Food and Chemical Toxicology xxx (2017) 1–8

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Innocuousness of a polyherbal formulation: A case study using a traditional Thai antihypertensive herbal recipe in rodents

Tossaton Charoonratana [a, *], Jirapa Puntarat [b], Sujinthara Vinyoocharoenkul [b], Teeratad Sudsai [c], Kingkan Bunluepuech [d]

[a] Department of Pharmacognosy, Faculty of Pharmacy, Rangsit University, Lak-Hok, Muang, Pathum Thani, 12000, Thailand
[b] Department of Pharmacology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand
[c] Faculty of Oriental Medicine, Rangsit University, Lak-Hok, Muang, Pathum Thani, 12000, Thailand
[d] Faculty of Traditional Thai Medicine, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand

ARTICLE INFO

Article history:
Received 2 June 2017
Received in revised form
24 July 2017
Accepted 25 July 2017
Available online xxx

Keywords:
Acute toxicity
Sub-chronic toxicity
Swiss albino mice
Tinospora crispa
Traditional Thai antihypertensive herbal recipe
Wistar rats

ABSTRACT

Recently, a traditional Thai antihypertensive herbal recipe has reportedly been used in Thailand. Its ingredients have long featured in traditional Thai medicine preparations; however, research indicates that the presence of one of them - Tinospora crispa - may have negative effects on the liver and kidneys. Thus, the safety data of this recipe must be proved in animal models prior to conducting any studies in humans. The present case study aims to evaluate the safety of this recipe in Swiss albino mice and Wistar rats through acute and sub-chronic toxicity studies, respectively. The quality control of this recipe was also achieved to guarantee the chemical consistency throughout the entire experiment. Results showed that this recipe did not cause death or any toxic signs in mice or rats. The oral LD50 value in mice was more than 5.0 g/kg. Some hematological and serum biochemical values of treated rats, such as hematocrit, hemoglobin, platelet, monocytes, aspartate aminotransferase, bilirubin, and creatinine, were found to be statistically different from the control group; however, all values were within the ranges of normal rats. Considering the histological study, no damage on liver and kidney tissues was observed in the treatment.

1. Introduction

A global consensus has emerged that herbal products or plants used as medicines are not necessarily safe because they are natural. Many traditional medicines and health supplements containing plants have been proven to cause toxicity at different levels, for example, hepatotoxicity by *Garcinia cambogia*, intestinal disorders by *Phaseolus vulgaris* lectins, or nephrotoxicity by *Aristolochia* spp. (García-Cortés et al., 2016; He et al., 2015; Cosyns, 2003). Thus, the use of herbs for ailment alleviation must be conducted with caution.

Because the demand for traditional medicines is increasing, many countries now recognize the need to set up a policy that allows people to utilize them safely. In Southeast Asia, efforts to harmonize the safety regulation of traditional medicines have been developing since 2004 (WHO, 2004), and a guideline on safety substantiation of traditional medicines was first published in 2015 (ASEAN, 2015).

In Thailand, various traditional medicine systems use several herbal recipes in therapeutic practices. Recent ethnopharmacological research revealed the usage of a traditional Thai antihypertensive herbal recipe (TTAH) in Nakhon Si Thammarat province (Charoonratana et al., 2014). This TTAH, comprising of *Acanthus ebracteatus* Vahl, *Aegle marmelos* (L.) Corrêa, *Boesenbergia rotunda* (L.) Mansf., *Cyperus rotundus* L., *Piper nigrum* L., and *Tinospora crispa* (L.) Hook. f. & Thomson, possesses the same constituents used in the Ya That Somdun recipe from the royal traditional Thai medicine collection (Chotchoungchatchai et al., 2012). Examination of the constituents of this TTAH confirmed the presence of antihypertensive substances, such as imperatorin from *A. marmelos*, piperine from *P. nigrum*, and adenosine from *T. crispa* (Cao et al., 2013; Praman et al., 2012; Taqvi et al., 2008). Other constituents were considered as auxiliaries proposed to improve effectiveness or reduce toxicity of the recipe.

Although TTAH components have been persistently used in Thailand for a long time, *T. crispa* has been reported to contribute to hepatotoxicity and nephrotoxicity in rats (Kadir et al., 2011; Chavalittumrong et al., 1997). Abolishment of herbal recipes

* Corresponding author.
E-mail address: peair_fanhoidonk@yahoo.com (T. Charoonratana).

http://dx.doi.org/10.1016/j.fct.2017.07.052
0278-6915/

containing *T. crispa* was recommended since hepatitis caused by *T. crispa* usage was reported in humans; recovery only occurred autonomously after discontinuation (Langrand et al., 2014). However, according to the World Health Organization Traditional Medicine Strategy, evidence-based traditional medicine promotion has been found to be more acceptable than disapproval of all traditional recipes containing one possibly toxic plant (WHO, 2013). Therefore, the present case study was undertaken to evaluate the toxicity of oral administration of TTAH, through clinical signs as well as biochemical, hematological and histological parameters of rodents. The information can also be used as part of the safety substantiation of the recipe prior to use in human clinical trials.

2. Material and methods

2.1. Test substances and chemicals

All plant materials were purchased from a Pan-Thai herbal drugstore and authenticated by Dr. Kingkan Bunluepuech, a traditional Thai doctor from the Traditional Thai Medicine Hospital at Prince of Songkla University, Thailand. All samples were dried and ground into powder. TTAH batch number 0606-57 was prepared by mixing the powder, then screening for the microbial limit and heavy metal test in accordance to approved criteria (Ministry of Public Health, 1995). The TTAH powder was stored at 22 ± 2 °C, 50% RH ± 10% RH, and protected from light. Adenosine, imperatorin, pinostrobin, and piperine were purchased from Sigma Aldrich (USA). HPLC grade acetonitrile and water were purchased from B&J (Korea). Analytical grade formic acid was purchased from Merck (Germany). Analytical grade ethyl acetate and hexane were purchased from J.T.Baker® Chemicals (USA). Water for plant extraction was from Puris, Expe-CB Ele10 Water System (Korea).

