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Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death globally, behind lung and stomach cancers and is increasing more than 626,000 new cases per year in the world (Rebecca et al., 2013). As one of the most common cancer prevalent in Asia and Africa, HCC is diagnosed in 30 to 40% of all patients at early stages and is amenable to potentially curative treatments, such as surgery, liver transplantation etc. Five-year survival rates of up to 60 to 70% can be achieved in well-selected patients. However, disease diagnosed at an advanced stage or with progression after locoregional therapy has a dismal prognosis, owing to the underlying liver disease and lack of effective treatment option (Ahn et al., 2011).

Recent studies have been carried out to find cancer chemo-preventive and/or chemo-therapeutic agents from edible and natural resources such as fruits, vegetables, and terrestrial plants. Some studies have reported that natural products have positive effects against cancer compared with chemotherapy. Therefore, many vegetables, fruits and medicinal herbs have been examined to identify new and effective anticancer compound (Kim et al., 2012). Most recently, pharmaceutical companies have started their search for new drugs from marine organisms including seaweeds. Seaweeds are one of the natural resources in the marine ecosystem. It contains various biologically active compounds which have been used as source of food, feed and medicine (Senthilkumar et al., 2013). The marine algae product fucoxanthin exerts anti-cancer potential (Muthuirulappan and Francis, 2013). Recent findings evidenced that seaweeds contained antiviral (Plouguerné et al., 2013), antibacterial (Manivannan et al., 2011) and antifungal (Li et al., 2006) potentials. Therefore, we selected Saccharina japonica which belongs to family of Phaeophyceae (brown algae) and extensively cultivated in China, Japan and Korea, to analyze its effect against hepatocellular carcinoma.

Marine brown algae contain several compounds with biological activities such as polysaccharides, iodine organic products, mannitol, macro- and micro elements, vitamins, unsaturated fatty acids, and other biogenic compounds. In the previous studies, researchers have found that brown algae and its extracts inhibited the proliferation of breast and prostate tumor cells, lung metastases, and leukemia in animal models (Ohigashi et al, 1992; Itoh et al., 1995; Funahashi et al., 1999; Jo et al., 2012). Funahashi et al. (1999) have shown that wakame extracts have a potent inhibitory effect on the progression...
of mouse mammary tumors. Similar extracts produced an equally profound apoptotic effect on breast cancer cells in vitro while the extracts were nontoxic to normal breast cells. When brown seaweed was included in the animals’ diet, it was very clear that there was an anticancer effect in ingestion although the active components have not been determined (Fitzon, 2003).

The apoptosis is a process of cell death that was originally described by its morphological characteristics including cell shrinkage and chromatin condensation (Khan et al., 2013). Apoptosis is essential for normal development and homeostasis in multicellular organisms and also serves as a defense mechanism to eliminate harmful cells, such as tumor cells and cells infected by viruses (Jacobson et al., 1997). It has shown that mitochondria play essential roles in apoptosis (Boland et al., 2013). Cytochrome c, an essential component of the respiratory chain of the mitochondria, is released in response to various apoptotic stimuli (Bossy-Wetzel et al., 1998; Allan and Clarke, 2009), and binds to apoptotic protease activating factor 1 (Apaf 1), leading to the formation of apoposome. Apoptosome then proteolytically activates caspase-9, and the activated caspase-9 cleaves the downstream caspases including caspase-3, 6, and 7, bringing about apoptotic cell death by digesting essential cellular proteins (Würtzle et al., 2012; Hu et al., 2013). On the other hand, mammalian cells in a certain circumstance can undergo caspase-independent apoptosis that is mediated by the disruption of the mitochondrial membrane potential and the translocation of AIF and endonuclease G (Endo G) to nucleus where they induce chromatin condensation and/or large-scale DNA fragmentation (Cregan et al., 2004; Sevioukova, 2011).

The ER is a continuous membrane system that consists of multiple domains that perform different functions (Wang et al., 2014). These include translocation of secretory proteins across the ER membrane, integration of proteins into the membrane, folding and modification of proteins in the ER lumen, synthesis of phospholipids and steroids, detoxification, storage of calcium ions in the ER lumen and their release in the cytosol as well as segregation of nuclear contents from the cytoplasm (Voeltz et al., 2002).

