Effect of Apoptosis Induction of *Ailanthus altissima* on Human Lung Carcinoma Cells

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**ABSTRACT**

We investigated the inhibitory effects of solvent extracts from *Ailanthus altissima* in A549 human lung cancer cell. *A. altissima* has been recognized as a traditional healthy food due to its various biological activities against hypertension, strokes, fever, pain, neuralgia, inflammation, and cancer effects. Recently, it has been reported that the extracts of various wild vegetables show strong anti-cancer properties by induction of apoptosis. However, the mechanisms of their cytotoxicity in human lung cancer cells have been poorly understood. The present study was investigated the effects of solvent extracts from *A. altissima* on cell growth and apoptosis on A549 human lung cancer cells. A treatment of *A. altissima* inhibited the growth of A549 cells in a dose-dependent manner by inducing apoptosis. Especially, the chloroform fraction showed the highest anti-cancer effect among five kinds of fractions. And also, induction of apoptosis by chloroform fraction were associated with down-regulation of Bcl-2, and up-regulation of pro-apoptotic Bax expression. From these results, *A. altissima* may have a therapeutic potential in human lung cancer cells and as a functional food.

**Key words** - *Ailanthus altissima*, Lung cancer, Apoptosis, Bax, Bcl-2

**I. INTRODUCTION**

Apoptosis, an important process in cell development and maintenance of tissue homeostasis, plays an essential role as a protective mechanism against carcinogenesis by eliminating damaged cells or abnormal excess cells (Kaufmann & Hengartner, 2001; Jin & El-Deiry, 2005). The relationship between apoptosis and cancer has been a recent focus. Apoptosis provides a number of useful clues upon developing effective therapies, and many chemotherapeutic agents exert their anticancer effects by inducing apoptosis in cancer cells (Jemal *et al.*, 2011). Therefore, induction of apoptosis has become a principal mechanism by which anticancer therapy is effective. Recent scientific efforts have focused on the potential roles of extracts of traditional herbs as alternative and complementary medications for cancer treatment.

*Ailanthus altissima* (Simaroubaceae) well-known 'tree-of-heaven', is used in Chinese traditional medicine. It has been used to treat cold and gastric diseases and anti-proliferation (Kim *et al.*, 1994), anti-inflammatory activity (Jin *et al.*, 2006), central nervous system depressants (Crespi-Perellino *et al.*, 1988), plant growth regulation and insecticidal effects (Pascual-villalobos & Robledo, 1998).

In several studies on *A. altissima* extracts, phenolic compounds, flavonoid compounds, merosin, tannin phlobaphen, ailanthone, amarolide, acetylamarolide were identified (Kazuya *et al.*, 1994; Barakat, 1998). The phenolic compounds are primarily composed of 3,4,5-trimethoxyphenol, p-coumaric acid, vanillin, vanillic acid. The flavonoid compounds are primarily composed

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of 5,7-dihydroxycromone-7-neohesperidoside, naringin. And these components have been reported their biological activities such as antimicrobial effect (Lee et al., 2002), cell cycle regulation (Hwang et al., 2002), improvement of liver function (Kim et al., 1994) and acute lymphocytic leukemia (Kim & Lee, 1997). Although many biological activities of A. altissima were reported, apoptosis induction of A. altissima on human cancer cell has not been reported. Thus, this study investigated how A. altissima fractions affects cell growth and apoptosis of A549 human lung cancer cell. The anticancer mechanism of A. altissima fractions was also elucidated by analyzing expressions of apoptosis-related molecules, including Bax and Bcl-2 gene.

II. Materials and Methods

2.1 Reagents

The reagent 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dulbecco’s Modified Eagle’s Medium (DMEM), RPMI1640 medium, fetal bovine serum (FBS), phosphate-buffered saline (PBS), penicillin-streptomycin and trypsin-EDTA were obtained from Invitrogen Life Technologies Inc. (Carlsbad, CA, USA). RNeasy plus mini kit and SYBR Green mix were purchased from Qiagen (Valencia, CA, USA). Other reagents were used of analytical grade.

2.2 Sample Preparation and Fractionation

The Korean wild edible vegetable, A. altissima was purchased from the Plant Extract Bank (Dae-jeon, Korea). The dried A. altissima was milled into powder of 40 mesh particle size and extracted with 70% ethanol by stirring for 24 hr at room temperature. The extract was filtered, and the residue was extracted in duplicate, under the same conditions. Subsequently, the filtrates were combined and evaporated under vacuum (EYELA N-1000, Tokyo Riakikai Co., Ltd. Japan) and then lyophilised with a Bondro Lyophpride freeze dryer (Ishine Lab Co. Ltd., Korea) at -70°C under reduced pressure (< 20 Pa). The dried ethanol extracts were then suspended in water (500 ml) and further fractionated, by additional extraction with n-hexane, chloroform, and n-butanol in a stepwise manner (Fig. 1.). Each fraction powder was dissolved in DMSO and diluted with PBS (pH 7.4) to the desired final concentration. And filtered through a 0.45 μm syringe filter (Advanced MFS. Inc., Dublin, CA, USA) before use.

![Fig. 1. Procedure of extract and fraction layers of Ailanthus altissima.](image-url)