Research Article

Anticancer efficacy of dry and fresh *Allium ascalonicum* (shallot) against HepG2 cell line

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Abstract

Background: Liver cancer is a deadly disease and devastating public health problem globally. Natural plant products with potent growth inhibition, and apoptosis induction properties are extensively being investigated for their cancer-preventive potential. *Allium ascalonicum*, being an important part of the diet across various countries, possesses numerous therapeutic effects and health-enhancing properties such as cancer prevention. **Aims and Objective:** To investigate anticancer activity of dry and fresh *A. ascalonicum.* **Materials and Methods:** The organic (ethanol) extracts of dry and fresh shallot bulb (*A. ascalonicum*) were prepared and tested for in vitro anticancer efficacy on liver cancer cell line HepG2 by MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. **Results:** The results showed that both dry and fresh shallot extracts have anticancer potential with inhibitory concentration (IC_{50}) of 50 μg/mL. **Conclusion:** The plant investigated possesses remarkable anticancer activity. Further studies are essential for the isolation of lead molecules from the plant to treat liver cancer.

KEY WORDS: *Allium ascalonicum*; HepG2 Cell Line; MTT Assay; Anticancer Activity

Introduction

Liver cancer (hepatic cancer) is the most frequent cause of death. Hepatocellular carcinoma is the fifth commonest liver cancer in the world.[1] Plants have a long history of use in the treatment of cancer.[2] However, the undeniable role of diet containing plants should not be disregarded in the prevention of many diseases such as cancer. Until recently, a number of biologically active phytochemicals have been identified in plant foods.[3] Plant-derived compounds have also been an important source of several clinically useful anticancer agents, which include vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide derived from epipodophyllotoxin, and paclitaxel (taxol). Moreover, several promising new agents, including flavopiridol and combretastatin A4 phosphate, are in clinical development based on selective activity against cancer-related molecular targets while some agents that failed in earlier clinical studies have been noticed again.[4] Alternate therapeutic strategies were essential to overcome the toxicity of chemotherapy drugs and side effects of current treatment. Shallot (*Allium ascalonicum*) is a member of the *Liliaceae* family, which has been used mainly as a spice traditionally from the ancient times. It has many benefits including antibacterial and antifungal properties,[4,5] beneficial hematological influences,[6] antioxidant properties,[7] anti-*Helicobacter pylori* potential effect,[8] and peroxynitrite-scavenging capacity.[9] Besides, the chemical composition of *A. ascalonicum* analyzed by researchers revealed the presence of effective components such as a mannose-specific lectin,[10] an antifungal peptide,[11] new furostanol saponins,[12] selenium and sulfur species,[13] and various flavonol glucosides[14] that inhibit proliferation and growth of tumor cell lines as HeLa and MCF-7 cell lines extract.[15] Cytotoxic effect of selenized odorless garlic and shallot against

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human leukemia cells (HL-60)[13] arresting cell cycle progression and inducing apoptosis in human cervical carcinoma HeLa cells[16] have been identified and isolated from it. Moreover, shallots contain considerable amount of polyphenols that are well-known anticancer compounds.[31] The aim of this study was to investigate the cytotoxic activity of *A. ascalonicum* against liver cancer cell line HepG2 with the prediction of the presence of lead molecule from natural source, which could lead to fewer side effects, which could be extracted and purified further for the prevention or cure of liver cancer.

Materials and Methods

Collection of Plant Samples

The bulbs of *A. ascalonicum* were brought from local vegetable markets, Thanjavur, Tamil Nadu, India.

Preparation of Sample and Extracts

The shallot bulbs were cut into small pieces and allowed to dry at room temperature to prepare dry extract, and fresh extract was prepared without drying.

Ethanolic extract of *A. ascalonicum* was prepared by grinding the bulbs (50 g) in a mortar with 50 mL ethanol and stirred overnight to complete extraction and was filtrated through a Whatman no.1 filter paper and centrifuged at 16,000 rpm at 4°C for 30 min. The collected ethanol extracts were dried in hot air oven at 80°C and 50°C for 20–30 min, respectively. One gram of plant sample (dry and fresh shallot extract) was taken and dissolved in 1 mL incomplete media for preparing various concentrations of plant extract.

MTT Assay

Collection of HepG2 cell lines: HepG2 cells were purchased from National Centre for Cell Science (NCCS), Pune. HepG2 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) medium (Gibco, Gaithersburg, MD) supplemented with 10% of fetal bovine serum (FBS: Gibco) and 2% of penicillin–streptomycin antibiotics (Gibco). Cultures were kept at 37°C in a humidified atmosphere of 95% air and 5% CO_2, and trypsin-EDTA.