Proper function of the ER is essential to cell survival and any perturbation of its function induces cellular damage and results in apoptosis. Various conditions can disturb ER functions, events collectively termed “ER-stress”. These stresses include inhibition of protein glycosylation, reduction of formation of disulfide bonds, calcium depletion from the ER lumen, impairment of protein transport from the ER to the Golgi, expression of mis-folded proteins, etc. The ER is regulated by signaling pathways that respond to accumulation of unfolded or mis-folded proteins in the organelle. To survive and adapt under ER-stress conditions, cells have a self-protective mechanism against ER-stress, which has been termed the ER-stress response (Kaufman, 1999).

To combat the deleterious effects of ER-stress, cells have evolved various protective strategies, collectively termed the unfolded protein response (UPR). This concerted and complex cellular response is mediated through three ER transmembrane receptors: pancreatic ER kinase (PKR)-like ER kinase (PERK), activating transcription factor 6 (ATF6) and inositol-requiring enzyme 1 (IRE1) (Oyadomari et al., 2002; Wang et al., 2014). At least four functionally distinct responses have been identified. The first one involves up-regulation of the genes encoding ER chaperone proteins to increase protein folding activity and to prevent protein aggregation. The second consists of translational attenuation to reduce the load of new protein synthesis and to prevent further accumulation of unfolded proteins. The third is degradation of proteins mis-folded in the ER and this is called ER-associated degradation (ERAD). The fourth is apoptosis which occurs when functions of the ER are extensively impaired (Oyadomari et al., 2002).

In this study, we evaluated the potential anti-cancer activities of S. japonica n-hexane extract in an aspect of apoptosis accompanied by cell cycle arrest and induction of ER-stress in SK-Hep1 hepatocellular carcinoma cells.

Materials and Methods
Seaweed material
S. japonica was harvested from Kijang aquaculture farm in Korea on May 201. The samples were dried in cold-air drier (60°C) for 40h and ground with a hammer mill. The dried powder was stored at -20°C until used.

Extraction and fractionation
The dried powder (2 kg) of S. japonica was refluxed with ethyl alcohol (95%, v/v) for 3h. The extract (446.0 g) was suspended in H2O:ethyl alcohol (9:1, v/v) and partitioned with n-hexane, dichloromethane, ethyl acetate (EtOAc), n-butanol (n-BuOH), and water in sequence, yielding the n-hexane (135.5 g), dichloromethane (18.1 g), EtOAc (39.6 g), n-BuOH (55 g), and water (162.8 g) fractions. The n-hexane fraction was subjected to preparative size exclusion column of Shim-pack PREP-ODS (500×21.2 mm, Shimadzu Co., Tokyo, Japan). An exclusion HPLC apparatus consisted of a pump (Shimadzu LC-6AD), a photodiode array detector (Shimadzu SPD-M20A), an online degasser (Shimadzu DUG-20A3), an auto sampler (SIL-20A), a fraction collector (Shimadzu FRC-10A), a system controller (CBM-20A), and a Shimadzu LC solution (ver. 1.22sp). The n-hexane fraction was chromatographed on a Shim-pack PREP-ODS column eluting with methanol at a flow rate of 5.0 ml/min and monitored at 240 nm. The fraction was separated into four fractions (GS1–GS4). The GS3 fraction was chromatographed over Phenomenex C18-ODS (Phenomenex Co., Tokyo, Japan). A preparative ODS HPLC system was similar to the exclusion HPLC system except for a binary pump (Phenomenex LC-6AD) and a column oven (Phenomenex CTO-20A). The separation of GS3 fraction was conducted using mobile phase water (solvent A) and methanol (solvent B). The elution profile consisted of a linear gradient from 20 to 70% solvent B for 90 min. The flow rate was 7.0 ml/min, and detection was performed at 216 nm. Fifteen subfractions (GS3-ODS1–GS3-ODS15) were tested for cell
viability and GS3-ODS2 sub-fraction is selected for the studies.

