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Single, repeated dose toxicity and genotoxicity assessment of herb formula KIOM2012H

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ABSTRACT

Background: Traditional medicine and herbal prescriptions are becoming more popular, and they account for a large share of the world's healthcare research studies, developments, and market demands. Increasing scientific evidence of the substantive efficacies such as preventive health keeping pharmaceutical materials and dietary supplements can be found elsewhere. Above all, safety should be the critical premise for considering developmental materials such as pharmaceuticals without side effects and toxicity.

Methods: The authors formulated KIOM2012H (K2H) using four herbs that were reported to have medicinal effects—including anticancer, antiaging, antimicrobial, inflammation, and neuroprotective properties. In order to examine the toxicity, single and repeated dose toxicity, and genotoxicities of bacterial mutation, micronucleus, and chromosomal aberration assays were conducted.

Results: All experimental observations and results showed normal findings. Toxicities or abnormal signs were not observed in all experimental assays, including oral administration, animal behavior, clinical findings, and changes in body weight *in vivo*. *In vitro* bacterial cultures produced no revertant colonies, and no increased numbers of structural or numerical aberrant metaphases were found in the metaphase chromosomes examined. Moreover, no significant increased frequency of micronucleus was observed in any of the doses used. Overall, no acute toxicity or genotoxicity was found in all analysis parameters in all the assays conducted.

Conclusion: Reviewing the results as a whole, K2H extract was regarded as a safe material with no toxicity, and can be applied for the research and development of complementary and alternative medicines with improved efficacy in current therapeutic healthcare, based on traditional medicine and herb resources.

1. Introduction

Asian countries and many other countries have their own traditional medicine that has been prescribed for a long time.

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Herbs and plants have also been used by the general public as folk remedies. This means that generally accepted and medicated herbal medicines have been empirically recognized as effective and safe, although their pharmacological safety and efficacies are not fully established and studies are still underway to understand them scientifically. Traditional medicine and herbs are becoming more common as practical complementary and alternative ways of modern preventive and therapeutic healthcare. It is reported that 70–95% of the population in many countries around the world use traditional medicine for primary healthcare.[1,2] Natural product sales in the United States alone reached US$14.8 billion in 2008, and global sales of Chinese medicine amounted to US$83.1 billion in 2012 with exponential increase, which is expected to reach US$115 billion by 2020 including all natural supplements and remedies.[3] Indeed, current traditional medicines are sold as medicinal herbs and functional dietary supplements, and are usually supplied in the form of tea, beverages, tablets, and capsules that come from aqueous extracts or lyophilized formulations. Pharmaceutical companies are trying to find and develop new drugs from natural and herb resources. Actually, many drugs are based on these natural compounds, whereas chemically synthetic compounds and drugs are more common and more frequently adopted for drug development. Just as many plants and herbs have efficacies and many active natural compounds, the component herbs of K2H in this study are also known to have the following activities. The seeds and leaves of *Arctium lappa* were reported to have anticancer and antiulcer activities,[4,5] antiaging,[6] neuroprotective,[7] and protective effect on atherosclerosis,[8] and on cadmium toxicity in the liver.[9] The root of *Glycyrrhiza uralensis* is known to have antiasthma,[10] antimicrobial,[11,12] antioxidant,[13] hepatoprotective,[14] neuronal cell protection,[15] and tyrosinase inhibition[16] effects, and has also been observed to stimulate osteoblast function[17] and detoxification system via Nrf2 activation.[18] The cortex of *Magnolia officinalis* has not only been shown to exhibit activities against inflammation,[19] tumor,[20] and memory deficiency,[21] but has also been observed to attenuate lipogenesis[22] and hyperglycemia.[23] The root of *Zingiber officinale* was reported as effective against rheumatoid arthritis,[24] allergic asthma,[25] Alzheimer's disease,[26] cancer,[27] and diabetes-induced heart abnormality.[28] It was also shown to exert a hepatoprotective effect against hepatic injury, lipid accumulation,[29] and liver fibrosis.[30] Improvement of cognitive function by ginger extract was identified in the mouse hippocampus.[31] As is well known, medicinal plants and herbs contain many natural compounds, which can be used for treatment of multiple diseases by combinatorial prescription, and development of multitarget drug, the so-called "multicomponent" drug.[32–34] In this respect, we have formulated K2H with four different herbs. K2H has already been identified earlier by the present authors for its lipid- and body weight-lowering activity in lipid- and high fat diet-induced cellular and animal model of nonalcoholic fatty liver disease.[35] Traditional medicine and folk medicines have been used for a long time and are considered effective and safe, at least in the empirical sense. Nowadays, in the face of growing demand for natural and traditional medicines as drugs or functional foods, toxicological examination and secured safety with scientific evidence should be considered a first priority. In this study, single and repeated dose toxicity studies were conducted using rats, and genotoxicity studies by bacterial reverse mutation, micronucleus assay, and chromosomal aberration assay for toxicity and safety evaluation of K2H. Based on the results, no toxicities were observed in single and repeated dose toxicities, bacterial reverse mutation, and micronucleus assays, except for chromosomal aberrations.

