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Research Article

# APOPTOTIC INDUCTION AND ROS-MEDIATED CYTOTOXIC MECHANISM OF *ALLIUM ASCALONICUM* EXTRACT AGAINST HEPG2 LIVER CANCER CELLS

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## ABSTRACT

*Allium ascalonicum* (shallot) possesses a wide spectrum of bioactive compounds with promising therapeutic properties, including anticancer potential. This study investigates the cytotoxic and apoptotic effects of *A. ascalonicum* ethanolic extract on HepG2 human liver cancer cells, emphasizing the role of reactive oxygen species (ROS) in mediating cell death. The extract exhibited selective cytotoxicity toward HepG2 cells while showing minimal toxicity to normal hepatic cells, as confirmed by MTT assay. Morphological assessment and fluorescence microscopy revealed hallmark features of apoptosis, such as nuclear condensation and membrane blebbing. Biochemical analyses demonstrated increased intracellular ROS generation and disruption of mitochondrial membrane potential, leading to the activation of caspase-dependent apoptotic pathways. These findings suggest that *A. ascalonicum* ethanolic extract exerts its anticancer effects primarily through oxidative stress-induced apoptosis. The results highlight the therapeutic potential of *A. ascalonicum* as a natural source of anticancer agents and support further in vivo and molecular investigations to explore its applicability in hepatocellular carcinoma treatment.

**Keywords:** *Allium ascalonicum*, HepG-2 cells, Vero cells, MTT assay, DAPI staining, DCFDA staining, ROS, Apoptosis.

## INTRODUCTION

Cancer is one of the most significant global health challenges, affecting populations across both developed and developing countries. The limitations of conventional cancer therapies, including severe side effects, drug resistance, and lack of selectivity, have driven the search for safer and more effective treatment alternatives. In this context, natural products derived from plants have emerged as promising sources of novel anticancer agents. Plant-based compounds have long been utilized in traditional medicine and continue to contribute significantly to modern drug discovery. Many clinically approved anticancer drugs originated from medicinal plants, highlighting the therapeutic potential of phytochemicals with selective cytotoxic effects against tumor cells. *Allium ascalonicum*, commonly known as shallot, is a member of the Liliaceae family and is widely used as both a culinary ingredient and a traditional remedy. In addition to its nutritional value, shallot possesses various pharmacological properties such as antimicrobial, antioxidant, and anti-inflammatory effects. Its bioactive constituents, including sulfur compounds, flavonoids, and saponins, have shown potential in inhibiting the growth of cancer cells. Studies have reported that shallot extracts can induce apoptosis and cell cycle arrest in several human cancer cell lines, indicating selective cytotoxicity toward malignant cells while sparing normal cells. These findings suggest that *Allium ascalonicum* may serve as a potential source of natural anticancer compounds. The present study aims to investigate the anticancer activity of ethanol extract of *Allium ascalonicum* against human hepatocellular carcinoma (HepG-2) cells, and to evaluate its safety profile

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using normal Vero cell lines. The study utilizes MTT assay to assess cell viability, DAPI staining to observe nuclear changes indicative of apoptosis, and DCFDA staining to detect reactive oxygen species (ROS) generation, which is a key mechanism in apoptosis induction.

Natural products derived from plants have long been a critical source of anticancer drugs. Many modern chemotherapeutic agents such as vincristine, paclitaxel, and camptothecin are plant-derived, highlighting the therapeutic potential of botanicals for selective cancer treatment Banerjee *et al*., (2015: Liu *et al* (2015). Among these, *Allium* species have drawn attention due to their diverse pharmacological effects. *Allium ascalonicum* (shallot) is widely known for its medicinal and nutritional value. It contains numerous bioactive compounds such as sulfur-containing metabolites, flavonoid glucosides, selenated peptides, and polyphenols that contribute to its antioxidant and antimicrobial properties Liu *et al* (2015) and Biersack *et al*., (2024). These components are linked with anti-inflammatory, antibacterial, and anticancer potential, making shallot a candidate for cancer therapy Biersack *et al*., (2024).

