Induction of Growth Arrest and Apoptosis in Human Lung Cancer Cells by Crude Saponin Extract Isolated from *Nigella sativa*

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Abstract: Lung cancer is a leading cause of cancer death worldwide. *De novo* and acquired resistance to chemotherapeutic agents and the toxicity to normal cells are the major causes of treatment failure in lung cancer. Therefore, there is a vital need for the development of optimal mechanism-based and targeted therapeutic strategies for lung cancers to alleviate treatment failure. The seeds and oil of *Nigella sativa* (*N. sativa*) have been reputed to have many curative properties in traditional medicine, and recent publications have also shown that many agents in *N. sativa* possess anticancer properties. However, the effects of *N. sativa*, (in particular, saponin fraction) on A549 human lung cells are still unknown. This study was carried out to evaluate the apoptotic effect of crude extract of saponins isolated from *N. sativa* (CSENS) on the human lung carcinoma cell line, A549, and to address its mechanism of action. The CSENS significantly inhibited proliferation and colony formation in A549 cells, in a dose- and time-dependent manner. CSENS induced apoptosis in A549 cells evidenced by apoptotic morphological changes and DNA fragmentation. Therefore, CSENS may be a promising agent for the treatment of human lung cancer.

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1.Introduction

Although having cancer of any type is devastating, lung cancer is particularly dreadful. This is because it is often diagnosed in advanced stage with no prior symptoms, one the most common type of cancer worldwide and the leading cause of cancer death for both men and women (Cranganu and Camporeale, 2009). Worldwide, case numbers are 1.04 million with 921,000 deaths annually (Cranganu and Camporeale, 2009). Approximately 85% of lung cancer cases are diagnosed as non-small cell lung cancer (NSCLC), and the 1-year survival rate for patients with untreated NSCLC is only 10-15%. Early detection and treatment can improve the prognosis, but only 15% of patients with lung cancer are diagnosed at an early stage (Cortes-Funes, 2002; Beadsmoore and Screaton, 2003; Jemal *et al.,* 2004).

Apoptosis is a multi-step and multi-pathway programmed cell death that is inherent in every cell of the body (Sarastea and Pulkki, 2000). It is a strictly regulated pathway responsible for the ordered removal of superfluous, aged, and damaged cells (Sarastea and Pulkki, 2000). Recently, the relationship between apoptosis and cancer has been emphasized, with increasing evidence suggesting that the related processes of neoplastic transformation, progression and metastasis involve the alteration of normal apoptotic pathways (Wong, 2011). Indeed, most tumor cells (including NSCLC) are naturally resistant not only to apoptotic-related cell death (type I programmed cell death), but are also resistant to nonapoptotic types such as necrosis, autophagy (type II programmed cell death), senescence, mitotic catastrophe, and paraptosis (Sperandio *et al.,* 2000; Okada and Mak, 2004; Debatin and Krammer, 2004). In addition to mechanisms enabling them to resist cell death, NSCLCs are also able to resist various cytotoxic insults because they possess a large set of intracellular signaling pathways that counteract chemotherapeutic insults including the constitutive activation of the phosphatidylinositide- 3-kinase (Lee *et al.,* 2003), Akt (Castillo *et al.,* 2004), and the nuclear factor-κB (NF-κB) signaling pathways (Mayo *et al.,* 2003; Wiener *et al.,* 2004). Morphological hallmarks of apoptosis includes loss of cell volume, hyperactivity of the plasma membrane, and condensation of peripheral heterochromatin, followed by cleavage of the nucleus and cytoplasm into multiple membrane-enclosed bodies containing chromatin fragments (Reviewed in Sarastea and Pulkki, 2000; Cruchten and Broeck, 2002; Wong, 2011). In turn, one essential strategy for cancer therapy is to target the lesions that suppress apoptosis in the tumor cells. Consistent with this notion, it has been found that many cancer chemotherapy drugs exert anticancer effects on malignant cells by inducing

apoptosis (Kaufmann and Earnshaw, 2000; Call *et al.,* 2008).

