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# Hexahydro-β-acids induce apoptosis through mitochondrial pathway, GADD153 expression, and caspase activation in human leukemia cells

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ABSTRACT

Hexahydro-β-acids (HBA) and β-acids (BA) displayed strong growth inhibitory effects against human leukemia HL-60 cells and were able to induce apoptosis in a concentration- and time-dependent manner and the morphological changes associated with apoptotic cell death; however, BA was less effective. Treatment with HBA caused a rapid loss of mitochondrial trans-membrane potential, release of mitochondrial cytochrome *c* into cytosol. The levels of Bad and Bax were dramatically increased in cells treated with HBA. In addition, the results showed that HBA promoted the up-regulation of Fas prior to the processing and activation of pro-caspase-8 and cleavage of Bid, suggesting the involvement of a Fas-mediated pathway in HBA-induced cells. Moreover, the changes occurred after single breaks in DNA were detected, suggesting that HBA induced irreparable DNA damage, which in turn triggered the process of apoptosis. HBA markedly enhanced the growth arrest DNA damage-inducible gene 153 (GADD153) protein in a concentration- and time-dependent manner. These findings suggest that HBA creates an oxidative cellular environment that induces DNA damage and GADD153 gene activation, which in turn triggers apoptosis in HL-60 cells. Our study identified the novel mechanisms of HBA-induced apoptosis and indicated that HBA may be used as a potential chemopreventive and chemotherapeutic agent.



## 1. Introduction

Hop (*Humulus lupulus* L.) is an essential ingredient of beer, where it provides the characteristic flavor and inhibitory effect on bacterial growth (Behr and Vogel, 2010). Hops extracts are currently marketed as botanical dietary supplements for the relief of hot flashes in menopausal women as an alternative to hormone replacement therapy, and they have also been used for treating insomnia and anxiety (Bowe et al., 2006). In addition to plant fibrous component and proteins, hops contain various small molecules, including flavonoids, volatile oils, and bitter resins or acids. The bitter acids of hops mainly consist of α-acids, β-acids (also called humulones and lupulones, respectively) and their oxidation products that contribute to the unique bitterness and aroma of beer (Chen and Lin, 2004b). Hop bitter acids, the prenylated derivatives of phloroglucinol, have been shown to have various potent biological activities, including anti-inflammation (Van et al., 2009), antioxidation (Tagashira et al., 1995), inhibition of tumor promotion (Yasukawa et al., 1995), and induction of cancer cell apoptosis (Chen and Lin, 2004b). Hop β-acids consist of humulone, cohumulone and adhumulone (Fig. 1), have a specific antibacterial action, however, they are very sensitive to oxidation during storage. On the other hand, hexahydro-β-acids, the reduced derivatives of β-acids, exhibit not only a stronger bacteriostatic action than that of β-acids, but also have an increased stability to oxidation (Liu et al., 2008).

Apoptosis is considered an emerging mechanism by which food bioactives could exert their anti-cancer properties (Kelloff et al., 2000; Sporn and Suh, 2000). It is defined as an active process leading to chromatin condensation, chromatin fragmentation and membrane blebbing, thereby maintaining the integrity of the cell membrane (Martin and Green, 1995). This definition distinguishes the apoptotic process from other forms of cell death, such as autophagy, oncosis, and necrosis (Nicotera and Melino, 2004; Shintani and Klionsky, 2004). A large number of chemopreventive compounds from natural products have been used as a promising strategy to fight against cancer by inducing apoptosis in malignant cells. The two main apoptotic pathways, the death receptor (extrinsic) and

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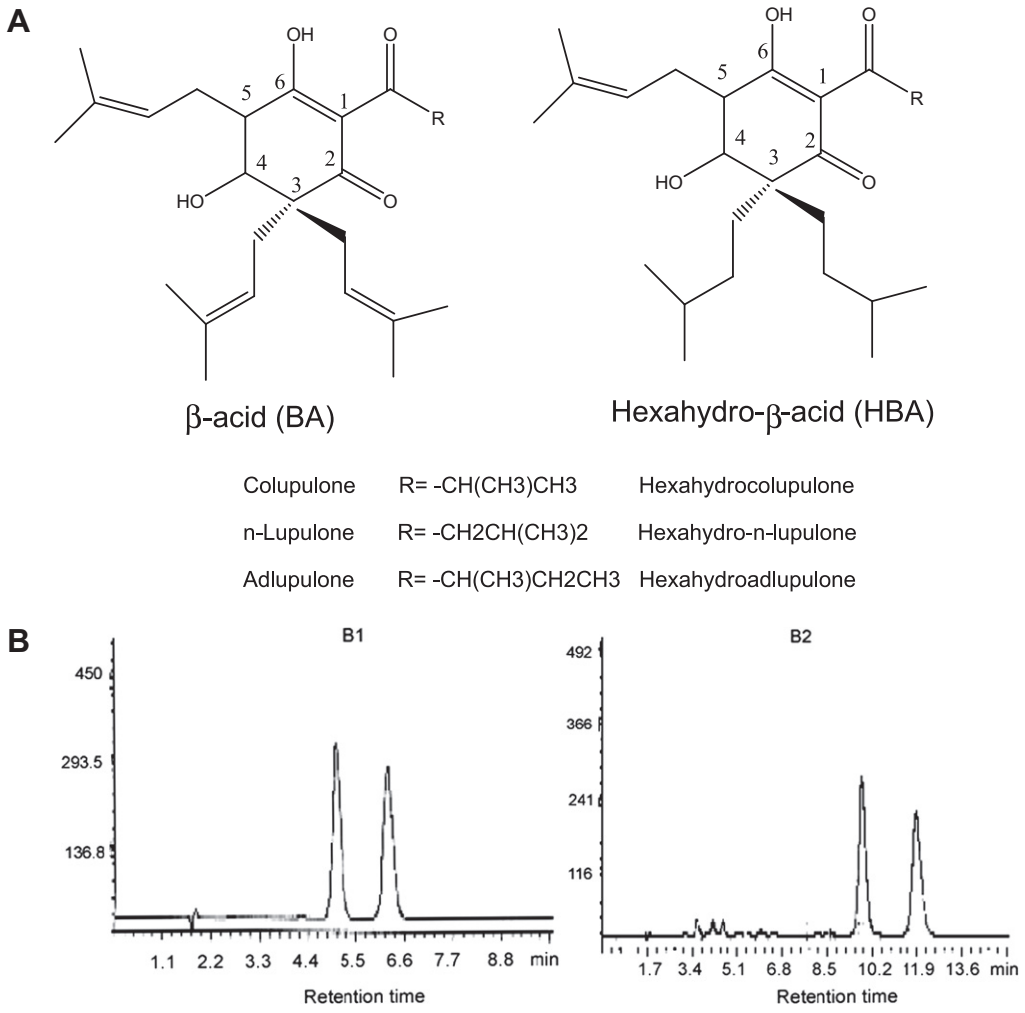