2. Materials and methods

2.1. *Preparation of herb extract*

To prepare K2H, four medicinal herb materials (Table 1) were purchased from a local distributor in Yeongcheon, Korea, and botanically identified by herb specialists and practitioners. Prior to extract preparation, all herb materials were immersed in distilled water for 1 hour at room temperature to enhance extraction yield, and boiled for 3 hours at 115 °C in the extractor (Cosmos 600; Kyungseo Machine, Korea). Extract was collected by filtering through a test sieve (106 μm; Retsch, Germany) to eliminate insoluble debris and residues, freeze-dried, and then stored at −20 °C until use. A voucher specimen (registration no. KIOM2012H) of the lyophilized extract was deposited in the institutional herbarium.

2.2. *Chemicals and reagents*

The following chemicals—2-aminoanthracene (2-AA), benzo[*a*]pyrene (B[*a*]P), sodium azide (SA), 2-nitrofluorene (2-NF), 4-nitroquinoline-1-oxide (4NQO), acridine mutagen ICR 191 (ICR-191), cyclophosphamide monohydrate (CPA), acridine orange solution (AO), ethyl methanesulfonate (EMS), dimethylsulfoxide (DMSO), potassium chloride (KCl), magnesium sulfate, citric acid monohydrate, potassium phosphate dibasic anhydrous, sodium ammonium phosphate, glucose, sodium chloride, tryptophan, histidine, biotin, methyl alcohol, and glacial acetic acid—were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Oxoid Nutrient Broth No. 2 and Bacto agar were purchased from Thermo Scientific Inc. (Waltham, MA, USA) and BD (Franklin Lakes, NJ, USA), respectively. S9 mix (5% for bacterial reverse mutation assay and 30% for chromosomal aberration assay, v/v) was prepared using Aroclor 1254-induced rat liver S9 (Molecular Toxicology Inc., Boone, NC, USA) supplemented with cofactor-I (8 μM $MgCl_2$, 33 μM KCl, 5 μM glucose-6-phosphate, 4 μM nicotinamide adenine dinucleotide phosphate, 4 μM nicotinamide adenine dinucleotide, 100 μM sodium phosphate buffer, pH 7.4; Wako Pure Chem. Ind., Osaka, Japan). S9 mix was prepared fresh prior to use and kept on ice during the experiment. Information on chemicals and reagents not described here is supplied in the relevant sections in "Materials and methods" section.

2.3. *Single dose toxicity study*

For single dose toxicity study, 7-week-old specific-pathogen-free (SPF) Sprague–Dawley (SD) rats (Samtako, Osan, Korea) were used after 7 days of acclimatization in the animal laboratory under the following conditions: temperature 23 ± 3 °C, humidity 55 ± 15%, ventilation 10–20 times/h, and 12-hour

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