Cell culture
All of the cell lines were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA). Human hepatocellular carcinoma SK-Hep1 cells were maintained in Minimum Essential Medium with Earle’s balanced salts (MEM/EBSS) (HyClone, Logan, UT, USA) supplemented with 10% heat-inactivated fetal bovine serum (HyClone), and 1% penicillin-streptomycin (PAA Laboratories GmbH, Pasching, Austria) at 37°C in humidified atmosphere at 95% air and 5% CO₂. The human embryonic kidney HEK293 cells were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) (HyClone) supplemented with 10% heat-inactivated fetal bovine serum, and 1% penicillin-streptomycin at 37°C in humidified atmosphere at 95% air and 5% CO₂. THLE-3 human normal liver cells were cultured in a flask coated with 0.01 mg/ml fibronectin, 0.03 mg/ml bovine collagen type 1 and 0.01 mg/ml bovine serum albumin for 24h and cultivated in Bronchial Epithelial Cell Basal Medium (BEBM) with BEGM SingleQuot kit except GA-1000 and Epinephrine (Lonza Group Ltd., Basel, Switzerland). 10% heat inactivated fetal bovine serum (Lonza Group Ltd.) and 1% penicillin-streptomycin (PAA Laboratories) at 37°C in a humidified atmosphere of 5% CO₂.

Cell viability assay
N-hexane fraction of S. japonica was dissolved in dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO, USA). The final concentration of DMSO in the culture medium was not exceeded 0.04% (v/v), and the same concentration of DMSO was added to the control dishes. For the cell viability assay, 1x10⁴ cells were re-suspended in 100 μl medium and seeded onto each well of a 96-well plate. The cells were then treated with 5, 10, 15 and 20 μg/ml of the fraction and were incubated for 24h. After the treatment, 10 μl of EZ-Cytox Cell Viability Assay Solution WST-1® (Daeil Lab Service, Seoul, Korea) was added onto each well and the cells were further incubated for 3h and then read the absorbance at 460 nm with ELISA reader (Molecular Devices, Sunnyvale, CA, USA).

Cell viability with caspase inhibitor
To compare the caspase-dependent and caspase-independent cell deaths, cells were treated as follows control, Z-VAD-FMK (20 μM), sample (10, 15, 20 μg/ml) and sample (10, 15, 20 μg/ml) containing Z-VAD-FMK (20 μM) group. After 24h, cells were changed to a fresh medium. After incubation, 10 μl of WST-1 solution was added to each well and further incubated for 3h. The absorbance of supernatant was determined at 460 nm using VersaMax microplate reader (Molecular Devices).

Western blot analysis
In order to identify the changes of protein expression levels by the treatment of n-hexane extract, SK-Hep1 cells were pre-incubated in 10% FBS/MEM for 48h. The medium was then replaced with the medium containing different dose of n-hexane extract followed by further incubation for 24h. To investigate the levels of protein expression at different treatment time, SK-Hep1 cells were cultured and treated with 20 μg/ml S. japonica n-hexane extract for 2, 4, 6, or 12h. The harvested cells were lysed in ice-cold lysis buffer [50 mM Tris-Cl (pH 7.5), 150 mM NaCl, 1 mM DTT, 0.5% NP-40, 1% Triton X-100, 1% Deoxycholate, 0.1% SDS and cocktail of protease inhibitors (Intron biotechnology, Gyeonggi, Korea)]. After incubation on ice for 30 min, the insoluble materials were removed by centrifugation at 14,000 rpm for 20 min at 4°C. The protein content of the cell lysates were determined by a Protein Quantification Kit (CBB solution®) (Dylight Molecular Technologies, Rockville, MD, USA) with bovine serum albumin (BSA) as standard. Each sample in Laemmli buffer was boiled for 5min, and then resolved by 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were electrotransferred onto a nitrocellulose membrane (PALL Life Sciences, Pensacola, MI, USA) and blocked in PBST buffer (135 mM NaCl, 2.7 mM KCl, 4.3 mM NaPO₄, 1.4 mM KH₂PO₄, 0.5% Tween-20) containing 5% skim milk. After blocking, the membranes were probed with primary antibodies (Cell Signaling Technology Inc., Danver, MA, USA) and then washed three times with PBST buffer followed by incubation for 1h with horseradish peroxidase-conjugated anti-rabbit IgG or anti-mouse IgG as secondary antibodies (Cell Signaling Technology Inc.). The blots were then washed in PBST buffer and visualized by enhanced chemiluminescent (ECL) detection solution (Pierce, Rockford, IL, USA).

Detection of intracellular Ca²⁺
SK-Hep1 cells were seeded in a 35x10 mm coverglass bottom dish (SPL life sciences, Gyeonggi, Korea) and cultured at 37°C. After 24h, the cells were treated with 20 μg/ml S. japonica n-hexane extract for 0, 1, 2, 3 and 4h. Then the cells were incubated with 1.5 μMFluo-3AM (Invitrogen, Eugene, OR, USA) at room temperature for 30 min in dark. These cells on the slides were mounted in Prolong Gold Antifade Reagent (Invitrogen) followed by observation under a Nikon ECLIPS 50i microscope equipped with charged-coupled device (CCD) camera (Nikon, Tokyo, Japan). The fluorescence intensity indicating the concentration of Ca²⁺ was captured and processed with High-Content Analysis Software (Cambridge Healthtech Institute, Needham, MA USA).