Currently, natural products, including plants, vegetables, herbs and spices used in folk and traditional medicine, have been found to be a potential source of novel anticancer drugs over the decades and have much contributed to cancer chemotherapy (Aggarwal *et al.,* 2009; Gupta *et al.,* 2010). These chemotherapeutic agents have been reported to exert their antitumor effects by inducing apoptosis (Khan *et al.,* 2007). Among natural products, the seeds and oil of *Nigella sativa* have attracted the interest of medical scientists. *N. sativa* belongs to the *Ranunculaceae* family, which is an annual herbaceous plant with black seeds; it is commonly known as black seeds, black cumin, black caraway seed, and *Habbatul* barakat; it grows in countries bordering the Mediterranean Sea, Pakistan and India (reviewed in Ali and Blunden, 2003). Its beneficial effects are related to their antioxidant, antidiabetic, antihistaminic, antiepileptogenic, antiinfective, antitumour and antiperoxidative properties (Mabrouk *et al.*, 2002; Ali and Blunden, 2003; Ilhan *et al.*, 2005; Kaleem *et al.*, 2006; Kanter *et al.*, 2006). Recently, we have demonstrated that crude *N. sativa* extract inhibited cell proliferation in cervical cancer cell line, HeLa (Elkady, 2012). In addition, numerous studies have shown that the seeds and oil of this plant are characterized by a very low degree of toxicity (Ali and Blunden, 2003). The major biologically active compound of *N. sativa* is thymoquinone notorious for its antitumour and antiinflammatory effects (Gali-Muhtasib *et al.*, 2006; El Gazzar *et al.*, 2006). Other various bioactive compounds have also been found in the seeds of N. sativa, such as both fixed and essential oils, proteins, alkaloids and saponin (Ali and Blunden, 2003).

Saponins are chemical structures consisting of triterpenoidal or steroidal aglycones with various carbohydrate moieties that are found in many plants. They have come into focus in recent years due to increasing evidence of their health benefits such as cholesterol lowering and anticancer properties (Gurfinkel and Rao, 2003). Recent research has established saponins as the active components in many herbal medicines (Alice *et al.,* 1991; Liu and Henkel, 2002). Saponins have been reported to possess a wide range of biological activities (Gl-stndag and Mazza, 2007). While crude isolates, extracts, and saponin containing plants have been utilized in the investigation of biological activity, especially in the earlier studies, developments in the isolation/purification and characterization techniques have enabled the investigation of the bioactivity of well characterized saponins and led to the emergence of structure and bioactivity relationships (Gurfinkel and Rao, 2003).

Anticancer activity has been reported for a number of saponins (in particular triterpenes and steroids) including but not limited to soya saponins. These include ginsenosides, saikosaponin-d, diosgenin and glycyrrhizic acid (reviewed in Gl-stndag and Mazza, 2007). Other saponins such as methyl protoneogracillin, methyl protogracillin (steroidal saponins isolated from the rhizomes of *Dioscorea collettii*), protoneodioscin and protodioscin (furastanol saponins isolated from the rhizomes of *Dioscorea collettii*) have been identified as potential anticancer agents by the National Cancer Institute's (NCI) anticancer drug screen program (reviewed in Gl-stndag and Mazza, 2007). Anticancer activities of saponin containing plants such as ginseng and licorice are also being investigated (Gl-stndag and Mazza, 2007). Other aglycones with anticancer activity include dammarane sapogenins from ginseng, betulinic acid and oleanolic acid (Gl-stndag and Mazza, 2007).

Despite knowledge about the potent anti-cancer, anti-oxidant and anti-inflammatory effects of the *N. sativa*, and particularly triterpenoid saponins, its activity against lung cancer has not been reported. Based on the aforementioned scientific data and considering the fact that in some cases herbal extracts are showing more potency than the purified components (Seeram *et al*., 2004, 2005), the present study was undertaken to investigate the anti-proliferative potentiality of the crude saponin extract of *N. sativa* on the human lung cancer cell line, A549, and further to elucidate the molecular mechanism underlying this action. The data herein demonstrate, for the first time, that the crude saponin extract of *N. sativa* strongly suppressed growth of A549 cells by induction of apoptotic cell death. These data provide a rationale for the treatment of lung carcinoma and other tumors.