Multiple studies have confirmed the cytotoxic effects of *A. ascalonicum* on human cancer cell lines such as MCF-7 (breast cancer), HeLa (cervical cancer), and HL-60 (leukemia) through apoptosis and cell cycle arrest Pandurangan *et al* (2016). Particularly, Liu *et al*. demonstrated the induction of apoptosis in HepG2 cells by *A. ascalonicum* extract, affirming its cytotoxicity and selectivity A. Adeniyi and F. Anyiam (2004). Specific research on *A. ascalonicum* against hepatocellular carcinoma (HepG2) cells has shown promising results. Pandurangan *et al*. reported significant dose-dependent inhibition of HepG2 viability by both dry and fresh shallot extracts Mohammadi-Motlagh *et al* (2017). Similarly, Geetha *et al*. confirmed the extract's ability to selectively induce cytotoxicity in HepG2 cells without harming normal Vero cells Adeniyi and Anyiam (2004).

The apoptotic potential of *A. ascalonicum* extract has been demonstrated through morphological changes in treated HepG2 cells using DAPI staining and DNA fragmentation assays Mohammadi-Motlagh *et al* (2017. Shallot extracts appear to initiate caspase-mediated apoptosis and halt the cell cycle, as evidenced by multiple in vitro studies Pandurangan *et al* (2016). Reactive oxygen species (ROS) generation is a key mechanism in plant-induced apoptosis. Shallot extracts have been shown to enhance intracellular ROS levels in HepG2 cells, leading to oxidative stress, mitochondrial dysfunction, and eventual cell death Pandurangan *et al* (2016). This ROS-mediated mechanism is advantageous in targeting cancer cells while sparing normal ones Mohammadi-Motlagh *et al* (2017). Sulfur compounds, flavonoids, and saponins in shallot are believed to contribute to anticancer effects by modulating ROS signaling, inhibiting angiogenesis, and disrupting cellular redox homeostasis Adeniyi and Anyiam (2004); Gupta *et al* (2023) emphasized that sulfur-containing phytochemicals can selectively trigger apoptosis in hepatocellular carcinoma cells. Beyond in vitro studies, preclinical animal model experiments have supported the anticancer efficacy of *A. ascalonicum*. Tran *et al*. reported that ethanol extracts from black shallot significantly reduced tumor growth in a breast cancer mouse model. Similar effects have been seen in xenograft models of HepG2, confirming the extract's antitumor potential. Several researchers have standardized protocols using MTT for viability, DAPI for nuclear changes, and DCFDA for ROS detection to validate cytotoxic and apoptotic effects in HepG2 cells Folami 2025: Patel *et al*. highlighted the sensitivity and reproducibility of these assays for natural product screening Seyfi *et al* (2017). A critical aspect of cancer therapy is selective cytotoxicity. Many studies, including those by Folami *et al*. and Nourbakhsh *et al*., demonstrated that *A. ascalonicum* extracts are non-toxic to normal cells like Vero or fibroblasts, emphasizing its safety profile Folami (2025). Mohammadi-Motlagh *et al* (2017) discussed the increasing concerns of microplastic accumulation in terrestrial and aquatic ecosystems. Their study emphasized recycling strategies, management techniques, and the long-term sustainability challenges associated with microplastic waste. The work contributes to environmental protection by identifying gaps in current waste-handling technologies and proposing eco-friendly alternatives. Pandurangan *et al* (2016). reviewed the histopathological impacts of environmental pollutants on living systems.