FACS analysis
SK-Hep1 cells were treated with different concentrations of S. japonica n-hexane extract for 24h. The cells were harvested by trypsinization, then washed with PBS and fixed in 70% ethanol at 4°C for overnight. Cells were stained with 40 μg/ml propidium iodide for 30 min and analyzed using a FACS Calibur apparatus (Becton Dickinson, Mountain View, CA, USA).

Immunofluorescence of cleaved caspase-3 protein
The cells were cultured on coverglass bottom dishes (SPL lifesiences) for 24h and fixed with 4% formaldehyde (Sigma) for 15 min at room temperature and then blocked for 1h in 5% mouse and rabbit normal serum
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Fixed and blocked cells were incubated with primary antibodies (cleaved caspase-3 and β-actin) (Cell Signaling Technology Inc.) for 2h and then with 0.1 μg/ml of anti-mouse IgG (H+L), F (ab') 2 fragment (Alexa Fluor® 555 Conjugate) and anti-rabbit IgG (H+L), F (ab') 2 fragment (Alexa Fluor® 488 Conjugate) (Cell Signaling Technology Inc.) for 1h. Stained cells on the slides were mounted in Prolong Antifade Reagent (Invitrogen) and observed in fluorescence microscope Nikon ECLIPS 50i microscope equipped with charged-coupled device (CCD) camera. Images were captured and processed with High-Content Analysis Software (Cambridge Healthtech Institute).

TUNEL assay

For in situ detection of apoptotic cells, the terminal deoxynucleotidyl transferase-mediated deoxy-uridine triphosphate (dUTP) nick-end labeling (Apoptag® Plus In situ Apoptosis Fluorescein Detection Kit) (Millipore, Billerica, MA, USA) was used to detect the DNA fragmentation. Cells were cultured on coverglass bottom dishes (SPL lifesciences) in MEM medium (Hyclone) containing 10% FBS and penicillin-streptomycin (PAA Laboratories) for 24h then fixed in 1% paraformaldehyde (pH 7.4) for 10 min at room temperature. The fixed cells were then incubated with cold ethanol and acetic acid mixture for 5 min at -20°C and then washed twice with ice cold PBST (135 mM NaCl, 2.7 mM KCl, 4.3 mM NaPO₄, 1.4 mM KH₂PO₄, 0.5% Tween-20). After that, the cells were incubated with terminal deoxynucleotidyl transferase (TdT) for 1h at a humidified atmosphere and were immersed in stop/wash buffer for 10 min at room temperature. The cells were then incubated with antidigoxigenin conjugate solution for 30 min in a humidified chamber to avoid exposure to light. For the counterstain, a mounting medium containing 0.5 μg/ml of propidium iodide was applied.

Statistical analysis

The GraphPad Prism 5.0 for Window was used to determine the statistical significance of the differences between the values of various experimental and control groups. Determinations were performed in triplicates and the results were presented as means±S.E.M. In cases where there was no error bar seen in the graph, it means the variation is small and thus, the bar is hidden behind. ANOVA post hoc test and subsequently, Dunnett’s multiple comparison tests were used for statistical analysis.

Results

Cell death of SK-Hep1 by n-hexane extract of S. japonica

To determine whether the extract of S. japonica n-hexane exerts anticancer effects on the proliferation of SK-Hep1, THLE-3 and HEK 293 cell lines, both dose-dependent and time-dependent studies were conducted. The result of the cell viability assay showed that the n-hexane extract of S. japonica inhibited proliferation of SK-Hep1 cells in a dose-dependent manner. However, the same dose of S. japonica n-hexane extract exhibited less anti-proliferation effect on both THLE-3 and HEK 293 cells compared to SK-Hep1 cells (Figure 1B). The treated cells were shrunken and were floated on the medium as time increases (Figure 1A). The results confirm that the extract of S. japonica exert anticancer activity on SK-Hep1 with less effect on non-cancer THLE-3 and HEK 293 cells (Figure 1B).

