Ⅱ-33

Gomisin N isolated from *Schisandra chinensis* was significantly induced anti-proliferative and pro-apoptotic effects in the hepatic carcinoma

[1)]Dept. of Horticultural Bioscience, [2)]Dept. of Biomaterial Science, Pusan National University, Miryang 627-706, Korea

Young Whan Choi[1*], You Jin Lee[1], Da Jung Park[1], Su Hyeong Park[1], and Dae Youn Hwang[2]

Objectives

To investigate the new function of compounds isolated from *Schisandria chinensis* on therapy of hepatic carcinoma, the killing ability of lignans were screened with cell proliferation assay.

Materials and Methods

Extraction and structure elucidation of compounds: The dried fruits of *Schisandra chinensis* (2.5kg) were ground to a fine powder and were successively extracted at room temperature with *n*-hexane, EtOAc, and MeOH. The hexane extract (308 g) was evaporated in vacuo and chromatographed on a silica gel (40 μm, J.T. Baker, NJ, USA) column (70x8.0 cm) with a step gradient 0,5%, 10%, 20%, 30% EtOAc in hexane (each 1 L) (Choi et al. 2006). The fractions were separated on a silicagel column (100x3.0 cm) to give Gomisin N (774mg), Schisandrin C (501 mg), Gomisin A (97 3mg) and Schisandrin (4,606 mg). Chemical structure of lignans was verified by LC-MS (Bruker BioApex FT mass spectrometer) and NMR analysis (Varian inova 500 spectrometer).

Cell culture and treatment: HepG2 cell, a human hepatoblastoma cell line, was purchased from Korean Cell Line Bank (Seoul, Korea).

Assay of cell proliferation: Firstly, the wells in 96-well plate were divided into four groups involving Vehichel, 40 uM, 80 uM, 160 uM and 320 uM and assign three well per each group. HepG2 cells were seeded at density of 4 x 10^4 cell/200 ul in 96-wel lplate and grown for 24 hrs at 37℃ incubator. Cell proliferation were evaluated using an tetrazolium compound MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide, Sigma M2128).

Flow cytometric analysis: The percentage of cell undergoing apoptosis process and dead cells was detected with staining methods of FITC Annexin V (BD Bioscience,

Corresponding author : Young-Whan Choi E-mail : ywchoi@pusan.ac.kr Tel : 055-350-5522

USA). HepG2 cells were seeded at density of 2 x 10^6 cells in 100 mm^2 dish and grown for 24-48 hrs at 37℃ incubator.

Western blot analyses: The HepG2 cell harvested from 100 mm^2 culture dish. The membranes were then incubated with the primary antibodies [anti-Bcl-2 (SC-7382), anti-Bax (SC-493), anti-p53 (SC-6243) and anti-α-tubulin (Sigma, MI)], which were used to detect Bcl-2, Bax, p53 and anti-α-tubulin. Each antigen-antibody complex was then visualized with a biotinylated secondary antibody (goat anti-rabbit)-conjugated HRP streptavidin (Zymed, Histostain-Plus Kit) diluted to 1:1,500 in PBS.

Results

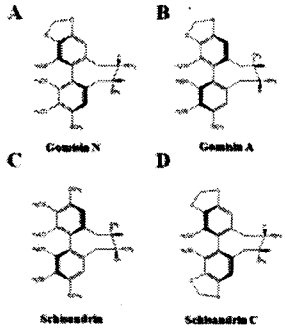

Fig. 1. Chemical structures of gomisin N (A), gomisin A (B), schisandrin (C), and schisandrin C (D) isolated from *Schisandra chinensis*.

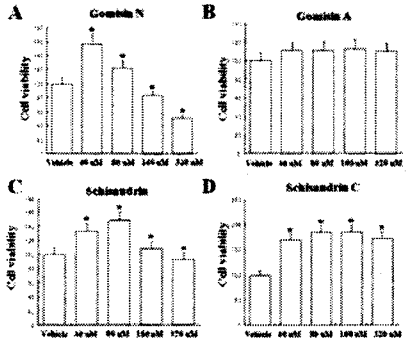

Fig. 2. Atin-proliferative effect of four compounds on HepG2 cell. Cells were cultured with four compounds at various concentrations for 24 hrs.

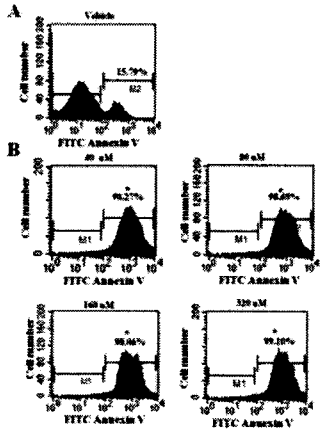

Fig. 3. Identifying cells that are undergoing apoptosis. HepG2 cell were incubated with Gomisin N at various concentrations for 24 hrs, and stained with FITC Annexin V to detect the apoptotic cells.

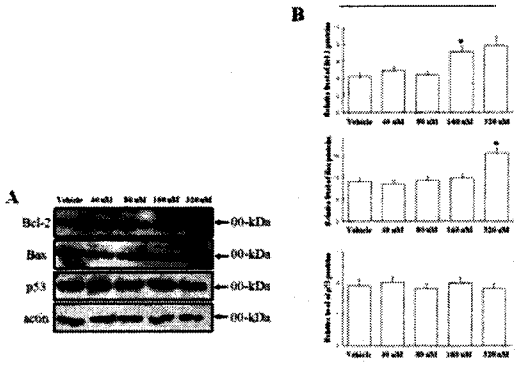

Fig. 4. Effects of Gomisin N on apoptotic pathway and the expression level of tumor suppressor gene.Expression levels of the Bcl-2, Bax and p53 proteins were analyzed using Western blot analysis.