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Journal of King Saud University – Science

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Original article

The protective effect of *Ammi visnaga* extract against human hepatic cancer



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ARTICLE INFO

Article history:

Received 2 March 2021

Revised 4 July 2021

Accepted 7 July 2021

Available online 21 July 2021

Keywords:

Apoptosis

Ammi visnaga

Cytotoxicity

HuH-7 cells

Liver

Cancer

ABSTRACT

Cancer is the most serious disease globally and it's the second in mortality rate after heart failure. Hepatic cancer is a common cancer disease worldwide. This investigation was designed to assess the cytotoxic and apoptotic effects of *Ammi visnaga* (*A. visnaga*) seed extract on human liver cancer cell line (HuH-7). Based on IC50 values, three doses (500, 1000, 1500 µg/ml) were selected. Treated cells with MTT assay reduced cell viability at 1000 µg/ml and higher after 48hrs. NRU test demonstrated significant increase at (1500 and 2000 µg/ml) in dose-dependent manner indicating lysosomal toxicity. The level of oxidative stress-mediated cytotoxicity was assessed using SOD, glutathione (GSH), catalase and ROS assays. The intracellular ROS was highly increased and loss of mitochondrial membrane potential (MMP) confirmed the damage of mitochondrial membrane in HuH-7 cells. The level of GSH significantly increased after treatment. In contrast, the levels of SOD, and catalase activities significantly decreased after treatment. The effects of MMP significantly decreased after treatment compared to control. Gene expression data reported significantly upregulation of apoptotic genes. In conclusion, this finding revealed that *A. visnaga* seeds extract has hepatoprotective potential against HuH-7 cells, signifying their promising clinical applications as a potential therapeutic agent for liver diseases.

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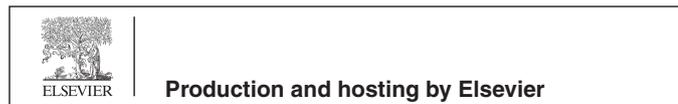
Abbreviations: Bax, Bcl-2 associated X; Bcl-2, B-cell lymphoma-2; Caspase, Cysteine dependent aspartate-specific proteases; Cas-3, Caspase-3; Cdk, Cyclin-dependent kinases; DISC, Death-inducing signaling complex; FADD, Fas associated via death domain; GSH, Glutathione; MMP, Mitochondrial membrane potential; NRU, Neutral red uptake; p53, Tumor suppressor gene; OD, Optical density; ROS, Reactive oxygen species; SOD, Super oxide dismutase; TNF, Tumor necrosis factor.

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Peer review under responsibility of King Saud University.



1. Introduction

Cancer comprises a huge number of diseases worldwide. Cancer cells may possess the ability to spread whole body organs. Hepatic cancer is one of the most common cancers globally. In Saudi Arabia, hepatic cancer increased three times in the years between 1990 and 2016 (Althubiti and Eldein, 2018). Plant products play an important role in traditional and pharmaceutical medicine. However, generally, liver cancer doesn't have a good prognosis, curative treatments can be presented to almost 30% of patients, but are complicated by a high rate of recurrence with patients (Mokdad et al., 2015). Understanding the treatment of hepatic carcinoma is essential for its prevention (Imawari, 2002). More than 80% of the population in the world counts on plant-based medicine nowadays for their primary healthcare (Majeed, 2017).

<https://doi.org/10.1016/j.jksus.2021.101540>

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Plants phytochemical studies are becoming popular because of their anticancer effects and natural source for promoting human health. More than 80% of the world's population relies on plant-based medicine for primary healthcare, a system that developed over time by dynamic interactions between people and their environment. Currently, plants and their products are important in traditional, botanical and pharmaceutical medicine (Albrahim et al., 2020). The *Ammi visnaga* plant is endogenous in the Middle East regions. This plant has a slight aromatic odour and a very bitter taste. The fruits of *A. visnaga* have been used in folk medicine for many years ago to relief kidney stones. In addition, *A. visnaga* extract showed marked antimicrobial activity against gram-positive (Beltagy and Beltagy, 2015) and used in the treatment of coronary diseases and bronchial asthma, reduces blood pressure and has anti-inflammatory effect in microglial cells (Kwon et al., 2010; Lee et al., 2010). Because of significant lack of cytotoxicity data of *A. visnaga*, the current study is designed to investigate the toxicity of *A. visnaga* in human liver cancer cell line (HuH-7), focusing on cells viability, oxidative stress-mediated cytotoxicity, evaluation of the genotoxic effects, and apoptosis.

2. Materials and methods

2.1. Preparation of aqueous plant extracts

The seeds of *A. visnaga* were crushed into fine powder using mortar and pestle, regular blender, and electric sieve system. After pulverization, the powder of seeds was stored at $-20\text{ }^{\circ}\text{C}$. Briefly, 30 g of the crushed material was soaked for 24 h in 300 ml distilled water (10% w/v) at room temperature. The soaked material was macerated with 50 ml distilled water (10% w/v) in a conical flask and kept in an orbital shaker (250 rpm at $45\text{ }^{\circ}\text{C}$ overnight). The extract was then concentrated and dried under reduced pressure at $40\text{ }^{\circ}\text{C}$ using a rotary evaporator (Rotavapor® R-215, BUCHI). All the filtered extract was preserved aseptically in glass bottles at $4\text{ }^{\circ}\text{C}$ until further use. The aqueous extract was prepared using sterile aqueous extracts and with distilled water (10% w/v). The reconstituted aqueous extract was passed through $0.45\text{ }\mu\text{m}$ bacterial filter papers (Millipore Inc., Riyadh, Saudi Arabia) before using them (Fatimah et al., 2019).

