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# Leachianone A as a potential anti-cancer drug by induction of apoptosis in human hepatoma HepG2 cells

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**Abstract**

The Chinese herbal medicine *Radix Sophorae* is widely applied as an anti-carcinogenic/ anti-metastatic agent against liver cancer. In this study, Leachianone A, isolated from *Radix Sophorae*, possessed a profound cytotoxic activity against human hepatoma cell line HepG2 *in vitro*, with an $IC_{50}$ value of 3.4 μg/ml post-48-h treatment. Its action mechanism via induction of apoptosis involved both extrinsic and intrinsic pathways. Its anti-tumor effect was further demonstrated *in vivo* by 17–54% reduction of tumor size in HepG2-bearing nude mice, in which no toxicity to the heart and liver tissues was observed. In conclusion, this is the first report describing the isolation of Leachianone A from *Radix Sophorae* and the molecular mechanism of its anti-proliferative effect on HepG2 cells.


*Keywords:* Leachianone A; *Sophora flavescens*; Liver cancer; Apoptosis

## 1. Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignant neoplasm of the liver worldwide. It accounts for about 6% of all human cancers annually [1]. The disease prevails in parts of Asia and Africa; yet appears rife in many European countries in recent years. Although substantial advances have been made in chemotherapy regimen for HCC, the efficacy of drugs is often hampered by a range of adverse side-effects imposed on patients. Accordingly, it is urged to explore a new approach for development of an effective therapy against this disease.

Apoptosis, a type of programmed cell death implicated in cellular homeostasis and numbers of physiological processes, is often linked with carcinogenesis, which is resulted from the abrogation of apoptotic process [6,7]. For instance, defects and/ or mutations of p53 delay cell-cycle arrest and abolish the DNA repair process, which otherwise render the cells to apoptosis. Besides, inadequate Bcl-2 and Bcl-$X_L$ protein expressions are also likely

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to enhance tumor cell survival. As such, apoptotic mechanisms are commonly exploited for tumor therapy. In general, initiation of apoptosis involves two major pathways, extrinsic (death receptor) and the other, intrinsic (mitochondria) pathways. They act in diverse routes, but ultimately come to converge and connect to each other on the caspase cascade. Apoptotic cells are usually characterized by several distinctive morphological and biochemical changes, including cell shrinkage, membrane blebbing, chromatin condensation, appearance of phosphatidylserine (PS) on cell membrane surface, DNA fragmentation, protein cleavage at specific locations and increased mitochondrial membrane permeability, etc. Recently, considerable attention has been devoted to the sequence of the events referred to as apoptotic cell death and the role of this process in mediating the lethal effects of anti-neoplastic agents towards cancer cells.

*Sophora flavescens* is a shrub cultivated widely in northeast Asian countries. *Radix Sophorae* (also known as *KuShen*), stipulated as the dried roots of this species, is a natural product used in traditional herbal preparations in China for centuries, and is prescribed as an anti-pyretic, anti-inflammatory and anti-ulcerative agent [2]. Previous phytochemical studies of *Radix Sophorae* have reported the isolation of quinolizidine alkaloids, flavonoids and triterpenoids [3]. Of them all, flavonoids are well-known for their anti-tumor activity, being able to bring about the differentiation and/or growth inhibition in various cancer cells, such as lung, esophageal, colorectal, breast and prostate cancers as well as osteosarcoma [4,5].

In the current study, we successfully purified and identified the bioactive constituent Leachianone A (LA) from this medicinal herb, and found that it exhibited a potent cytotoxicity on human hepatoma cell line HepG2 *in vitro*. Combining both the *in vitro* and *in vivo* experiments, it demonstrated that LA possessed the ability to induce apoptosis in HepG2 cells, and retard tumor growth in nude mice without leaving apparent toxicity to the hosts.

## 2. Materials and methods

### 2.1. Preparation of LA

*Radix Sophorae* was obtained in the Guangzhou province of mainland China (voucher number 2004–2531). The dried root of the herbal species (about 1 kg) was soaked and boiled in 1 L absolute ethanol for 2 h. The sample was then filtered, and the residue was further extracted under the same condition twice. The filtrates collected from three separate extractions were combined, concentrated and partitioned between hexane:ethyl acetate (1:1) for three times. Ethyl acetate fraction of totally 420 ml was collected and evaporated to dryness under a reduced pressure; power extract weighing about 34 g was thus resulted. The extract was subsequently subjected to silica gel column chromatography ($20 \times 8.5$ cm; 8.5 ml/min), and the purified LA-containing fractions were allowed to dry under vacuum to confer a yield of about 931 mg. The structure and molecular weight of LA were resolved by $^{1}H$ NMR and $^{13}C$ NMR spectroscopies and mass spectrometry, respectively (Fig. 1). The lyophilized powder of LA was dissolved in absolute ethanol, filtered and stored at −20 °C. For all experiments, the final concentrations of the tested compound were prepared by diluting the stock with RPMI-1640 or DMEM culture medium. Control cultures received the carrier solvent (1% ethanol).

### 2.2. Cell culture

Human hepatoma cell line HepG2 and hepatic epithelial cell line WRL-68 were purchased from American Type Culture Collection (ATCC, Rockville, MD). WRL-68 was originated from the normal liver tissue of a fetus, and was used as a normal cell model here to examine the non-specific cytotoxicity of LA. Both cell lines were cultured as a monolayer with RPMI-1640 or DMEM medium (Invitrogen), supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 μg/ml streptomycin and 100 unit/ml penicillin. The cultures were maintained at 37 °C in a humidified atmosphere of 5% $CO_2$. Cells in the exponential growth phase were used for all experiments.

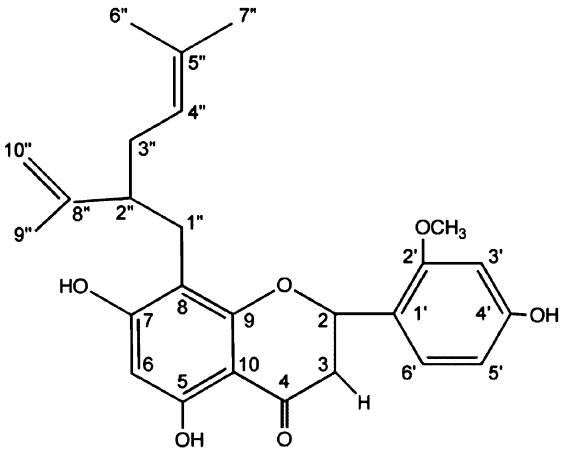


Fig. 1. Chemical structure of LA from *Radix Sophorae*.