratory Practice (GLP) regulations of the China State Food and Drug Administration (CFDA). The Animal Ethics Committees of Yantai University approved the standard operating procedures.

2.2.2. Animals and treatment

Sprague-Dawley rats were divided into four groups and received once-daily oral (gavage) of astilbin at 50 (low dose), 150 (middle dose), and 500 (high dose) mg/kg (The recommend dose volume is 10 ml/kg). The concentration of the test substance is 5, 15, and 50 mg/ml in the low-, middle-, and high-dose groups, respectively. The negative control received the same volume of the solvent. Since we did not plan to compare the toxicity of the test substance to that of other compounds, we did not use a positive control group. All animals were dosed 7 days a week for a minimum of 4 weeks, followed by a recovery period of 28 consecutive days. The dose selection for the study was based on the outcomes from a pilot study.

We observed all animals daily for general appearance (e.g., changes in skin, eyes, fur, mucous membranes) and excretions or secretions. They were weighed before treatment (Day 0) and weekly thereafter; we calculated individual body weight changes. Food consumption was measured once a week. After the last dosing, we fasted 12 animals (six males and six females from each group) for 12 h, after which they were euthanized. We used blood samples to perform hematology and blood biochemistry studies, and dissected their organs to estimate organ/body weight ratios and conduct histopathological analysis. Another 12 animals (six males and six females) from each group were euthanized at the end of the 28-day recovery period.

We used ethylene diamine tetraacetic acid (EDTA) anticoagulant for hematological analyses of blood samples. Tests included red blood cell count (RBC), white blood cell count (WBC), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), hematocrit (HCT), platelets (PLT), thrombin time (TT), prothrombin time (PT), and activated partial thromboplastin time (APTT).

We evaluated clinical chemistry parameters. These included alkaline phosphatase (ALP), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), alanine aminotransferase (ALT), blood urea nitrogen (BUN), total bilirubin (T-BILI), creatinine (CRE), glucose (GLU), cholesterol (CH), creatinine kinase (CK), triglycerides (TG), potassium (K), sodium (Na), and chloride (CL). We also collected individual overnight urine samples from the same animals under the same conditions. The urinalysis included specific gravity, volume, appearance, protein, ketones, bilirubin, pH, glucose, blood, and microscopic investigations.

All animals that completed the scheduled test periods were euthanized by exsanguination under chloral hydrate anesthesia and subjected to necropsy. We studied the clinical history of each animal and conducted a detailed postmortem examination that included external surface and orifices. We noted changes and weighed requisite organs. These were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). We preserved tissue samples in fixative and processed them for histopathological examination.

2.3. Genotoxicity studies

2.3.1. Bacterial reverse mutation assay (Ames test)

We purchased five strains of *Salmonella typhimurium* (TA97a, TA98, TA100, TA102, and TA1535) from Moltox Molecular Toxicology Inc., and treated them according to plate incorporation methods. The tester strains were exposed to astilbin in the presence and absence of an exogenous metabolic activation system (S9). To evaluate the toxicity of astilbin, we carried out a pilot experiment to determine the highest concentration using 50, 100, 300, 600, 1250, 2500, and 5000 μg/plate. Results indicated that the number of revertant colonies for the five tester strains did not increase to more than twice the value observed in the control. However, positive controls showed at least a 2-fold increase in the number of revertant colonies (Table 1). The recommended maximum test concentration for soluble noncytotoxic substances is 5000 μg/plate or 5 μl/plate (OECD, 1997a). We selected 5000 μg/plate as the maximum