2.2. Cytotoxicity assessment by MTT assay

The HuH-7 cells were obtained from (ATCC, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) Sigma-Aldrich (St Louis, MO, USA) added to 10% Fetal Bovine Serum (FBS) (Sigma-Aldrich (St Louis, MO, USA) and 1% penicillin–streptomycin (Invitrogen (Carlsbad, CA, USA)). In brief, HuH-7 cells were seeded in plates at a density of 1×10^4 cells per well and allowed to grow up to 90–95% confluence at $37\text{ }^{\circ}\text{C}$ humidified cabinet of 5% CO_2 . Then, in order to determine the IC50, cells were exposed to various concentrations (375, 500, 750, 1000, 1500, 2000 and 3000 $\mu\text{g/ml}$) of *A. visnaga* seeds extract for 48 hrs. The MTT assay was performed as described by Mosmann (1983). The formazan crystal dissolved in dimethylsulphoxide, and measured at 595 nm (multi-mode Microplate Reader-Gen5™, BioTek Cytation 5™, USA).

2.3. Neutral red uptake (NRU) test

Neutral red uptake (NRU) test was performed according to the method described Ali et al. (2011). In brief, (2×10^4) cells/well in 96-well plates and kept in the 5% CO_2 incubator for 24 h at $37\text{ }^{\circ}\text{C}$ prior to experiment for the proper growth of the cells. After incubation the cells were exposed to *A. visnaga* extract for different time points. The cells were incubated for 3 h in complete medium

containing neutral red dye (50 $\mu\text{g/ml}$). The accumulated dye was extracted with 50% ethanol containing 1% (v/v) acetic acid and incubated 20 min at $37\text{ }^{\circ}\text{C}$. finally, plates were read at 540 nm (Microplate Reader-Gen5™ BioTek Cytation 5™, USA).

2.4. Oxidative stress biomarkers

The levels of oxidative stress biomarkers in HuH-7 cells were measured. In brief, HuH-7 cells (6×10^6) were cultured and treated with different doses of *A. visnaga* (500, 1000, and 1500 $\mu\text{g/ml}$) for 48hr. After respective exposure cells were washed with PBS and scraped. The scraped cells were incubated in lysis buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM Na₂ EDTA, 1% Triton, and 2.5 mM sodium pyrophosphate). HuH-7 cells were centrifuged at 15,000 rpm at $4\text{ }^{\circ}\text{C}$ for 15 min. The supernatant was kept at $-80\text{ }^{\circ}\text{C}$ in freezer to measure the oxidative stress biomarkers such as glutathione (GSH), superoxide dismutase (SOD), and catalase. GSH was measured by using a GSH kit (item no. 703002, Cayman chemical). SOD was measured by using a SOD kit (item no. 706002, Cayman chemical), and catalase was measured by using a catalase kit (item no. 707002, Cayman chemical).

2.5. Reactive oxygen species (ROS) generation

In brief, cells were exposed to seeds extract for 48 h. Culture media was aspirated off and carefully add $\sim 150\text{ }\mu\text{l}$ of included cell-based assay buffer. Cell-based assay buffer carefully aspirates, leaving a small amount (20 μl) of liquid in the well. 130 μl of ROS staining buffer was added to each well. 10 μl N-acetyl cysteine assay reagent was added to negative control wells. The plate was covered and incubated in dark place for 30 min at $37\text{ }^{\circ}\text{C}$. Plate was incubated for 30 min in 10 μl of the antimycin. Working reagent was added to positive control wells and incubated for an additional one hour at $37\text{ }^{\circ}\text{C}$ in the dark. The ROS Staining Buffer was aspirated and 100 μl of cell-based assay buffer was aspirated. Assay plate placed on fluorescent plate reader and measured the fluorescence using an excitation wavelength between 480 and 520 nm and an emission wavelength between 570 and 600 nm. The fluorescence of cells was analyzed using a fluorescence microscope (Olympus).

2.6. Mitochondrial membrane potential (MMP)

Cells were cultured in 96-well black plate at a density of 5×10^4 -cells/well in 100 μl culture medium and incubated in 5% CO_2 incubator overnight at $37\text{ }^{\circ}\text{C}$. Control and treated cells were washed twice with PBS. Then the cells were further treated with 10 mg/ml of Rhodamine-123 fluorescent dye for 15–30 min at $37\text{ }^{\circ}\text{C}$ in dark. The plate was centrifuged at 400xg for 5 min and supernatant was discarded. Then assay buffer (200 μl) was added to each well and quantified by fluorescent Microplate Reader-Gen5™ (BioTek Cytation 5™, USA). Also, HuH-7 cells were evaluated by Rhodamine-123 fluorescence dye. The fluorescence of Rhodamine-123 intensity was determined by fluorescence microscope by grabbing the images at 20X magnification.

2.7. Quantitative assay of apoptotic genes by RT-PCR

HuH-7 cells were grown in 6-well plates and exposed with seeds extract. RNA was isolated using a RNeasy Mini Kit (Cat No./ID: 74104). The purity and concentration of RNA were measured using a Nanodrop (DS-11; Bio-Rad Laboratories Inc., Hercules, California). The purity of RNA (A260/A280 ratio) sample was ~ 2 . We have synthesized cDNA from RNA (250 ng) using a cDNA reverse transcription kit (Revert Aid First Strand cDNA Synthesis Kit; Thermo Fisher Scientific). Analysis of *Bax*, *Bcl-2*, *caspase-3* and

p53 mRNA levels was done by RT-PCR (PE Applied Biosystems, Foster City, California). The primer sequences targeting apoptotic genes. The RT-PCR was performed; 5 min at 95 °C for initial denaturing, followed by 30 cycles, involving denaturation at 95 °C for 15 s, annealing at 60 °C for 20 s and elongation at 72 °C for 20 s. The cycle threshold (Ct) values were standardized to the house-keeping gene (GAPDH), and data were analyzed using the comparative the $\Delta\Delta C_t$ method [23] and the expression of target genes was normalized to GAPDH. Each experiment was performed with three replicates.