2. Material and Methods

Preparation of Extracts

Fresh black seeds of *N. sativa* (500 g) were purchased from the local market of Jeddah, Kingdom of Saudi Arabia. They were properly washed and used to prepare crude saponin extract. The dried seeds were extracted by cold percolation with 70% (2L) ethanol for 72 h at room temperature and then filtered. The extraction was repeated twice. The combined filtrates were concentrated in a vacuum evaporator to afford a syrupy brown residue. This residue was suspended in water (250 mL) and defatted by using petroleum ether (250 mL x 3) by using separating funnel. The aqueous portion was then separated and collected. The aqueous portion was then fractionated with N-butanol saturated water (250 mL x 3). The N-butanol portion was then

separated and collected. The aqueous portion was discarded. The N-butanol portion was then fractionated with 1% KOH. The N-butanol portion was collected, dried to obtain a crude extract of saponins. Before use, the stock was further diluted in DMSO to give the final indicated concentrations and termed as crude saponin extract of *N. sativa* (CSENS).

Phytochemical examination for testing the presence of saponin in CSENS was carried out using Froth test (Tiwari *et al.,* 2011). The extract was diluted with distilled water to 20mL and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Cell Culture

The human lung cancer cell line, A549 was obtained from King Fahd Center for Medical Research, King Abdulaziz University, Kingdom of Saudi Arabia. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin antibiotics in tissue culture flasks under a humidifying atmosphere containing 5%CO_2 and 95% air at 37 °C. The cells were subcultured at 3–4 day interval. They were seeded at a density of $5x10^4$ cells/mL and passaged by washing the mono-layers with phosphate-buffered saline (PBS) followed by a brief incubation with trypsin/EDTA. The washed cells were isolated by centrifugation and re-suspended in culture medium for plating or counting. Cells growing at the exponential phase were used to perform all the experiments.

Cell Viability and Colony Formation Assays

The dose-dependent effects of the CSENS on the viability of the A549 cells were determined by trypan blue dye exclusion assay. Early log phase cells were trypsinized and re-grown in 24-well cell culture plates at the concentration of 50×10^3 cells/mL in 1 mL of complete culture medium. 24 h post-treatment, the medium was removed and replaced with fresh medium containing (otherwise indicated) increasing concentrations (50, 100, 150 and 200 µg/mL) of the CSENS for the indicated time intervals. At end of these treatment interval, both floating and adherent cells were collected (taking care that none of the floating cells were lost during washes), and pelleted by centrifugation at 700 *g* for 5 min. The cells were re-suspended in 25 mL phosphate-buffered saline (PBS), mixed with 5 mL of 0.4% trypan blue solution and counted using a hemocytometer under an inverted microscope. The effect of CSENS on growth inhibition was assessed as percent cell viability, where control treated cells were taken as 100% viable. For these studies, all experiments were repeated three or more times in triplicate.

Colony forming assays were performed as described earlier (Elkady, 2013). Briefly, log growth phase A549 cells were trypsinized and plated onto 6-well plates at initial cell concentrations of $1x10^3$ cells/mL. Twenty-four hours later, the medium was removed and fresh medium was added with the indicated concentrations of the CSENS for 12 days to allow cells to form colonies. The resulting colonies were stained with 0.4% trypan blue solution for 30 min and counted. Colonies with >50 cells were counted under a dissection microscope. Colony formation was calculated as a percentage of untreated control cultures. Each condition was repeated in at least duplicate.

Apoptotic Assay

The nuclear morphological changes associated with apoptosis was analyzed using DAPI staining as described previously (Elkady, 2012). Briefly, A549 cells (3×10^4) were plated on coverslips, allowed to attach overnight, and exposed to vehicle or indicated concentrations of CSENS for 24 h. The cells were washed with PBS and fixed with cold methanol for 20 min at -20° C. Fixed cells were washed with PBS, and stained with 4,6- diamidino-2-phenylindole (DAPI; Sigma-Aldrich) solution for 20 min at room temperature. The cells were washed twice more with PBS and analyzed; the cells with condensed and fragmented DNA (apoptotic cells) were scored under a fluorescence microscope (Carl Zeiss, Germany) at x40 objective lens magnification.