2.2. Product quality control

To acquire a wide variety of compounds, the TTAH powder (5 g) was sonicated five times with hexane (15 min per one extraction). The solution was then filtrated, pooled, and evaporated under reduced pressure to produce the hexane extract. The same TTAH powder was further extracted with ethyl acetate using the same method described above to obtain the ethyl acetate extract. The TTAH powder was finally extracted with water and lyophilized to obtain the water extract.

Dionex Ultimate TM^{2000} Liquid Chromatography systems coupled with a Bruker Amazon SL Mass Spectrometer were used to analyze all extracts. Acclaim® 120-C18 column (3 μm, 2.1 mm × 150 mm) was a stationary phase. Analysis was performed in a gradient mode using 0.1% formic acid-acetonitrile ratio from 95:5 to 60:40 (0–30 min), an isocratic mode at a ratio of 60:40 (30–35 min), the gradient mode at 60:40 to 20:80 (35–50 min), the isocratic at a ratio of 20:80 (50–60 min), and re-equilibrated at 95:5 for 5 min. The mobile phase flow rate was maintained at 0.15 mL/min, and the column temperature was 25 °C. The mass spectrometer was equipped with an ESI ion source and a quadrupole-ion trap. The analyses were performed in positive mode, recorded on a mass range of *m/z* 100-1000. Nitrogen was used as the desolvation gas with a flow rate of 7.0 L/min at 200 °C. Capillary voltage was 4500 V while nebulizer pressure was set at 2 bars. Adenosine, imperatorin, pinostrobin, and piperine were identified by comparing the parent and fragment ions with the reference standards. The constituent amount was measured using extracted ion quantification and data were processed by Compass 1.3 SR2. To check the quality of the TTAH powder, the stability of all mentioned markers was also determined monthly during sub-chronic toxicity experiment.

2.3. Experimental animals and treatments

Both sexes of 6-week-old Swiss albino mice and 8-week-old Wistar rats were used for the acute and 90-day toxicity studies, respectively. All animals were obtained from the Laboratory Animal Center at Prince of Songkla University, Thailand. The animals were housed divided by sex under standard conditions (22 ± 2 °C; 50% RH ± 10% RH; 12 h light/dark cycle), with free access to food and water. The animals were acclimatized for at least 7 days before the experiments. The study protocol was reviewed and approved by Rangsit University Animal Ethics Committee (Protocol number RSEC 02/2556).

The animal doses were translated, based on K_m value, from an established dose of TTAH administered per day to 60 kg adult hypertensive human (Reagan-Shaw et al., 2008). The values of animal dose were calculated using the equation: human dose x [human K_m]/[animal K_m]. Thus, the starting doses in mice and rats were 0.4 g/kg and 0.2 g/kg, respectively. The suspension of TTAH powder in distilled water was freshly prepared and mixed vigorously before oral gavage. All control groups received distilled water as a vehicle.

2.4. Acute toxicity

To estimate LD_{50}, the acute toxicity was performed consistently with the OECD guideline no. 425 (OECD, 2008). Both male and female Swiss albino mice (five mice/sex/group) were divided equally into six groups (control, 0.4, 2.0, 3.0, 4.5, and 5.0 g/kg, p.o.). Toxicity was assessed based on mortality and signs such as general activities, reflexes (corneal and headset), or activities on the central and autonomic nervous system (anesthesia, sedation, tremor and convulsion), or activities on motor system (straightening, response to tail touch and grip). The animals were closely observed during the first 30 min, and then 1, 2, 4, 6, 24 h after dosing. The number of survivors was recorded once a day for 14 consecutive days.

2.5. Sub-chronic toxicity

The 90-day sub-chronic toxicity was performed consistently with the OECD guideline no. 408 (OECD, 1998). Both sexes of Wistar rats (10 rat/sex/group) were divided into four groups (control, 0.04, 0.2, and 1 g/kg, p.o.). A further five males and five female rats were added in the control group and the highest dose group serving as the control satellite group and 1 g/kg satellite group, respectively. After the 90-day treatment period, the satellite groups were kept untreated for 30 more days to observe persistence or delayed incidence of toxic effects. The rats were weighed weekly and observed daily for signs of toxicity. At the end of the experiment, all survivors were fasted overnight before anesthetization. Blood samples were collected via cardiac puncture for the measurement of hematological parameters including hematocrit, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, neutrophils, lymphocytes, monocytes, eosinophils, and white blood cell (WBC) count. Serum biochemical parameters including cholesterol, triglyceride, total bilirubin, aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), creatinine, and glucose were also determined using an Automatic Clinical Chemistry A25 Analyzer (BioSystems).

Immediately after blood sample collection, visceral organs such as heart, kidneys, livers, lungs, spleen, and stomach were removed, weighed, and fixed in 10% neutral buffered formalin. After fixation, the kidneys and livers of all rats were dehydrated continually with 95% alcohol and isopropanol. The samples were then diaphanized in xylene and infiltrated with paraffin using Automatic Tissue Processor (Excelsior ES). Paraffin-embedded tissues were cut to

Please cite this article in press as: Charoonratana, T., et al., Innocuousness of a polyherbal formulation: A case study using a traditional Thai antihypertensive herbal recipe in rodents, Food and Chemical Toxicology (2017), http://dx.doi.org/10.1016/j.fct.2017.07.052