Aerial Parts and Roots of *Pulsatilla koreana* Affect the Viability of HSC-T6 Hepatic Stellate Cells

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**Abstract** – During liver fibrosis, hepatic stellate cells (HSCs) undergo a complex activation process characterized by increased proliferation and extracellular matrix deposition, which is the major pathological feature of hepatic cirrhosis. Therefore, suppression of HSC's activation has been proposed as therapeutic strategies for hepatic fibrosis. We tried to screen the antifibrotic activity of natural products employing HSC-T6, hepatic stellate cell lines as an *in vitro* assay system. In the present study, we investigated the antiproliferative activity of aerial parts and roots of *Pulsatilla koreana* Nakai (Ranunculaceae). Our present study shows that roots of *P. koreana* exerted more strong inhibitory activity compared to its aerial parts. In addition, among the fractions of the aqueous methanolic extract of *P. koreana* roots, both n-hexane and CHCl₃ fraction showed the strong inhibitory activity on HSC proliferation. Further study also demonstrated that the n-hexane and CHCl₃ fraction of *P. koreana* roots significantly inhibited the HSC proliferation in time- and concentration-related manners.

**Keywords** – *Pulsatilla koreana*, Ranunculaceae, antifibrotic, HSC-T6, hepatic stellate cells, viability

**Introduction**

Liver fibrosis is a wound-healing response to various chronic liver injuries caused by toxic, infectious or metabolic agents. An early event in the development of hepatic fibrosis is the activation of hepatic stellate cells (HSCs). HSCs play important functions in normal liver, such as retinoid storage, remodeling of ECM (extracellular matrix) and production of growth factors. During liver fibrosis, HSCs undergo a complex activation process characterized by increased proliferation and extracellular matrix deposition, which is the major pathological feature of hepatic cirrhosis (Li and Friedman, 1999; Tsukada et al., 2006). Therefore, suppression of HSC activation and proliferation, and induction of apoptosis in activated HSCs have been proposed as therapeutic strategies for the treatment and prevention of hepatic fibrosis (Wu and Zern, 2000; Bataller and Brenner, 2005).

Though severe problems in health, no effective anti-fibrogenic therapy is available for the treatment of fibrosis in chronic liver diseases. Recently, there is a growing interest in searching for anti-fibrotic compounds from natural products with lower adverse effects.

*Pulsatilla koreana* Nakai belongs to the family Ranunculaceae and is an endemic species in Korea. Previous phytochemical studies of *Pulsatilla* species have reported the isolation of diverse triterpenoids and saponins, and their antitumor activity (Mimaki et al., 1999; Bang et al., 2005a, 2005b). Interestingly, various parts of *P. koreana* have been used to treat different diseases in traditional medicine. For example, the roots of *P. koreana* have been used for blood-cooling and detoxifying effects. The flowers of *P. koreana* have been used for the treatment of smallpox and leaves for edema (Bae, 2000). Natural products have been used as different parts considering diverse conditions such as toxicity, efficacy, yield and etc. Recently, the constituents and biological activity of each part of same plants are reported to be somewhat different in many cases (Kim et al., 2008). Therefore, we investigated and compared the antifibrotic activity of aerial parts and roots of *P. koreana*, employing...
HSC-T6, a rat hepatic stellate cell line as an in vitro assay system. We have also attempted to evaluate antifibrotic activity of *P. koreana*.

**Methods and materials**

**Plant material**—The aerial parts and roots of *P. koreana* were collected from Gyunggi province, Korea in April 2008. They were identified by the herbarium of College of Pharmacy at Chungbuk National University, where a voucher specimen was deposited (CBNU200804-PK).

**Extraction and isolation**—The dried roots of *P. koreana* (200 g) were extracted 3 times with 80% MeOH, which yielded the methanolic extract (46.1 g). The methanolic extract was then suspended in H$_2$O and partitioned successively with *n*-hexane, CHCl$_3$, EtOAc and *n*-BuOH to yield *n*-hexane (4.6 g), CHCl$_3$ (0.7 g), EtOAc (1.2 g), *n*-BuOH (18.0 g) and aqueous fraction, respectively. The dried aerial parts of *P. koreana* (150 g) were extracted and partitioned as described above, which yielded the methanolic extract (15.9 g), *n*-hexane (0.8 g), CHCl$_3$ (0.3 g), EtOAc (0.4 g), *n*-BuOH (1.5 g) and aqueous fraction, respectively.

**Culture of HSC-T6 hepatic stellate cells**—An immortalized rat hepatic stellate cell line, HSC-T6 was kindly provided by Prof. SL Friedman (Columbia University, New York). HSC-T6 cells were maintained in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/ml penicillin and 100 µg/ml streptomycin at 37 °C in a humidified atmosphere of 95% air-5% CO$_2$.

**Measurement of cell viability**—Samples to be tested were dissolved in dimethyl sulfoxide (DMSO). Our preliminary study showed that DMSO at a final concentration of 0.1% in media did not affect the cell viability. HSC-T6 cells were treated with vehicle or samples to be tested for 48 hr or as indicated. Cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. HSC-T6 cells were incubated with 0.5 mg/mL of MTT in the last 2 hr of the culture period tested. Reduction of MTT to formazan was assessed in an ELISA plate reader.

**Statistical analysis**—The evaluation of statistical significance was determined by the Student’s *t*-test with a value of *p* < 0.05 or less considered to be statistically significant.

**Results and discussion**

Although liver fibrosis has been regarded as irreversible, recent studies in animal models and patients have suggested that hepatic fibrosis is, at least to some degree, a reversible process (Iredale *et al.*, 1998; Issa *et al.*, 2004). During fibrosis, HSCs undergo a complex activation process characterized by increased proliferation and extracellular matrix deposition, which is the major pathological feature of hepatic cirrhosis (Li and Friedman, 1999; Tsukada *et al.*, 2006). Elimination of the activated HSCs has been linked to the reversal of liver fibrosis and treatments that induced HSC apoptosis and/or reduced proliferation are currently under investigation as the potential treatment for liver fibrosis. Recently, there is a growing interest in searching for antifibrotic compounds, especially from natural products. We have tried to search for antifibrotic compounds from natural resources.