DNA Fragmentation Assay

DNA gel electrophoresis was used to determine the presence of internucleosomal DNA cleavage as described previously (ElKady, 2013). Briefly, A549 cells ($3x10^6$ cells/100 mm dish) treated with increasing concentrations of CSENS for 24 h were collected, washed in PBS and centrifugated at 12,500 × *g* for 5 min. Cell pellets were then lysed in 600 µl lysis buffer (10mM Tris (pH 7.4), 150mM NaCl, 5mM EDTA and 0.5% Triton X-100), kept on on ice for 30 min, and centrifuged at 12,500×*g* for 20 min. The supernatant with DNA fragment was transferred into 1.5 ml tubes which contained 2 µl RNase A (20 mg/ml) and incubated at 37 ◦C for 1 h, then kept with 2 µl proteinase K (20 mg/ml) at −20 ◦C over night. After centrifugation at 12,500×*g* for 20 min, the sediment was dissolved in 30 µl TE buffer (10mM Tris (pH 7.4) and 1mM EDTA (pH 8.0)) and the concentration of DNA was determined spectroscopically. Then DNA was resolved by electrophoresis on 1.5 % agarose gel. After electrophoresis at 80 ~ 100 V, the gel was stained with ethidium bromide, and DNA was visualized by a UV trans-illuminator (BIO-RAD).

Statistical Analyses

All experiments were performed in triplicates and repeated at least five times and the data were presented as mean ± SD. Statistical analyses were

evaluated by Student's *t*-test. Probability values $P < 0.05$ were considered statistically significant.

3. Results

CSENS Inhibits Proliferation of A549 Cells

Initially, the effect of *N. sativa* extract on the cell survival was determined using a well-characterized human cervical carcinoma cell line, A549, as a model. The cells were incubated with increasing concentrations (0.00, 50, 100, 150 and 200 µg/mL) of CSENS for 12, 24, 48 and 72 h, before being harvested and assayed for cell viability by trypan blue dye exclusion assay, as explained in Materials and Methods. The results are summarized in Figure 1(A). Generally, the extract consistently exhibited a does- and time-dependent anti-proliferative effect on the cell viability. Inhibition of proliferation was observed as early as 12 h when cells were treated with CSENS at a concentration of 50 µg/mL, and the strongest inhibition was detected when cells were treated with CSENS at a concentration of 200 µg/mL for 72 h. The IC_{50} values (the concentration of test compound that inhibits 50% of the cell growth) for 100, 150 and 200 µg/mL were observed after 60, 20 and 9 h of treatment, respectively. To find out whether the anti-proliferative potentiality of CSENS is unique to A549 cells, the above experiments were repeated using human cervical carcinoma cell line, HeLa, as a model. We observed similar results (data not shown). Collectively, these results indicated that the CSENS markedly inhibited growth of the human lung A549 cells.

CSENS Suppresses Colony Formation In A549 Cells

Next, the anti-proliferative and cytotoxic effects of the CSENS on A549 cells were further determined and verified by using anchorage-dependent colony formation assay (also referred to as clonogenicity). This assay measures the ability of tumor cells to grow and form foci in a manner unrestricted by growth contact inhibition as is characteristically found in normal, untransformed cells. As such, clonogenicity provides an indirect assessment of the propensity of tumor cells to undergo neoplastic transformation. As shown in Figure 1(B), the CSENS suppressed colony formation in a dose-dependent manner. The histogram in Figure 2B demonstrates that, treatment with 50 µg/mL CSENS, colony formation was inhibited to 20 % of control, the ability of the cells to form colonies was reduced to 40, 20 and 15 % at concentrations of 100, 150 and 200 µg/mL, respectively.