2.8. Statistical analysis

The present data were analyzed by one-way analysis of variance (ANOVA), and the significant value was set at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

3. Results

3.1. Cytotoxicity assessment by MTT and NRU assays

In the MTT assay, results showed that *A. visnaga* was exhibited cytotoxicity. The cells viability decreased in a dose and time dependent manner. HuH7 cells significantly ($p < 0.05$, $p < 0.01$, $p < 0.001$) decreased after treatment with 1000, 1500, 2000 and 3000 $\mu\text{g/ml}$ by 75%, 62%, 49% and 34% respectively, compared to the control (Fig. 1A). HuH-7 treated with *A. visnaga* shown cell toxicity by the NRU test. Lysosomal toxicity significantly ($p < 0.05$, $p < 0.01$) increased after treatment with 1500 and 2000 $\mu\text{g/ml}$ compared to the control (Fig. 1B). The reduction in cell survival was clear in both MTT and NRU results.

3.2. ROS generation activity and oxidative stress biomarkers

Generation of ROS and levels of GSH, SOD and catalase were examined to show the potential of seeds extract of *A. visnaga* to increase oxidative stress in HuH-7 cells. The results revealed that HuH-7 cells exposed to seeds extract had higher intracellular ROS generation. The production of (ROS) was exceedingly later exposing various doses (500, 1000 and 1500 $\mu\text{g/ml}$) of plant extract as stained with fluorescence dye DCFDA. However, the generated ROS was significantly ($*p < 0.05$) increased at 1500 $\mu\text{g/ml}$ compared with untreated control (Fig. 2A). To confirm ROS activity, treated HuH-7 cells were examined by fluorescence microscope. Images showed the production of ROS was exceeding after treating with different concentration (500, 1000 and 1500 $\mu\text{g/ml}$) of plant extract as stained with fluorescence dye. The density of fluorescence dye was increased in dose dependent manner (Fig. 2B); (A): control. (B): HuH7 cells at 500 $\mu\text{g/ml}$. (C): HuH7 cells at 1000 $\mu\text{g/ml}$. (D): HuH7 cells at 1500 $\mu\text{g/ml}$.

The level of GSH in HuH-7 after treating with different concentration of *A. visnaga* extract for 48 h significantly ($*p < 0.05$ and $**p < 0.01$) increased at (500 and 1000 $\mu\text{g/ml}$) compared with untreated control (Fig. 3A). In contrast, current results showed a significantly ($p < 0.05$, $p < 0.01$) depletion in the levels of SOD at (500, 1000 and 1500 $\mu\text{g/ml}$) compared with untreated control (Fig. 3B). For catalase activity the results revealed clear and strong significant ($p < 0.05$, $p < 0.01$ and $p < 0.001$) depletion in enzyme concentration at three doses and this reduction was found in a concentration-dependent manner (Fig. 3C).

3.3. Determination of MMP ($\Delta\Psi_m$).

The effect of *A. visnaga* exposure on MMP was evaluated in HuH-7 cells by JC-1 dye as shown in (Fig. 4A). The level of MMP

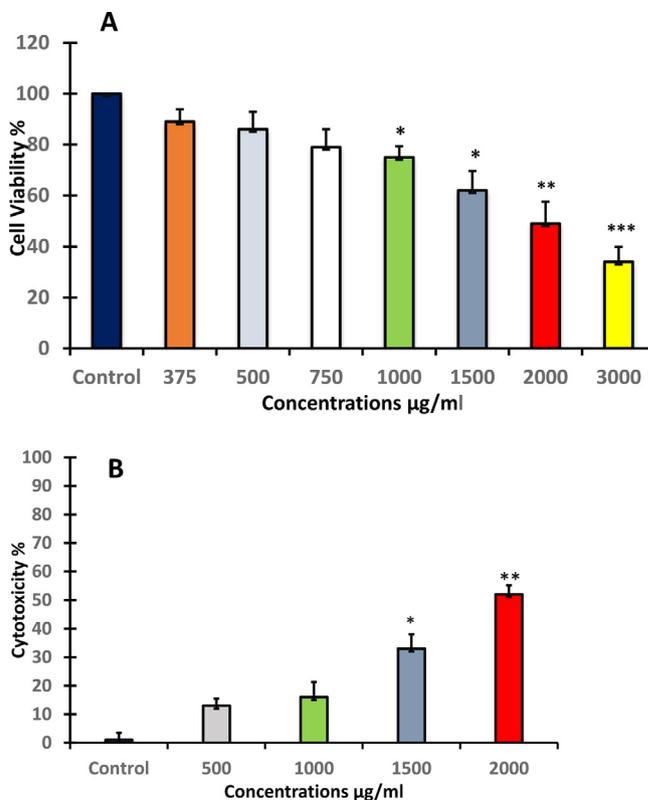


Fig. 1. The effect of *A. visnaga* seeds extract on treated HuH-7 cells for 48 h as evaluated by A: cell viability (MTT assay), and B: cytotoxicity (NRU assay). Each value represents the mean \pm SE of three experiments. $n = 3$, ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$) compared with control.

was significantly ($p < 0.05$, $p < 0.01$) decreased as concentration increased. For reconfirm the result, the intact MMP was also evaluated by rhodamine fluorescence dye. Result showed the intact MMP was found in control cells as rhodamine dye was penetrated to the cells and caused dye aggregates, with deep red fluorescence. In contrast, treated cells showed significant loss of MMP (Fig. 4B).