**Fig. 1.** HPLC profile and chemical structures of β-acids (BA) and hexahydro-β-acids (HBA).

mitochondrial (intrinsic) pathways, the mitochondrial pathway is regulated by the Bcl-2 family of proteins, including anti-apoptotic proteins such as Bcl-2 and Bcl-$X_L$ and pro-apoptotic proteins such as Bad, Bid, Bim, Bax, and Bak (Li et al., 2004). Recent studies of the endoplasmic reticulum (ER) as a third subcellular compartment containing caspases were implicated in apoptotic execution induced by ER stress (Kaufman, 1999; Nakagawa et al., 2000; Rao et al., 2004). The ER stress-induced cell death modulator is a CCAAT/enhancer-binding protein (CEBP) homology protein (CHOP)/growth GADD153, known as CHOP, is a member of the CEBP family of transcription factors (Wang et al., 1996).

In this research, we first examined the antiproliferative effects of HBA and structurally related compound, BA on human leukemia cells. Our results demonstrate that HBA can induce apoptosis in a dose- and time-dependent manner in HL-60 cells. We further evaluate the molecular mechanisms of apoptotic effects induced by HBA. It is suggested that HBA modulates the production of ROS, the release of cytochrome *c*, and the activation of caspases in HBA-induced apoptosis.

## 2. Materials and methods

### 2.1. β-acids and hexahydro-β-acids

β-acids were extracted, fractionated and purified from hop (*Humulus lupulus*) of Tsingdao variety as described by Liu et al. (2007). It contains 56% colupulone (Fig. 1B1 left peak), and 42% lupulone and adlupulone (Fig. 1B1 right peak). Hexahydro-β-acids was derived from β-acids by way of hydrogenation as described by Liu et al. (2008). It contains 57% hexahydrocolupulone (Fig. 1B2 left peak) and 41% hexahydrolupulone and hexahydroadlupulone (Fig. 1B2 right peak).

### 2.2. Cell culture and chemicals

Human promyelocytic leukemia (HL-60) cells obtained from American Type Culture Collection (Rockville, MD) were grown in RPMI 1640 medium and 10% fetal bovine serum (Gibco BRL, Grand Island, NY) supplemented with 2 mM glutamine (Gibco BRL) and 1% penicillin/streptomycin (10,000 units of penicillin/mL and 10 mg/mL streptomycin) and kept at 37 °C in a humidified 5% $CO_2$ incubator. Human polymorphonuclear cells (PMNs) were obtained from healthy male donors and were separated by Ficoll–Hypaque density gradient. Human PMNs were washed twice in 0.9% NaCl and resuspended in RPMI-1640 medium. Pan-caspase inhibitor (z-Val-Ala-Asp-fluoromethyl ketone, z-VAD-FMK) was purchased from Calbiochem (La Jolla, CA). Propidium iodide was obtained from Sigma Chemical Co. (St. Louis, MO). Propidium iodide was obtained from Sigma Chemical Co. (St. Louis, MO).

### 2.3. Cell survival assay

Cell viability was assayed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). Briefly, HL-60 cells were plated at a density of $1 \times 10^5$ cells/mL into 24 well plates. After overnight growth, cells were pretreated with a series of concentrations (0.5, 1, 2.5, 5, and 10 μg/mL) of BA and HBA for 24 h. The compounds were dissolved in dimethyl sulfoxide (DMSO) and the final concentration of DMSO in the culture medium was <0.05%. At the end of treatment, 200 μL of MTT was added, and cells were incubated for further 4 h. Finally, the absorbance was monitored by a microplate reader at a wavelength of 570 nm.