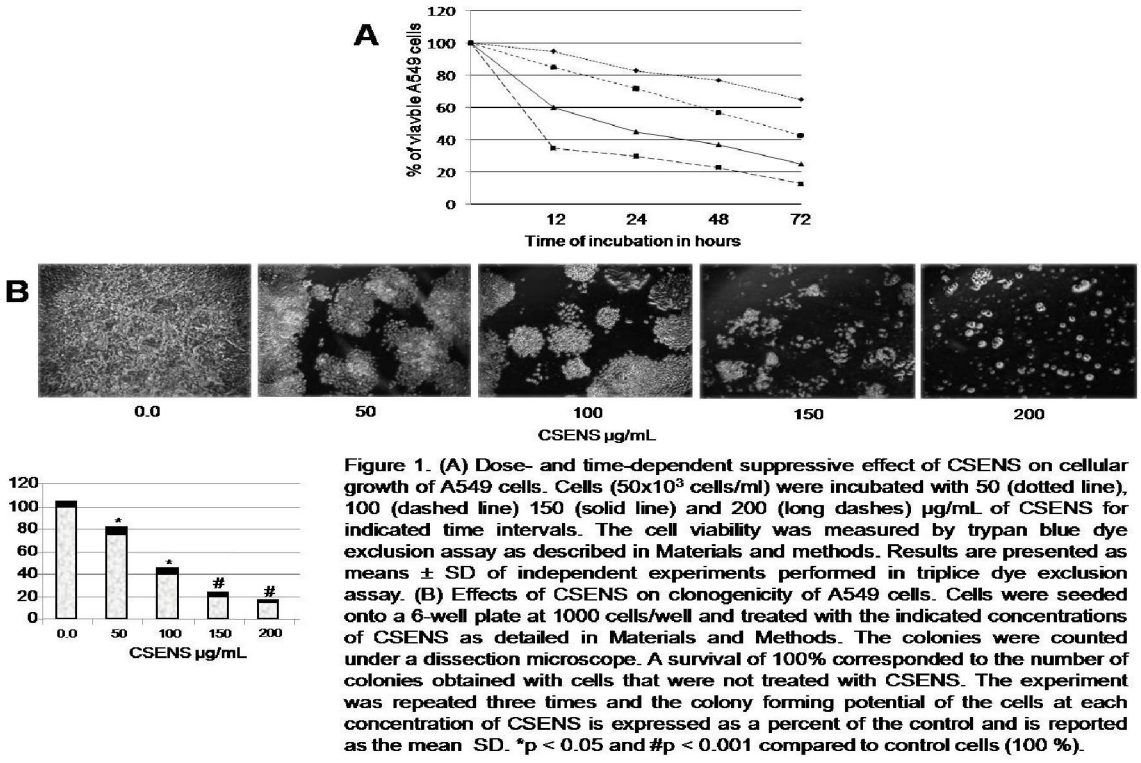

Figure 1. (A) Dose- and time-dependent suppressive effect of CSENS on cellular growth of A549 cells. Cells ($50x10^3$ cells/ml) were incubated with 50 (dotted line), 100 (dashed line) 150 (solid line) and 200 (long dashes) µg/mL of CSENS for indicated time intervals. The cell viability was measured by trypan blue dye exclusion assay as described in Materials and methods. Results are presented as means ± SD of independent experiments performed in triplice dye exclusion assay. (B) Effects of CSENS on clonogenicity of A549 cells. Cells were seeded onto a 6-well plate at 1000 cells/well and treated with the indicated concentrations of CSENS as detailed in Materials and Methods. The colonies were counted under a dissection microscope. A survival of 100% corresponded to the number of colonies obtained with cells that were not treated with CSENS. The experiment was repeated three times and the colony forming potential of the cells at each concentration of CSENS is expressed as a percent of the control and is reported as the mean SD. *p < 0.05 and #p < 0.001 compared to control cells (100 %).

CSENS Induces Apoptosis in A549 Cells

A growing body of evidences support that suppression of cancer cell growth can be caused either by arrest of cell cycle progression and/or induction of apoptosis (Gupta *et al*., 2010) and that