3.4. Quantitative real time PCR (qRT-PCR) of apoptotic marker genes

After exposure to *A. visnaga* quantitative real-time PCR was used to detect changes in mRNA levels of different apoptotic-related genes (*Bax*, *Bcl-2*, *Cas-3*, and *p53*) in treated HuH-7 cells. The level of mRNA expression for *Bax* as a pro-apoptotic gene was significantly ($p < 0.05$) up-regulated after 48 h (Fig. 5A). Also, treated HuH-7 cells showed significantly ($p < 0.05$ and $p < 0.01$) increasing in the level of *Cas-3* as an executioner gene at three doses compared with untreated cells (Fig. 5B). In contrast, the anti-apoptotic gene *Bcl-2* as an anti-apoptotic gene was significantly ($p < 0.05$) increased only at 1000 $\mu\text{g/ml}$ (Fig. 5C). Tumor suppressor gene is one of the most important gene that has ability to activate apoptosis. The level of *p53* was measured after exposing to *A. visnaga* seeds extract. Current study illustrated that treated HuH-7 cells were significantly ($p < 0.05$) increased the level of *p53* after 48hrs at all tested doses Fig. 5D).

4. Discussion

Liver cancer became a common cancer disease globally. In Saudi Arabia, hepatic cancer increased three times in the years between 1990 and 2016 (Althubiti and Eldein, 2018). However, researcher continually investigate the effective remedy including plant prod-

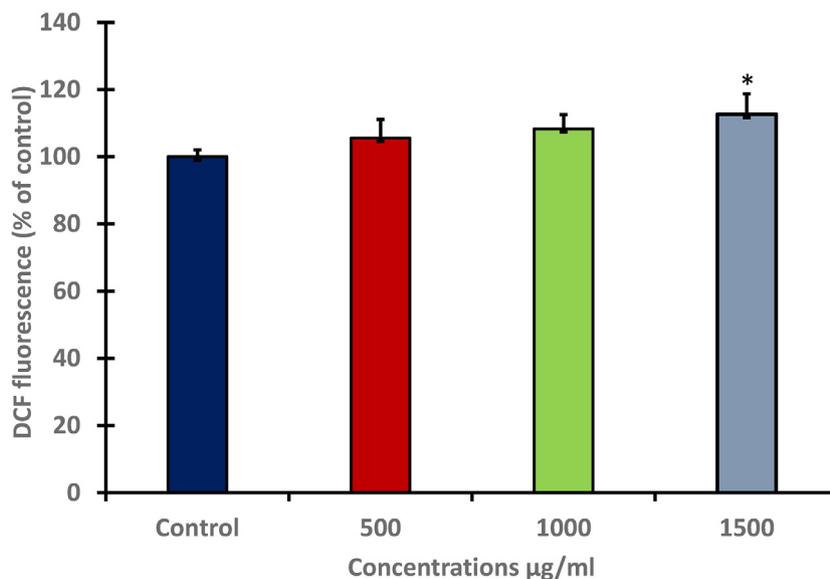


Fig. 2A. Generation of (ROS) levels in HuH-7 after treating with different concentration of *A. visnaga* seeds extract for 48 h. Data represents the mean \pm SE of three experiments. n = 3, (* $p < 0.05$) compared with untreated control.

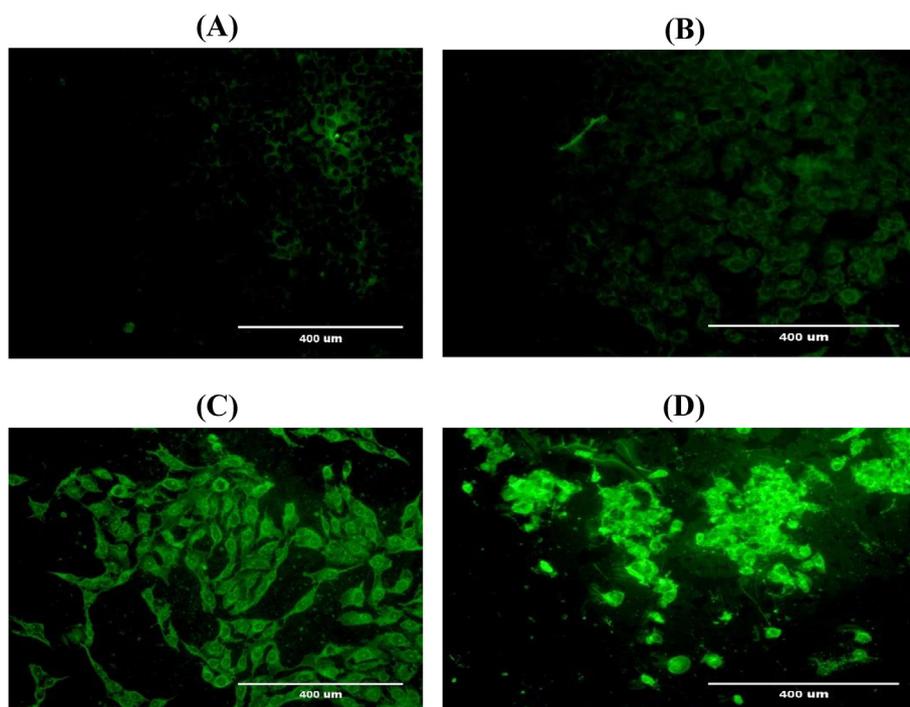


Fig. 2B. Shows the production of (ROS) was exceeding after treating with different concentration (500, 1000 and 1500 $\mu\text{g/ml}$) of plant extract as stained with fluorescence dye. (A): control. (B): HuH7 cells at 500 $\mu\text{g/ml}$. (C): HuH7 cells at 1000 $\mu\text{g/ml}$. (D): HuH7 cells at 1500 $\mu\text{g/ml}$.

ucts (Mokdad et al., 2015). Recently, medicinal plants have attracted research worldwide due to their unique and fascinating properties. Wang et al., (2018a) has reported that flavonoids have used as anti-aging, anti-viral/bacterial and cardio protective compounds. These agents protect cells by acting as antioxidants to neutralize the ROS. In healthy cells antioxidant enhance cell signaling to activate defense system of cells against oxidative stress and regulate the proliferation and apoptotic rate (Tait and Green, 2013; Ali and Ali, 2014; Halliwell and Gutteridge, 2015).