Induction of caspases mediated apoptosis in the extract treated cells

As shown in Figure 2A, Western blot analysis revealed that n-hexane extract of S. japonica releases cytochrome c with the possible involvement of the increased expression of both Bax and Bak as an initial signal to induce apoptosis. Caspase-9 was activated by cytochrome c released from the mitochondria and the activated caspase-9 cleaves caspase-3. Cellular death receptors may also initiate caspase cascade by the activation of caspase-8. As cleaved caspase-8 was dramatically increased in a dose-dependent manner, induced expression levels of Bad and cleaved caspase-3 by the extract was detected in a time-dependent manner (Figure 2B). In addition, we observed a time dependent increase in the expression level of cleaved caspase-3 in SK-Hep1 cells using immunofluorescense (Figure 2C). Both Western blot analysis and immunofluorescence suggest that the S. japonica n-hexane extract induces not only mitochondria but also death receptor mediated apoptosis in SK-Hep1 cells.

Induced apoptosis is mediated by both caspase-dependent and independent pathways

In order to verify the involvement of caspases in S. japonica n-hexane extract induced cell death, we examined the cell viability with the treatment of Z-VAD-FMK, a caspase inhibitor. The cells were divided into two groups. One is treated with different concentration of the extract and the other is with a combination of both n-hexane extract and Z-VAD-FMK (20 μM). In SK-Hep1 cells, between 40% and 60% of cell death was induced by the treatment of 15 μg/ml n-hexane extract. Whereas, the combination of Z-VAD-FMK and

Figure 1. Morphological Changes and the Cell Viability by n-hexane Extract of S. japonica. After treatment of the extract, morphological changes of SK-Hep1 cells were examined at various times. Morphology of the cells was visualized (x100) using an inverted microscope (A). Cell viability was examined by WST-1 assay after treatment of 5~20 μg/ml S. japonica n-hexane extract on SK-Hep1, THLE-3 and HEK 293 cells for 24h (B). For the control, non-treated cells were used. The bars represent means±S.E.M. of three experiments (**p<0.001)
S. japonica n-hexane extract showed less effect on the viability of SK-Hep1 cells. There was no difference in non-treated and Z-VAD-FMK only treated samples. The results suggest that the extract partly enhances caspase-independent cell death (Figure 3A). Moreover, PARP cleavage and AIF activation were also demonstrated by Western blot analysis in a dose-dependent manner in SK-Hep1 cells (Figure 3B). Caspase-independent apoptosis is mediated by the disruption of the mitochondrial membrane potential and the translocation of AIF to nucleus where they induce chromatin condensation and/or large-scale DNA fragmentation. The results suggest that the increased expression of cleaved PARP and AIF reveals the partial involvement of caspase independent cell death in SK-Hep1 cells treated with S. japonica n-hexane extract.

**Induction of ER-stress and cell cycle arrest by S. japonica n-hexane extract**

In order to study the effect of S. japonica on ER-stress, we used fluo-3/AM to measure the relative Ca\(^{2+}\) concentration. Disruption of calcium homeostasis induces endoplasmic reticulum stress. As shown in Figure 4A, the density of fluorescence gradually increased in SK-Hep1 cells treated with S. japonica n-hexane extract in a time-dependent manner. The degree of inducted Ca\(^{2+}\) density was getting increased at 2h of treatment. The expression levels of ER-stress-associated proteins were investigated by Western blot analysis. The obtained data demonstrated that time dependent expression of ER-stress associated proteins Bip, TRAF2, phospho-JNK, ATF6\(\alpha\), CHOP, Calnexin and phospho-eIF2\(\alpha\) (Figure 4B). CHOP proteins were delayed somewhat compared with the onset of the expression of ATF6\(\alpha\). This is consistent with the known placement of these proteins downstream of ATF6\(\alpha\). Additional evidence for activation of the ER-stress response is the induction of the ER chaperone protein BiP and calnexin. The phosphorylation of eIF2\(\alpha\) is a stress signal and is a negative regulator, thereby attenuating translation of most mRNAs while selectively increasing translation of the ATF4 (Donnelly et al., 2013). Also, expression of TRAF2 and phospho-JNK is increased for stress responses as shown Figure 4B. The observations indicate that S. japonica n-hexane extract triggers ER-stress.