the anti-proliferative effect of many naturally occurring cancer chemopreventive agents is tightly linked to their ability to induce apoptosis (Khan *et al*., 2007). To determine whether the proliferation-inhibitory effect of CSENS is related to the induction of apoptosis, cells were treated with escalated doses of CSENS for 24 h and incidents of apoptotic cell death was assessed by several approaches. Firstly, apoptotic cell death was detected by phase microscope. As shown in Figure 2(A), under the inverted light microscope, the untreated A549 cells grew well forming confluent monolayer. On the other hand, the CSENS-treated cells exhibited marked morphological changes including cell shrinkage, cellular detachment and lost of the originally confluent monolayer; all these events characterize apoptotic cell death (Dini *et al*., 1996). To further examine the morphological changes in responding to CSENS treatment, both control and CSENS treated cells were stained with the fluorescent dye DAPI and visualized by a fluorescent microscope. As depicted in Figure 2(B), the control cells appeared normal with round and homogenous nuclei. By contrast, the CSENS-treated cells exhibited the typical characteristics of apoptotic death, such as nuclear condensation and fragmentation. Scoring numbers of apoptotic cells with condensed and fragmented DNA from control and CSENS-treated cultures revealed that the percentage of apoptotic cells was 10, 35 and 55 % upon a 100, 150 and 200 µg/mL CSENS treatment, respectively.

DNA fragmentation is a hallmark of apoptosis and the detection of DNA ladder in agarose gel electrophoresis is commonly used as a biochemical marker for the measurement of apoptosis (Kroemer, 1995). Therefore, DNA gel electrophoresis was used to determine the presence of DNA fragmentation. Exposure of A549 cells to the CSENS resulted in marked DNA fragmentation, as indicated by the appearance of DNA ladders upon agarose gel electrophoresis in Figure 2(C). Meanwhile, the control indicated no evident DNA ladder.