Current study was designed to assess the cell toxic and apoptotic effects of *A. visnaga* seeds extract on HuH-7 cells. The result

of MTT test exposed that *A. visnaga* decreased the viability HuH-7 cells. In addition, NRU test showed the toxic effects of plant extract in the treated cells. These findings may be attributed to the evident lysosomal damage. However, destabilization of lysosomes is considered to be an earlier sign of mitochondrial injury (Zhao et al., 2003). For further investigation, fluorescence microscopic analyze was done to confirm the loss of MMP. Fluorescence enhancement is related with function of the mitochondria, which swells during apoptosis and generate cellular ROS (Murphy, 2009). The most common pathway revealed that once mitochondrial functions affected, a reaction cascade enhanced by the release

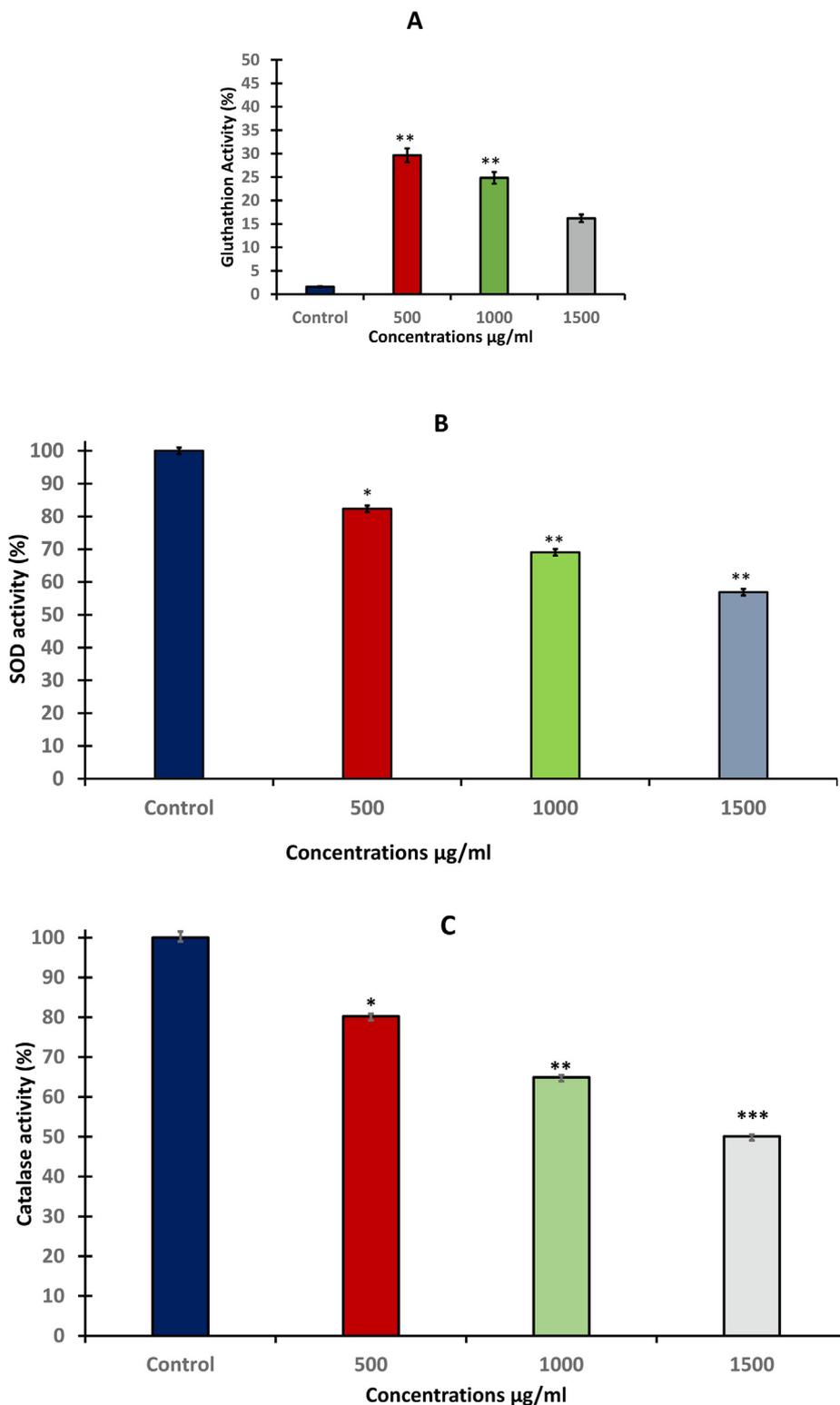


Fig. 3. Oxidative Stress Biomarkers after treating HuH-7 cell line with different concentration of *A. visnaga* seeds extract for 48 h. **A:** level of GSH. **B:** SOD activity, and **C:** level of catalase. Data represents the mean \pm SE of three experiments. n = 3, (**p < 0.01) compared with untreated control.

of cytochrome c and some proteins which produce the apoptosome complex, activates caspases (Garrido et al., 2006) as a consequence it promotes the generation of intracellular free radicals. These oxidant factors affect cell organelles and cellular compounds (Lobo et al., 2010). Furthermore, ROS promote the caspase-3 activity which in turn results in chromatin condensation in treated cell

lines. Furthermore, changes in the structure of lysosomes and mitochondria membranes increase their permeability, resulting in a release of their contents (Mathiasen and Jäättelä, 2002).