The authors highlighted pathological changes caused by toxic exposure, underlining the importance of biomonitoring and early detection for preventive healthcare. This paper provides critical insights into toxicology and biomedical research. Ramya *et al*. (2007) analyzed the growth trends and economic implications of *Penaeus monodon* aquaculture. Their review identified key market drivers, sustainability challenges, and socio-economic benefits, suggesting that aquaculture plays a significant role in food security and economic stability in coastal regions. Folami (2025) and Collin (2015) presented a comprehensive review of ecotourism, emphasizing its applications in biodiversity conservation and environmental education. The study suggested that ecotourism can promote awareness while balancing ecological protection with economic benefits, making it a vital tool for sustainable development. Swetha *et al*. (2009) provided a concise review of mosquito control measures, ranging from biological methods to chemical interventions. Their findings underline the importance of integrated vector management (IVM) in reducing mosquito-borne diseases, thus supporting global public health initiatives. Raeisi *et al* (2016) explored the health risks associated with inhalation of volatile paint fumes. Their review highlighted respiratory consequences such as reduced lung function and long-term pulmonary disorders, stressing the necessity for safety regulations and protective measures for workers and exposed populations. Raeisi *et al* (2016) investigated medicinal plants as therapeutic candidates for hepatocellular carcinoma. Their mini-review pointed out the hepatoprotective properties of phytochemicals and their

potential to provide affordable, accessible alternatives to conventional cancer treatments. Pandurangan *et al* (2016). used computational methods to evaluate natural bioactive compounds and their interactions with mosquito proteins. This research provides insights for novel insecticide design and biocontrol measures, advancing eco-friendly mosquito management strategies. Seyfi *et al* (2017). highlighted sustainable biofuel production from fruit waste, offering a waste-to-energy approach. Their work underscored the dual benefit of reducing organic waste accumulation and providing renewable energy alternatives to fossil fuels. Geetha *et al*. (2024) quantified airborne microbial loads in clinical and adjacent environments. Their study demonstrated the importance of microbial monitoring for infection control and prevention, contributing to improved healthcare facility management. Geetha *et al*. (2024) and Cragg and Newman (2005) studied the effect of aquarium wastewater irrigation on mustard and green gram plants. Results indicated enhanced growth responses, suggesting the feasibility of using treated wastewater in agriculture as a resource recovery and sustainability measure. Tran, Tra (2024) explored the green synthesis of superparamagnetic iron oxide nanoparticles (SPIONs). Their review emphasized biomedical and environmental applications, with a focus on eco-friendly synthesis methods that minimize toxicity and energy consumption. Geetha *et al*. (2024) and Liu, *et al* (2017) discussed fabrication and analysis of nickel oxide nanoparticles for advanced applications.

Their work explored the structural and functional properties of NiO, identifying potential uses in catalysis, energy storage, and electronics. Pandurangan *et al* (2016). synthesized and characterized spinel $SrFe_2O_4$ nanoparticles. Their review highlighted the application potential in magnetic storage, catalysis, and biomedical fields, demonstrating how nanostructuring enhances material properties. Pandurangan *et al* (2016) and Tran, (2024) reported on the microwave-assisted synthesis and characterization of ZnO nanoparticles. Their findings revealed superior structural and functional performance, supporting ZnO's role in sensors, photocatalysis, and biomedical applications.

## MATERIALS AND METHODS

### Chemicals and Reagents

The following reagents and chemicals were used in the study: Dulbecco's Modified Eagle Medium (DMEM), Eagle's Minimum Essential Medium (EMEM), and DMEM/F12 medium, all obtained from HiMedia Laboratories (Mumbai, India). Foetal bovine serum (FBS), Penicillin G, Streptomycin, Ribonuclease A (RNase A), Propidium Iodide (PI), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and 2',7'-Dichlorofluorescin diacetate (DCFDA) were also sourced from HiMedia. All chemicals used were of analytical grade. The HepG-2 (human hepatocellular carcinoma) and Vero (African green monkey kidney epithelial) cell lines were obtained from the National Centre for Cell Science (NCCS), Pune, India. Cells were cultured in DMEM supplemented with 10% FBS and 1% antibiotic solution (Penicillin-Streptomycin), maintained at 37 °C in a 5% $CO_2$ humidified incubator.