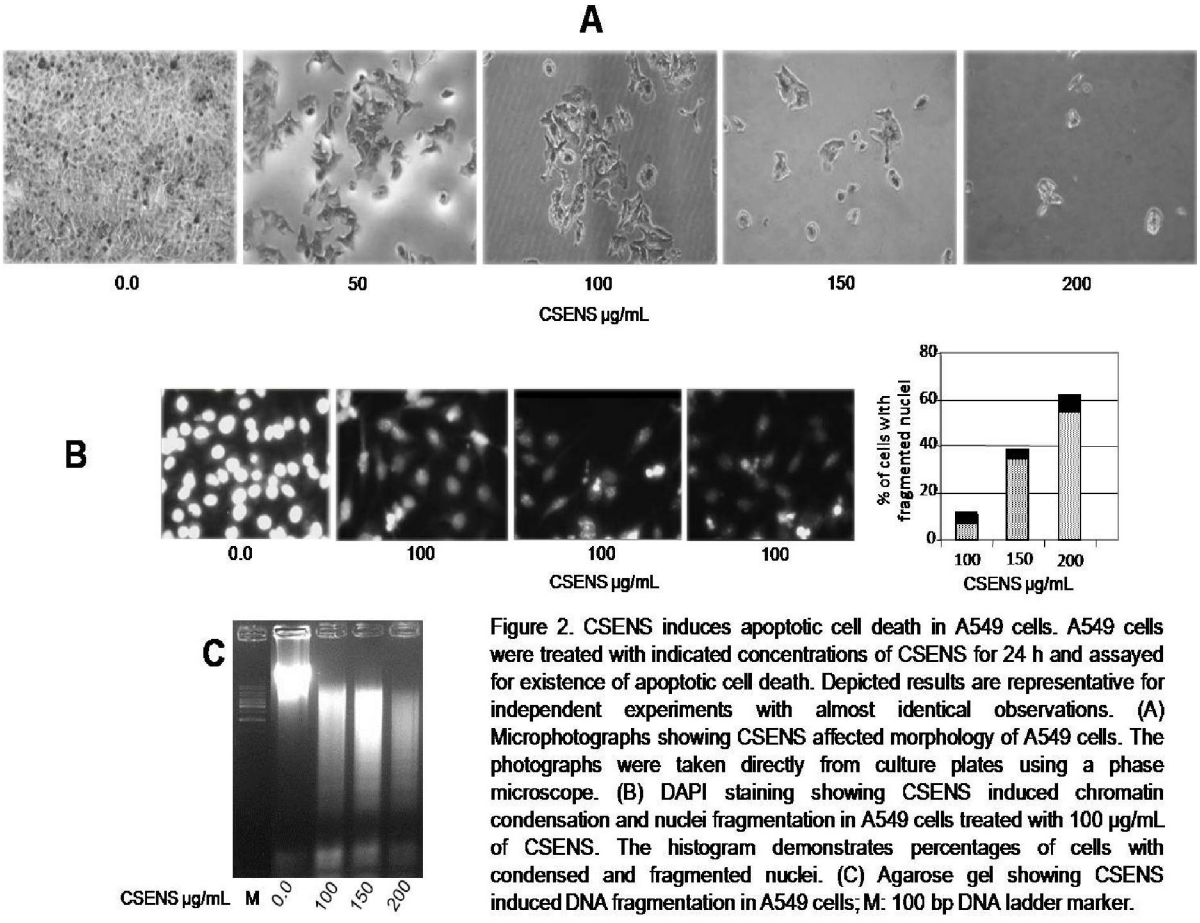

Figure 2. CSENS induces apoptotic cell death in A549 cells. A549 cells were treated with indicated concentrations of CSENS for 24 h and assayed for existence of apoptotic cell death. Depicted results are representative for independent experiments with almost identical observations. (A) Microphotographs showing CSENS affected morphology of A549 cells. The photographs were taken directly from culture plates using a phase microscope. (B) DAPI staining showing CSENS induced chromatin condensation and nuclei fragmentation in A549 cells treated with 100 µg/mL of CSENS. The histogram demonstrates percentages of cells with condensed and fragmented nuclei. (C) Agarose gel showing CSENS induced DNA fragmentation in A549 cells; M: 100 bp DNA ladder marker.

4. Discussion

An important aim of cancer research is to find therapeutic compounds having high specificity for cancerous cells/tumor and fewer side effects than the presently used cytostatic/cytotoxic agents. In this regard, a number of biologically active

phytochemicals have been identified in plant foods (Lampe, 1999). In reality, some phytochemicals derived in spices and herbs as well as other plants possess substantial cancer preventive properties (Surh, 2002; Surh, 2003 and Lai and Roy, 2004). This is supported by the fact that several therapies used today trace their origin to plants, such as Vinca alkaloids (vincristine and vinblastine) isolated from *Catharanthus roseus* (Johnson *et al*., 1963), taxanes (paclitaxel and docetaxel) derived from the Pacific yew *Taxus brevifolia* (Wani *et al*., 1971), and camptothecins from the Chinese tree *Camptotheca acuminata* (Wall *et al*., 1966). Based on that, the target of much current research has been focused on the discovery of natural compounds that can be used in the prevention and/or treatment of cancer (Orsolic *et al*., 2005). A primary interest of our laboratory is to obtain experimental evidence that supports the cancer growth-suppressive potentiality of phytochemicals and its plausible application in preventing and treating cancer. The hallmarks of cancer include tumor cell proliferation and survival (Hanahan and Robert Weinberg, 2011). The present study was carried out to examine anti-proliferative potentiality of a crude saponin extract derived from N. *sativa*. We demonstrated for the first time that the CSENS has strong dose- and time-dependent anti-proliferative activity on A549 cells in culture, with an IC_{50}, 100, 150 and 200 µg/mL, respectively. This dose-dependent growth inhibitory effect was also confirmed in the colony formation assay, which showed statistically significant reduction in the number and size of CSENS-treated colonies, compared to the control colonies. Since this assay measures the ability of tumor cells to grow and form foci in a manner unrestricted by growth contact inhibition as is characteristically found in normal, untransformed cells, therefore CSENS could be proposed to be a promising candidate for restricting the growth of A549 lung cancer cells. These data strongly suggest that at least some of the active components of this extract can be exploited for chemoprevention of cancer cell growth. Since a crude saponin extract was used in this study, it is not possible to attribute the reported effects to specific ingredient. In addition, it cannot be ruled out that the overall reported effects were contributed by the interactions between the various compounds in the extract. Thus, future work involving separation, purification and bioassays on purified ingredients may disclose the true nature and extent of their potency for anti-cancer effects observed *in vitro*.