Superoxide dismutase SOD plays a dynamic role against ROS by converting antioxidant to harmless molecules. This enzyme mainly exists in the cytosol with different forms but also it is localized in

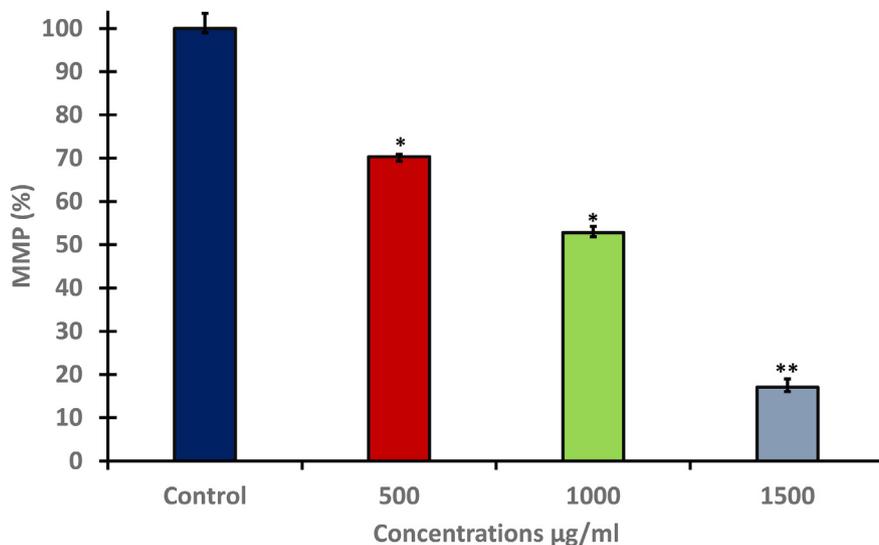


Fig. 4A. Level of MMP in HuH-7 after treating with different concentration of *A. visnaga* seeds extract for 48 h. Data represents the mean ±SE of three experiments. n = 3, (*p < 0.05 and **p < 0.01) compared with untreated control.

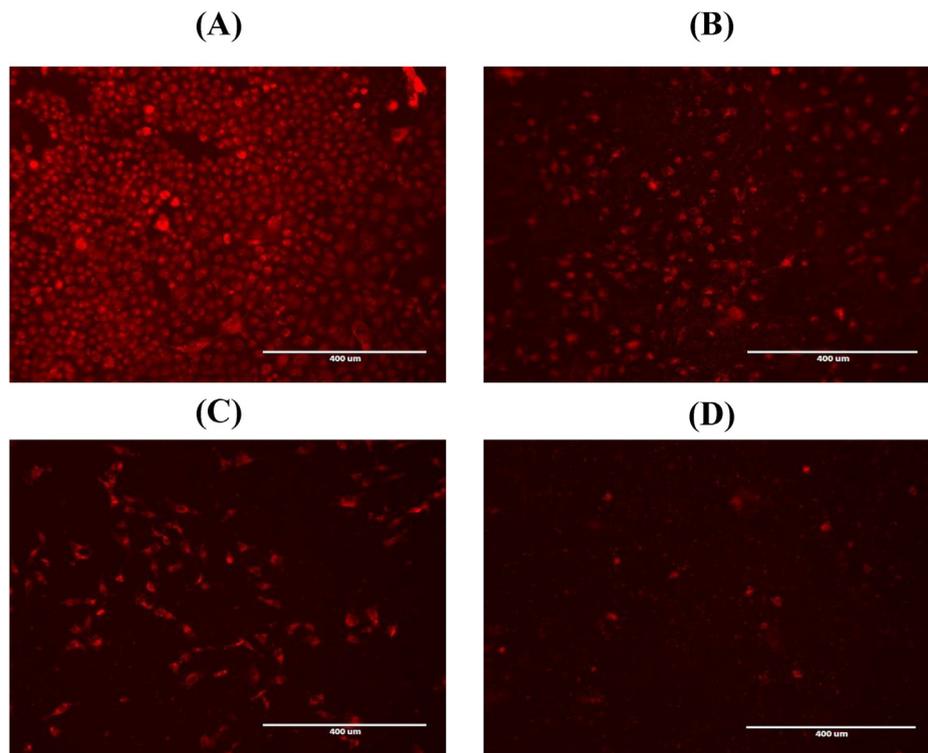


Fig. 4B. MMP as evaluated by rhodamine stain the strong MMP was found in control cells as dye penetrate to cells and produced aggregated (deep red fluorescence). Cells at 1500 µg/mL of *A.visnaga* exposure decreased their MMP and showed low density stain.

peroxisomes (Wang et al., 2018b). The expression of catalase enzyme varies in tissues; this variation reflects on the cellular stress (He et al., 2019). Downregulation of catalase may enhance oncogenesis by elevating ROS levels in transformed cells to take place and further, in metastatic tumor cells. Isolated cancer cells from circulating blood and secondary tumor sites have displayed greater cytoplasmic and mitochondrial-derived ROS than those from their primary tumors (Glorieux and Calderon, 2018). These results indicate that the increased levels of SOD are dynamic responses to ROS and/or oxidative damage and may relate to a defensive response to elevated intracellular H₂O₂. However, the

regulation of catalase expression under oxidative stress is not predictable (Choi et al., 2009). The action mechanisms of *A. visnaga* for the prevention of cell damage has not fully understood (Vanachayangkul et al., 2010). Our result showed that the lower and medium concentrations of *A. visnaga* induced a significant raise in the oxidative stress and GSH enzyme at 48 hr (Zalewska-Ziob et al., 2019). However, the high expression level of SOD was connected with survival in various types of cancerous cell lines (Robbins and Zhao, 2014). In contrast, it showed that reduced SOD expression is related with mortality of hepatocellular carcinoma (Wang et al., 2016). Our results demonstrated *A. visnaga*

extract induced apoptosis and oxidative stress, this result support previous study by Aydoğmuş-Öztürk et al., 2019 that revealed visnagin has the ability to induce ROS (Aydoğmuş-Öztürk et al., 2019).

