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Original Research

Induction of reactive oxygen species–mediated apoptosis by purified Schisandrae semen essential oil in human leukemia U937 cells through activation of the caspase cascades and nuclear relocation of mitochondrial apoptogenic factors

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ABSTRACT

The aim of this study was to evaluate the beneficial effects of Schisandrae semen essential oil (SSeo) on apoptosis events and the mechanisms associated with these effects in human leukemia U937 cells. The treatment of U937 cells with SSeo significantly inhibited survival and induced apoptosis. Schisandrae semen essential oil treatment increased the levels of death receptors and Fas, and activated caspases accompanied by proteolytic degradation of poly(ADP-ribose)-polymerase, which was associated with the downregulation of members of the inhibitor of apoptosis protein family protein expression; however, a pan-caspase inhibitor reversed SSeo-induced apoptosis. Treating the cells with SSeo also caused truncation of Bid, translocation of proapoptotic Bax to the mitochondria, and loss of mitochondrial membrane permeabilization, thereby inducing the release of cytochrome c into the cytosol. Subsequently, SSeo upregulated the translocation of mitochondrial

Abbreviations: AIF, apoptosis-inducing factor; ANOVA, analysis of variance; COX IV, cytochrome oxidase IV; DAPI, 4,6-diamidino-2-phenyllindile; DCFH-DA, 2,7-Dichlorodihydrofluorescein diacetate; DR, death receptor; EndoG, endonuclease G; IAP, inhibitor of the apoptosis proteins; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide; MMP, mitochondrial membrane potential; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; NAC, *N*-acetylcysteine; PARP, poly(ADP-ribose)-polymerase; PBS, phosphate-buffered saline; PI, propidium iodide; pNA, *p*-nitroaniline; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; SSeo, Schisandrae semen essential oil; tBid, truncated Bid; z-VAD-fmk, z-Val-Ala-Asp-fluoromethyl ketone.

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apoptogenic factors, such as endonuclease G and apoptosis-inducing factor, into the nucleus during the apoptotic process. Notably, SSeo immediately increased the generation of intracellular reactive oxygen species (ROS); however, pretreatment with *N*-acetylcysteine, a common ROS quencher, almost completely blocked SSeo-induced apoptosis. Taken together, these findings indicate that SSeo caused ROS- and caspase-dependent cell death involving mitochondrial dysfunction and nuclear translocation of mitochondrial proapoptosis proteins. Based on our data, the consumption of Schisandrae semen or its essential oil is a good natural therapeutic agent for anticancer activity and regression.

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1. Introduction

Leukemia is a cancer of the blood and bone marrow that is characterized by uncontrolled proliferation of immature white blood cells. Leukemia cells are unable to undergo growth arrest, terminal differentiation, and apoptosis in response to appropriate environmental stimuli and disseminate from the bone marrow into peripheral tissues [1,2]. Despite recent advances, this disease has an extremely poor prognosis; and treatment for leukemia has generally consisted of a combination of cytarabine and an anthracycline [3–5]. However, the toxicity of these drugs in patients limits their dosage; and recurrences of leukemia are frequent [6,7]. Therefore, efforts have been made to identify new therapeutic strategies to overcome resistance and identify treatments that possess apoptotic potential.

Apoptosis, which is characterized by a series of morphological changes involving cell shrinkage and chromatin condensation, is a physiological mechanism for elimination of malignant or cancer cells without eliciting damage to normal cells or surrounding tissues. Therefore, the induction of apoptosis in target cells is the best strategy for anticancer therapy. In general, 2 signaling pathways are involved in apoptosis: the intrinsic mitochondrial-mediated pathway and the extrinsic death receptor (DR)–mediated pathway, both of which involve multiple steps and complex interactions of molecular events. The intrinsic pathway involves disruption of the mitochondrial membrane and the release of cytochrome *c* into the cytosol, thereby activating caspase-9 [8,9]. The extrinsic pathway is initiated through interaction with DRs and their corresponding ligands to promote caspase-8 activation [10,11]. Activated caspase-8 induces cleavage of cytosolic Bid to truncated Bid (tBid), which induces proapoptotic protein Bax oligomerization and interaction of the mitochondrial permeability transition pore, leading to the loss of mitochondrial membrane potential (MMP) and release of cytochrome c[10,12,13]. In the final stage of apoptosis, both the intrinsic and extrinsic pathways induce the activation of executioner caspases such as caspase-3 and -7, which results in cell apoptosis. In addition, the caspase-independent pathway occurs in the mitochondria, leading to the translocation of mitochondrial apoptogenic factors such as endonuclease G (EndoG) and apoptosis-inducing factor (AIF) from the mitochondria to the nucleus. Nuclear relocation of EndoG and AIF induces DNA fragmentation and apoptotic cell death. However, the release of EndoG and AIF from the mitochondria in response to proapoptotic stimuli also occurs in a caspase-dependent manner [14–16]. Moreover, previous studies have shown that reactive oxygen species (ROS) and disruption of the MMP contribute to apoptosis induced by chemotherapeutic agents [17,18].

Bioactive compounds in plant sources have been recognized as effective agents for preserving human well-being by preventing or even treating several pathological conditions. Among plant-derived natural products, essential oils, which are concentrated hydrophobic liquids containing volatile aroma compounds from plants, have recently drawn scientific interest for their medicinal applications [19–22]. Essential oils are endowed with various pharmacological effects, including antibacterial [23], antioxidant [24], antifungal [25], and antimutagenic [26]; and their assorted therapeutic potential has attracted the attention of many researchers to explore their anticancer activities [27–30].

Schisandrae semen (*Schisandra chinensis* [Turcz.] Baill) is a medicinal herb that is widely used for treating various inflammatory and immune diseases in East Asian countries [31,32]. The pharmacological activities of the main chemical components, including antioxidant and antimicrobial activities, have been found in the essential oil [33–36]. Nevertheless, the biological activities and underlying molecular mechanisms of the potential anticancer effects of Schisandrae semen essential oil (SSeo) remain unclear. Therefore, we examined the molecular mechanisms of SSeo underlying this phenomenon as a trial to develop a novel functional anticancer food. Although SSeo is a potent antioxidant, the current results showed that SSeo-induced apoptosis is mediated by generating intracellular ROS and triggering mitochondria-dependent caspase activity.

2. Methods and materials

2.1. *Preparation of SSeo*

Reddish brown, clear SSeo was prepared by Dr KY Park (The Medical Research Center for Globalization of Herbal Formulation, College of Korean Medicine, Daegu Haany University, Gyeongsan, Republic of Korea) as follows. Briefly, dried seeds of Schisandrae semen (Turcz.) Baillon were collected around Mungyeong-city (Gyeongbuk, Republic of Korea) and then were completely dried at 180°C in a furnace (Daihan Scientific Co, Seoul, Republic of Korea), pulverized, and lyophilized in a programmable freeze dryer (Freezone 1; Labconco Co, Kansas, MO, USA). The lyophilized material was extracted with 100% ethanol at room temperature for 24 hours, filtered, and then concentrated using a rotary vacuum evaporator (Buchi Rotavapor R-144; BÜCHI Labortechnik, Flawil, Switzerland). Finally, the SSeo was isolated by hydrodistillation using a Clevenger-type apparatus for 3 hours according to a method

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