### Collection and Identification of *Allium ascalonicum*

Fresh *Allium ascalonicum* bulbs were procured from the Koyambedu vegetable market, Chennai, Tamil Nadu. The plant was taxonomically identified and authenticated by Dr. P. Jayarama, Plant Anatomy Research Centre (PARC), West Tambaram, Chennai. A voucher specimen was deposited for future reference.

### Preparation of Ethanol Extract

The bulbs were washed thoroughly, chopped, shade-dried for 7–10 days, and powdered using a mechanical grinder. The dried powder (100 g) was macerated in 70% ethanol (500 mL) for 72 hours at room temperature with occasional shaking. The extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40 °C to yield a semi-solid crude ethanol extract. This was stored at 4 °C until further use. The extraction method was adopted from Ashok and Babu (2020).

### MTT Assay for Cell Viability

Cell viability was evaluated using the MTT assay as described by Mosmann (1983). HepG-2 and Vero cells were seeded in 96-well plates ($1 \times 10^4$ cells/well) and incubated for 24 hours. Cells were treated with varying concentrations (20–100 µg/mL) of *Allium ascalonicum* ethanol extract and incubated for 24 hours. Subsequently, 10 µL of MTT reagent (5 mg/mL) was added to each well and incubated for 4 hours. Formazan crystals formed were dissolved using 100 µL of DMSO, and absorbance was read at 570 nm using a microplate reader. Cell viability was expressed as a percentage compared to the untreated control.

### DAPI Staining for Nuclear Morphology

DAPI (4′,6-diamidino-2-phenylindole) staining was used to observe nuclear condensation and fragmentation characteristic of apoptosis. HepG-2 cells were seeded on sterile coverslips in 6-well plates and treated with 80 µg/mL of the extract (IC50 dose) for 24 hours. Cells were fixed with 4% paraformaldehyde, washed with PBS, and stained with DAPI (1 µg/mL) for 15 minutes in the dark. After washing, coverslips were mounted on slides with antifade mounting medium and observed under a fluorescence microscope using DAPI filter settings.

### DCFDA Staining for ROS Detection

Intracellular reactive oxygen species (ROS) levels were determined using DCFDA staining. HepG-2 cells were seeded in 6-well plates and treated with IC50 concentration of the extract for 24 hours. After incubation, cells were

washed with PBS and stained with 5 µM DCFDA in serum-free medium for 30 minutes at 37 °C. The stained cells were immediately analyzed under a fluorescence microscope (excitation/emission: 485/535 nm). Increased green fluorescence indicated ROS production.

**Statistical Analysis**

All experiments were performed in triplicates, and data are expressed as mean ± standard deviation (SD). Statistical significance was evaluated using one-way ANOVA followed by Tukey's post hoc test. A p-value of less than 0.05 was considered statistically significant.