Apoptotic genes are an essential marker to monitor cytotoxicity and genotoxicity that may have existed as a result of oxidative stress. *Bcl-2* gene plays a regulatory role as an anti-apoptotic factor, which maintain cell against apoptosis and allow DNA repair mechanisms to save cell life. Previous study showed that the low level of *Bcl-2* protein was involved in the apoptotic pathways of GCD II PCFs disturbs the engulfment of ROS. On the other hand, upregulation of catalase caused an increase in *Bcl-2* expression. Thus, ROS and *Bcl-2* levels have a reciprocal relationship where an increase in ROS correlates with a decrease in *Bcl-2* levels and vice versa. This finding suggests that increased ROS may reduce *Bcl-2* levels (Choi et al., 2009). In addition, it has been shown that ceramide treatments induce oxidative damage through proteolytic cleavage of catalase by activated *Cas-3*. Activated *Cas-3* reduced the levels of catalase protein because of its proteolysis, not because of inhibition of mRNA levels (Choi et al., 2009). The loss of mitochondria membrane potential has considered as the specific intrinsic mitochondrial pathway of in apoptosis as consequence the release of cytochrome c, in that way activating *Cas-9* followed by *Cas-3*, in the process of apoptosis (Tait and Green, 2013; Ali and Ali, 2014; Abdullah et al., 2018).

Current study reported the dysfunction of MMP due to *A. visnaga* extract that has been influenced by the upregulation of *Bax* and *Bcl-2* at transcriptional level (Wang et al., 2019). Moreover, the *Cas-3* was upregulated in a time- and dose-dependent manner. Previously, it was reported that *p53* promote the upregulation of *Bax*. Thus, it presumed that *p53* plays a crucial role in *Bax* upregulation by incorporating *Bax* into the mitochondrial membrane, and thus activates apoptotic pathways. However, in some types of cancer *Bcl-2* expression was negligible, and the rate of apoptosis was markedly higher than expected. In contrast *Bax*, has apoptosis-promoting activity and can counter the anti-apoptotic effects of *Bcl-2*. The *p53* is the tumor suppressor gene and the essential regulator of DNA repair, once *p53* activated, the homo-oligomerize of *Bak* and *Bax* occurs and leads to a different event like; mitochondrial outer membrane permeabilization (MOMP) (Sheikh and Fornace, 2000). Also, current study showed the *p53* gene was upregulated compared to the control as shown in the results section, this illustrated both *Cas-3* and *p53* were activated and induced apoptosis. The activation of *p53* arises due to cellular stress that eventually led to DNA damage which in turn induces pre-apoptotic gene expression on the mitochondrial membrane and induces caspase enzyme to promote death of cell (Sheikh and Fornace, 2000). Moreover, the *p53* turns to add various stress into different anti-proliferative responses such as its capability to