Lung cancers are classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC, including adenocarcinoma, squamous cell carcinoma, and large cell carcinoma). NSCLC is the more common phenotype (~80% of lung cancers) and the less sensitive to chemotherapeutic agents. Survival statistics are dismal with an average 5-year survival of 10–15% (National Cancer Institute, 2009). Thus, it is crucially important to develop better therapeutic strategies for the management of NSCLC. We used in this study an A549 cell line, which is a non-small cell lung cancer cells. Thus, the findings herein suggest that CSENS could potentially be a new therapeutic option in anticancer treatment for NSCLC. It is a note of worth to mention that, recently, we found CSENS induced apoptosis too in a colon cancer cell line, HCT116 (Manuscript submitted). More importantly, HCT116 cells showed more sensitivity to CSENS-induced growth suppression than A549 cells. Since A549 cells and HCT116 cells are different cell lines derived from different tissues, it is reasonable to suggest that CSENS has a general function in suppressing cancerous cell growth but may act through multiple pathways in these two cell lines.

Apoptosis is an active form of cell suicide controlled by a network of genes and is an essential process during development as well as playing a key role in the pathogenesis of diseases including cancer (Wong, 2011). Tumor cells do not undergo apoptosis easily because they have defects in their ability to activate the death signaling pathways. Thus, one effective cancer therapy is to activate the tumor cell's apoptosis pathway (Finkel, 1999; Evan and Vousden, 2001; Marx, 2002). Indeed, when it comes to the successful eradication of cancer cells by non-surgical means, ultimately, all roads lead to apoptosis-programmed cell death (Khan *et al.,* 2007). Consistent with this notion, the findings in the present study indicated that the proliferation inhibitory activity of CSENS was related to the induction of apoptosis. The common features of cells committing apoptotic cell death include chromatin condensation, DNA fragmentation to nucleosome-sized pieces, membrane blebbing, cell shrinkage and compartmentalization of dead cells into membrane-enclosed vesicles or apoptotic bodies (Reviewed in Sarastea and Pulkki, 2000; Cruchten and Broeck, 2002). The data herein demonstrate that the CSENS-treated A549 cells exhibited typically morphological features of apoptosis, such as a loss of cell viability, cell shrinkage, irregularity in cellular shape, and cellular detachment. In addition, DAPI staining assay revealed occurrence of nuclear condensation, and apoptotic bodies in CSENS-treated A549 cultures. Furthermore, DNA laddering assay, which is widely used as biochemical marker of apoptosis (Nagata, 2000), demonstrated fragmentation of DNA isolated from CSENS-treated cells. Collectively, these findings demonstrate that the cytotoxic effect

observed in response to the CSENS treatment is associated with the induction of apoptosis in the A549 cells. Consistent with our finding, Swamy and Huat previously reported that a bioactive principle, α-hederin, belonging to saponin stuffs and isolated from the *N. sativa* seeds, has an *in vivo* anti-tumor activity, being inhibited growth of implanted LL/2 (Lewis lung carcinoma) cells in BDF1 mouse tumors *in vivo* (2001). Similar to CSENS, *in vitro* treatment with the monodesmosidic, triterpene saponin, α-hederin (extracted from *Hedera helix*) inhibited proliferation of mouse B16 melanoma cells and non-cancer mouse 3T3 fibroblasts (Danloy *et al.,* 1994) and also induced apoptosis, intracellular glutathione depletion and reactive oxygen species generation in P388 murine leukemia cells (Swamy and Huat, 2003).

In conclusion, the present results provided evidence for the potential use of CSENS in the prevention, or even treatment of human lung cancer. The findings indicated that CSENS significantly inhibited the proliferation of A549 cells *in vitro*, confirmed by the cell viability and colony formation assays. The growth-suppression potentiality of CSENS might be due to the induction of apoptosis, as demonstrated by the stereotypical morphologic changes and DNA laddering assay results. These combined results suggest that the CSENS may be an attractive candidate to beat human lung cancer. Our ongoing study will further validate the effective cancer-preventive potentiality of CSENS in other cancer cell lines as well as in a xenograft assays. Additional analytical and molecular investigations are in progress to identify the active component(s) in CSENS that induced growth inhibition in A549 cells and to further explore the underlying molecular mechanisms.

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9/2/2013