On the other hand, S. japonica n-hexane extract induces cell cycle arrest. The results shown that when the cells were treated with 10, 15 and 20 µg/ml of S. japonica n-hexane extract for 24h, it leads to changes in cell cycle progression and the expression of cell cycle related proteins such as CDK2, phospho-Rb and phospho-Cdc2. As shown Figure 4C, down-regulation of CDK-2 resulted in the expression of phospho-Cdc2 following inhibition of CDK-2. Figure 4C, down-regulation of CDK-2 resulted in the expression of phospho-Cdc2 following inhibition.
Figure 4. ER-stress is Involved in n-hexane Extract Induced Apoptosis. Concentration of cellular Ca\(^{2+}\) was gradually increased in SK-Hep1 cells, treated with 20 µg/ml of *S. japonica* n-hexane extract (A). Western blot analysis of ER-stress related proteins in SK-Hep1 cells treated with 20 µg/ml of the extract for indicated time periods (h) (B). Cells were treated with the extract at 0, 10, 15 and 20 µg/ml for 24h. Expression levels of proteins in cell cycle were examined by Western blot analysis. Down-regulation of CDK-2 resulted in the expression of phospho-cdc2 following inhibition of phosphorylated Rb (C).

Figure 5. Induction of Apoptosis by *S. japonica* n-hexane Extract on SK-Hep1 Cells. Flow cytometry analysis for quantification of sub-G1 DNA content of SK-Hep1 cells treated with 15 and 20 µg/ml for 24h (A). Detection of nuclear DNA fragmentation of SK-Hep1 cells by *in situ* TUNEL assay (B).

Discussion

Apoptosis is the best characterized form of programmed cell death and it is the major strategy for the development of anti-cancer drugs. Mitochondria are one of the most susceptible organelles to apoptotic stimuli. In this study, we evaluated the potential anti-cancer activity of *S. japonica* n-hexane extract in hepatocellular carcinoma. The observed results demonstrate that *S. japonica* n-hexane extract induces not only caspase-dependent/independent cell death but also both ER-stress and cell cycle arrest. The caspase-dependent cell death is mediated by cell surface death receptors such as Fas. This receptor recruits the adaptor protein Fas-associated death domain (FADD) and caspase-8, leading to cleavage and activation of caspase-8. Activated caspase-8 induces the mitochondrial release of cytochrome C or activation of caspase-3 directly (Ashkenazi and Dixit, 1998; Jin and El-Deiry, 2005). Our results indicate that the extract induced activation of caspases (caspase-8, 9 and-3) attesting the possibility of caspase-dependent apoptotic effect on human hepatocellular carcinoma SK-Hep1 cells. The Bcl-2 family comprises a group of structurally related proteins that plays a vital role in the regulation of intrinsic apoptosis. Bcl-2 and Bcl-xL maintains the integrity of the mitochondrial outer membranes and prevent apoptosis. Our data demonstrated that *S. japonica* n-hexane extract increases the expression of pro-apoptotic Bcl-2 family protein Bad, Bax and Bak. The extract also induced caspase-independent cell death by the activation of AIF. AIF (a mitochondrial flavoprotein) translocates to the nucleus when it induce caspase independent chromatin condensation and DNA fragmentation. The release of AIF from mitochondria is largely depends on activation of caspase-3 and-9 (Zhao et al., 2009). The results in this study reveal that *S. japonica* n-hexane extract induces the higher expression of cleaved PARP and AIF. The large-scale DNA fragmentation and cell cycle arrest were confirmed by FACS and Western analysis. These results indicate that the extract induces the caspase-independent cell death and cell cycle inhibition in SK-Hep1 cells.

In addition to mitochondria, other organelles like ER, Golgi and lysosome are also involved in the apoptotic initiation. Recently, many researchers have focused on ER stress induced apoptosis in tumor cells. Two distinct phenomena involved in ER stress induced apoptosis: the accumulation of unfolded protein and Ca\(^{2+}\) signaling. The disruption of calcium homeostasis induces ER-stress, resulting in the up-regulation of ER-stress related genes Bip, TRAF2, phospho-JNK, ATF6\(\alpha\), and CHOP. In this study, the results shown that the extract of *S. japonica* alters the calcium homeostasis and induced ER-stress followed by the up-regulation of many ER specific.
mediators leading apoptosis on hepatocellular carcinoma cells.

In summary, the n-hexane fraction of S. japonica induces ER-stress by disturbing the calcium homeostasis and activation of both caspase-dependent and independent cell death in SK-Hep1 cells.

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References