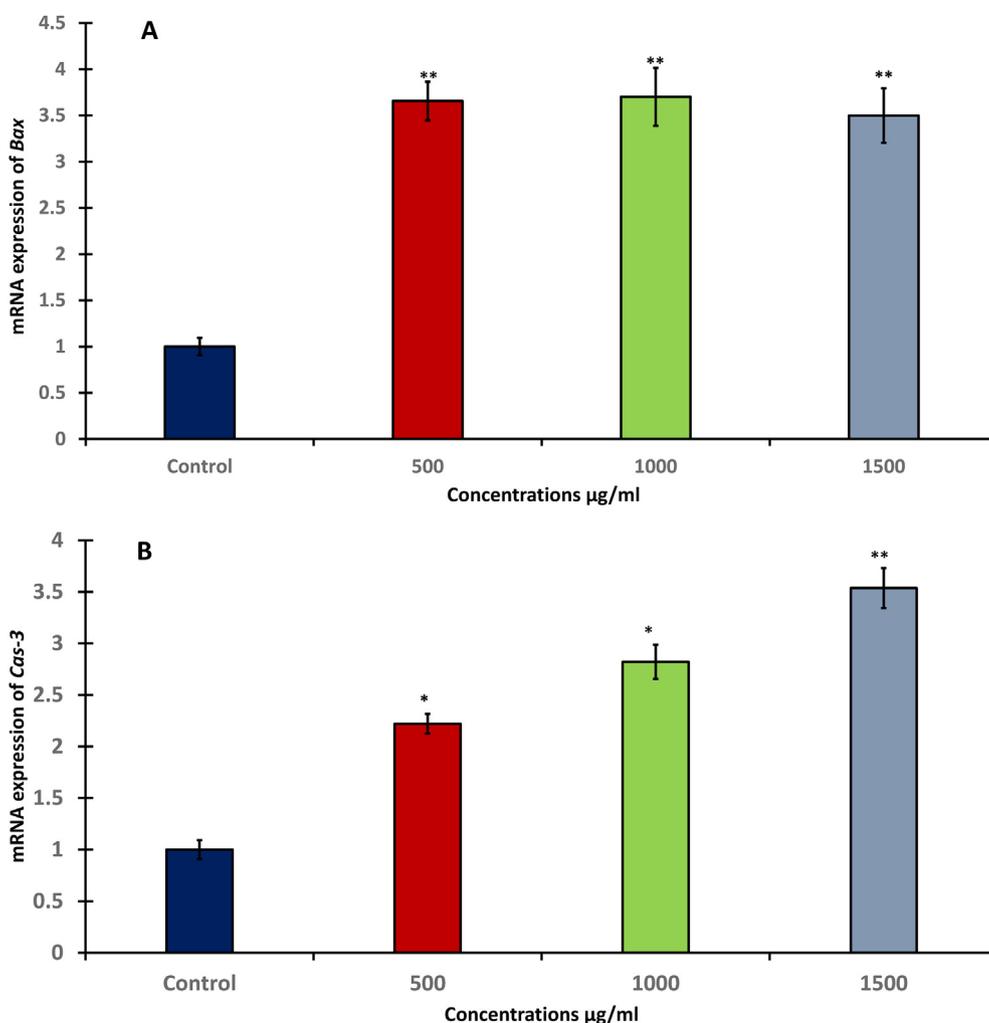


Fig. 5. RT-PCR of apoptotic genes in HuH-7 cells after treating with different concentration of *A. visnaga* seeds extract for 48h; **A**: *Bax* gene expression, **B**: *Cas-3* gene expression, **C**: *Bcl-2* gene expression, **D**: *p53* gene expression. Data represents the mean ±SE of three experiments. n = 3, (**p < 0.01) compared with control.

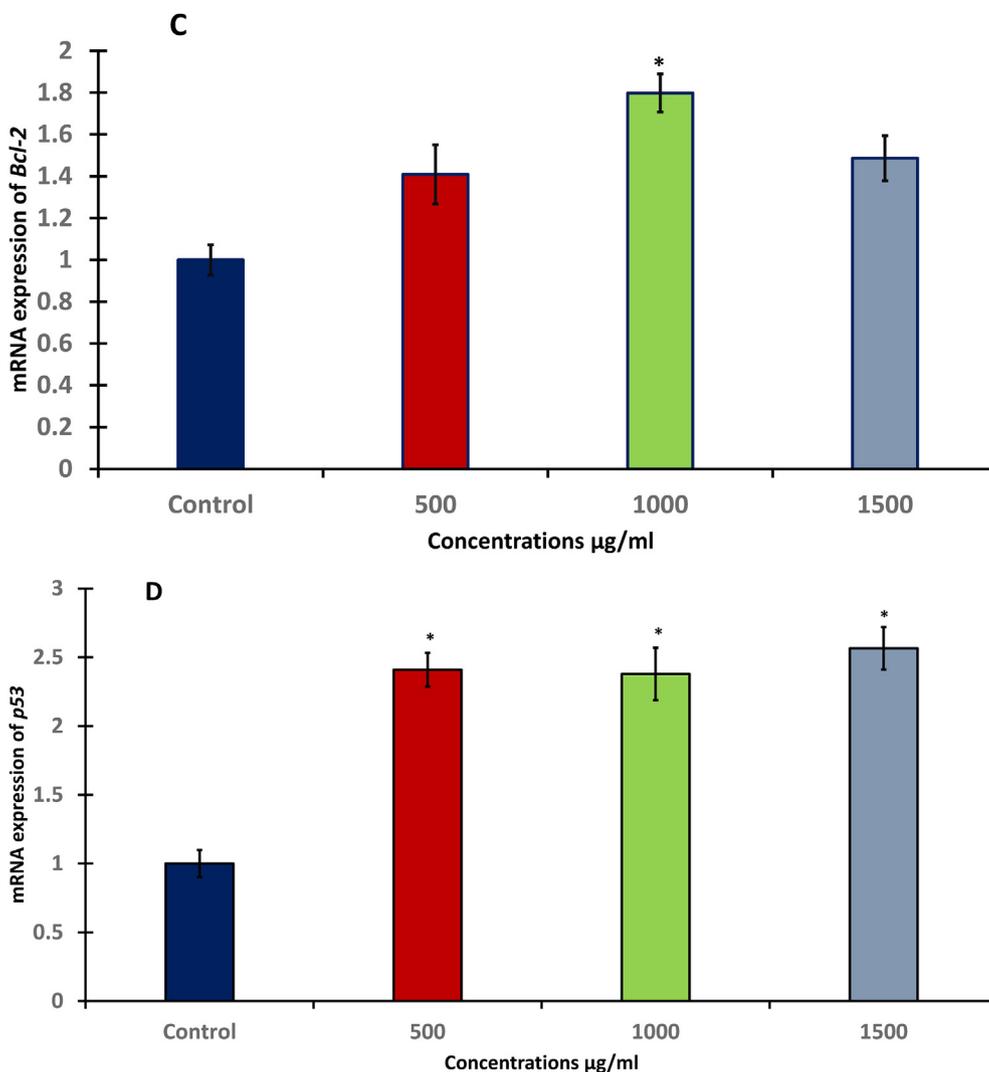


Fig. 7 (continued)

induce apoptosis, and interrupt promote tumor growth. Apparently *p53* induce apoptosis by transcription dependent and nondependent mechanisms.

5. Conclusion

The *A. visnaga* seeds extract has showed anti-cancerous properties and significantly decreased viability of HuH-7 cells. Moreover, toxicity of lysosomal was seen, indicating the toxicity of plant extract on treated cells. The plant extract also significantly increased the level of oxidative stress and altered the mitochondrial function, which lead to the onset of apoptosis in the HuH-7 cells. Upregulation of apoptotic; *Bax*, *Bcl-2*, *Cas-3* and *p53* genes expression was reported. Taken together, current findings revealed that *A. visnaga* seeds extract have hepato-protective potential against human hepatic cancer cell line HuH-7, signifying their promising clinical applications as a potential therapeutic agent for liver diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group No (RG-1441-018).

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