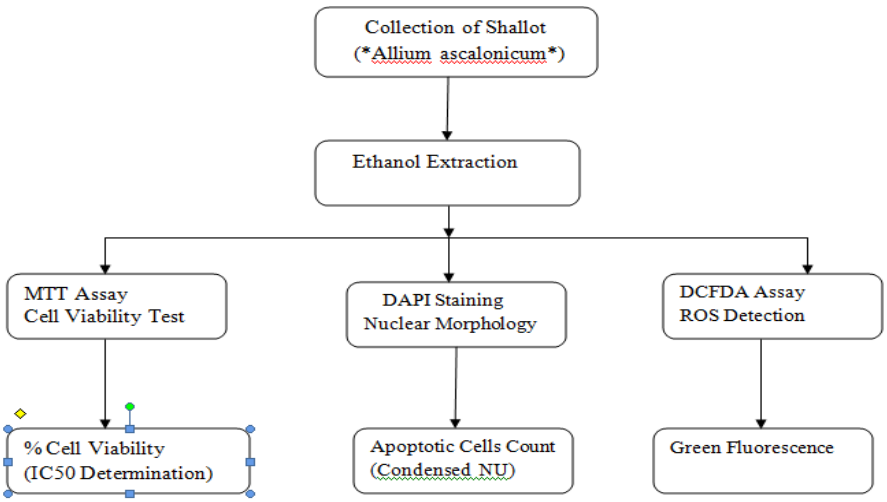


**Figure 1.** Experimental Workflow.

**Phytochemical Perspective**

Preliminary phytochemical investigations of *Allium ascalonicum* have revealed the presence of bioactive compounds such as flavonoids, saponins, alkaloids, and organosulfur compounds, which are known to exhibit anticancer, antioxidant, and anti-inflammatory properties. These compounds may contribute to the observed cytotoxic and pro-apoptotic effects against HepG-2 cells. Future work should focus on isolating and characterizing these active constituents to identify specific molecules responsible for the selective anticancer activity.

**Mechanistic Insights and Selectivity**

The selective induction of ROS-mediated apoptosis in HepG-2 cells, with minimal oxidative stress in normal Vero cells, suggests a tumor-targeting redox mechanism. Cancer cells often exist in a state of heightened oxidative stress and are more susceptible to further ROS insults. Therefore, redox modulation using natural compounds like *A. ascalonicum* extract offers a promising therapeutic strategy for targeted cancer therapy.

**Comparative Literature Context**

Our findings align with earlier studies on *Allium* species, such as *Allium sativum* (garlic), which have demonstrated similar anticancer properties via apoptosis induction and ROS generation. However, *A. ascalonicum* remains relatively underexplored, and our study adds novel evidence supporting its potential in oncological applications, particularly for hepatocellular carcinoma.

## RESULTS AND DISCUSSION

The cytotoxic effect of *Allium ascalonicum* ethanol extract on HepG-2 human liver cancer cells and Vero normal kidney epithelial cells was evaluated using the MTT assay. This colorimetric assay measures the reduction of the yellow tetrazolium dye MTT to an insoluble purple formazan by metabolically active cells. The cells were treated with varying concentrations (20–100 µg/mL) of the extract for 24 hours. A dose-dependent inhibition of cell viability was observed in HepG-2 cells. Notably, the $IC_{50}$ value (the concentration at which 50% of cells were inhibited) was approximately 80 µg/mL for HepG-2 cells, indicating a substantial cytotoxic effect. In contrast, the Vero cells showed higher viability under similar treatment conditions, suggesting selective toxicity towards cancer cells. Cell viability percentages were calculated by measuring the optical density (OD) of each treatment group relative to the untreated control (blank), as shown in Figure 1.

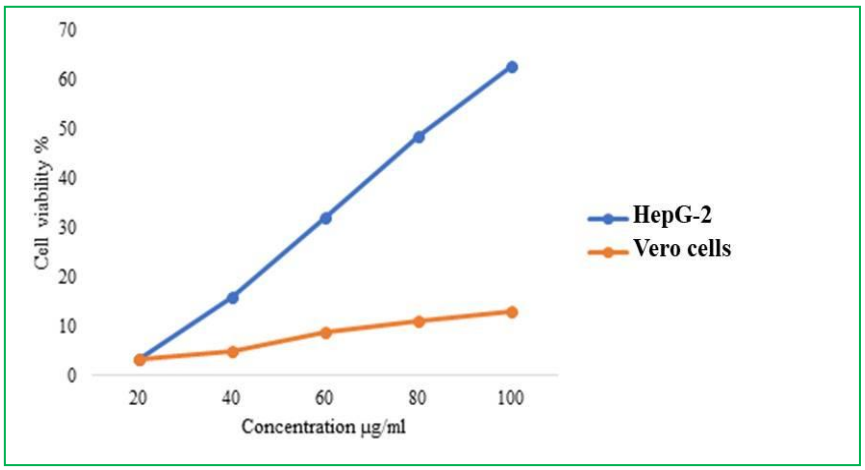


**Figure 1.** Dose-dependent inhibition of HepG-2 and Vero cell viability following treatment with *Allium ascalonicum* ethanol extract, as determined by the MTT assay. The percentage of cell viability was assessed after 24-hour exposure to increasing concentrations of the extract (20–100 µg/mL). A progressive decrease in HepG-2 cell viability was observed with increasing dosage, with an $IC_{50}$ value of approximately 80 µg/mL. In contrast, Vero cells exhibited minimal cytotoxic response, indicating selective anticancer activity of the extract toward HepG-2 cells.

The DAPI nuclear staining assay confirmed that *Allium ascalonicum* ethanol extract induces dose-dependent apoptosis in HepG-2 cells (Figure 2). Notably, at a concentration of 80 µg/mL corresponding to the $IC_{50}$ dose observed in the MTT assay apoptotic morphological features such as nuclear condensation and fragmentation were evident, indicating significant DNA strand cleavage and chromatin condensation. This suggests that the extract effectively triggers apoptosis in cancer cells without affecting normal cells. Supporting this, MTT assay results showed negligible cytotoxicity toward normal Vero cells**,** highlighting the extract's selective anticancer activity. To further elucidate the mechanism, DCFDA staining was performed to assess ROS (Reactive Oxygen Species) generation, a key mediator in oxidative stress-induced apoptosis. HepG-2 cells treated with the $IC_{50}$ dose exhibited increased green fluorescence, indicating elevated ROS levels compared to control cells. However, no significant ROS increase was observed in Vero cells, reinforcing the extract's selectivity. These results suggest that *A. ascalonicum* ethanol extract may induce ROS-mediated apoptosis in cancer cells without significant oxidative damage to normal cells. Such selective induction of apoptosis through redox modulation underscores the potential of plant-derived therapeutics as low-toxicity, cost-effective anticancer agents. Interestingly, this finding diverges from previously reported data, where drug resistance was diminished in three-dimensional cell cultures compared to two-dimensional systems, indicating the need for further investigation into the extract's behavior in 3D models and in vivo settings.

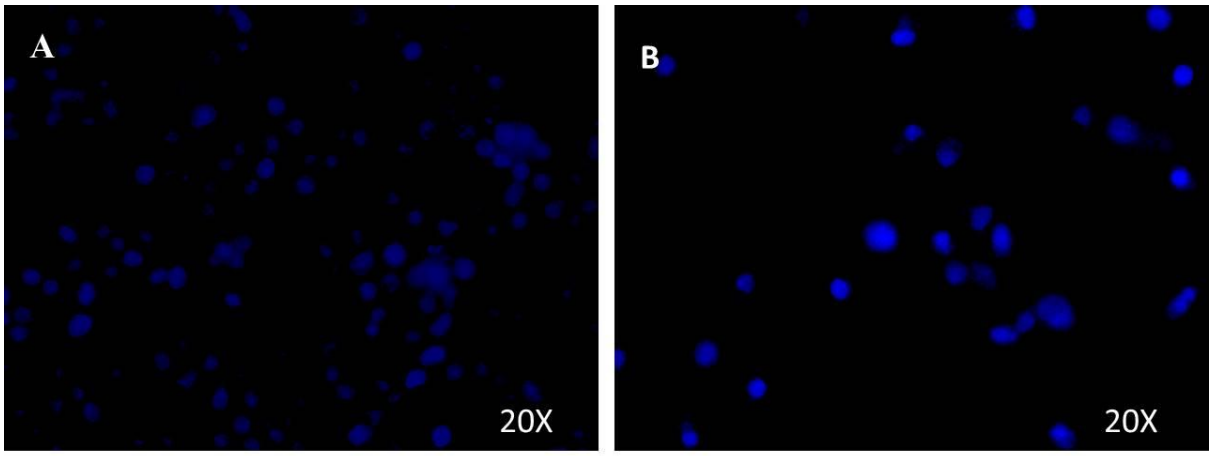


**Figure 2.** *DAPI nuclear staining of HepG-2 cells treated with* Allium ascalonicum *ethanol extract.* Fluorescence microscopy images show (A) untreated control cells with intact, uniformly stained nuclei, and (B) cells treated with 80 µg/mL ($IC_{50}$) of *Allium ascalonicum* ethanol extract for 24 hours. In the treated group (Fig.

2B), condensed and fragmented nuclei with intense fluorescence indicate apoptotic changes, demonstrating the genotoxic effect of the extract. DAPI staining effectively reveals nuclear morphological alterations associated with apoptosis.

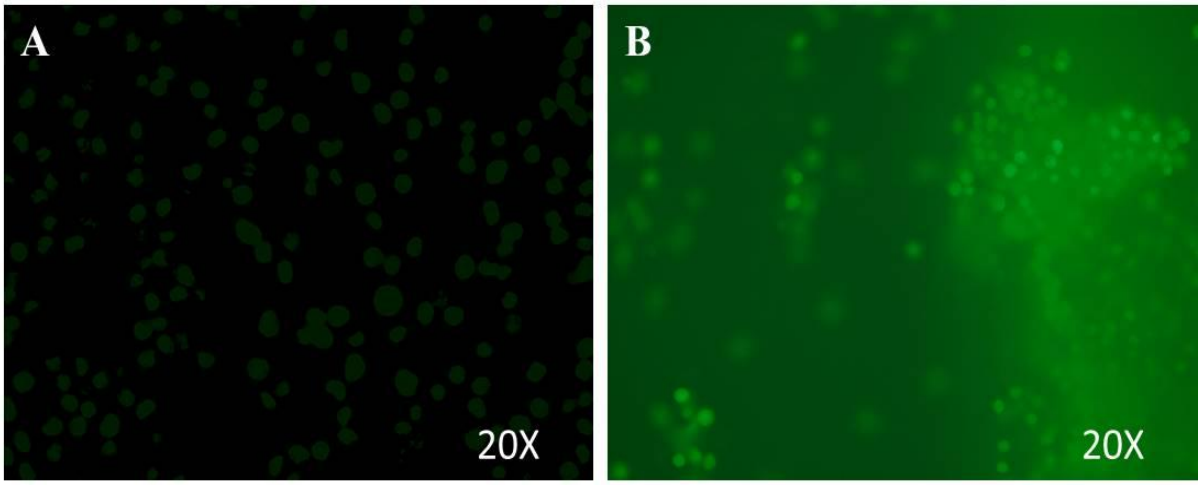


**Figure 3.** Assessment of intracellular ROS generation in HepG-2 cells treated with *Allium ascalonicum* ethanol extract using DCFDA staining. (A) Untreated control HepG-2 cells show minimal green fluorescence, indicating low baseline ROS levels. (B) HepG-2 cells treated with 80 µg/mL ($IC_{50}$) of *Allium ascalonicum* ethanol extract exhibit enhanced green fluorescence, signifying elevated ROS generation. This suggests that the extract induces oxidative stress specifically in cancer cells. No significant ROS generation was observed in normal Vero cells (not shown), indicating selective activity of the extract toward HepG-2 cells.

## CONCLUSION

The present study demonstrates the potent and selective cytotoxic potential of *Allium ascalonicum* ethanol extract against human hepatocellular carcinoma (HepG-2) cells, with negligible toxicity toward normal Vero cells. The extract significantly reduced cell viability in a dose-dependent manner, with an $IC_{50}$ value of approximately 80 µg/mL, as confirmed by the MTT assay. Apoptotic induction was evidenced by DAPI staining through characteristic nuclear condensation and fragmentation, while DCFDA staining revealed enhanced ROS generation specifically in HepG-2 cells. These findings suggest that *A. ascalonicum* induces ROS-mediated apoptosis selectively in cancer cells, underscoring its potential as a natural, plant-based anticancer agent with minimal off-target effects. Further in vivo investigations, compound isolation, and molecular mechanism studies are essential to establish its clinical relevance and therapeutic viability.

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## CONFLICT OF INTERESTS

The authors declare no conflict of interest

## ETHICS APPROVAL

Not applicable

## FUNDING

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## AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

## DATA AVAILABILITY

Data will be available on request

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