Cytotoxicity Assay

The cytotoxicity assay was performed by the MTT method. HepG2 cells (1×10^4) were seeded onto a 96-well plate and incubated overnight. The cells were then treated with fresh and dry shallot extract (at 0, 5, 10, 25, 50, 100, 200 μg) for 24 h. An untreated group was used as a control. Following 24 h of cell treatment, the MTT dye (thiazolyl blue tetrazolium bromide: (3-(4, 5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was added at 5 mg/mL into each well and incubated for 3 h in the dark. The formazan crystal products formed were dissolved by the addition of 100 μL of Dimethyl sulfoxide (DMSO) . After 15 min, the amount of purple formazan was determined by measuring the optical density (OD) using the ELISA microplate reader at 595 nm. The experiment was performed in triplicate and the percentage of cell viability was calculated. The cell growth was expressed as a percentage of absorbance in cells with (fresh and dry shallot extract) treatment to that in cells without (fresh and dry shallot extract) treatment (100%). The inhibitory concentration (IC_{50}) was calculated using the formula as follows:

$$IC_{50} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

Result

The seeded 25 μg of HepG2 (liver cancer) cell lines were treated with the various concentrations of plant extracts and the results were obtained as follows:

Shallot *(A. ascalonicum)* Induces Dose-Dependent Growth Inhibition of HeLa Cells

MTT assay was used to determine the cytotoxic effect of *A. ascalonicum* on HepG2 cells. Cells were treated with different concentrations of the extract (5, 10, 25, 50, 100, and 200) for 24 h. *A. ascalonicum* effectively inhibits HepG2 cell proliferation in a dose-dependent manner. The percentage of cell viability was found to gradually decrease from lower concentration to higher concentration. The IC_{50} of the dry shallot extract *(A. ascalonicum)* was found to be around 50 μg/mL for HepG2 cells (Figure 1 and Table 1).

Shallot *(A. ascalonicum)* Induces Dose-Dependent Growth Inhibition of HeLa Cells

MTT assay was used to determine the cytotoxic effect of *A. ascalonicum* on HepG2 cells that where treated with different concentrations of the extract (5, 10, 25, 50, 100, and 200) for 24 h. *A. ascalonicum* effectively inhibit HepG2 cell proliferation in a dose-dependent manner. The percentage of cell viability is found to gradually decrease from lower concentration to higher concentration. The IC_{50} of the fresh shallot extract (*A. ascalonicum)*

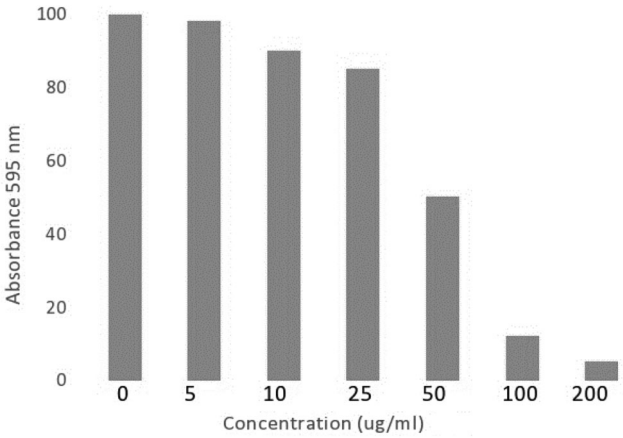

Figure 1: Percentage of cell viability in dry ethanol extract treated HepG2 (liver cancer) cells.

Table 1: HepG2 cell viability with different concentration of dry *Allium ascalonicum* ethanolic extract

Concentration (mg/dl)	0 (control)	5	10	25	50	100	200
Row-1	3.33	2.67	2.671	1.944	1.113	1.097	0.097
Row-2	2.718	2.823	1.9	2.234	1.813	0.174	0.174
Row-3	2.254	2.501	2.573	2.208	1.685	1.092	1.092
Average	2.767333	2.664667	2.381333	2.128667	1.537	0.787667	0.454333

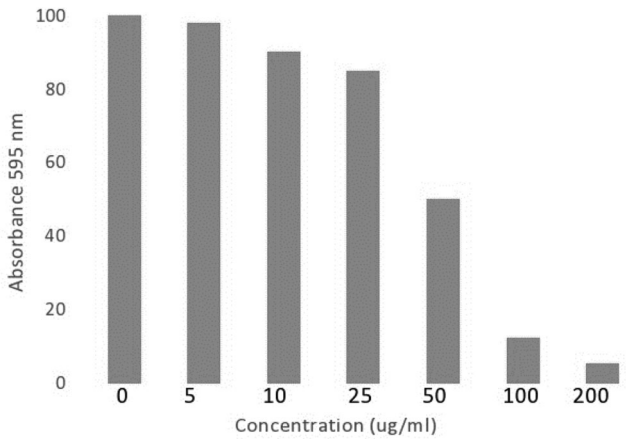

Figure 2: Percentage of cell viability in fresh shallot extract treated HepG2 (liver cancer) cells.

was found to be around 50 μg/mL for HepG2 cells (Figure 2 and Table 2).

Discussion

Diet plays a major role in cancer etiology and prevention. Although inconsistencies exist across studies that have investigated the relationship between diet and cancer, dietary factors undoubtedly influence cancer risk.[17] There is increasing evidence that fruits and vegetables have chemopreventive properties because of the supplementary and synergistic effects of various phytochemicals present in these nourishments.[18,19] From another point of view, herbs and spices are generally considered safe and proved to be effective against various human ailments, and their medicinal uses have increased gradually in many countries. In this regard, to assess the anticancer properties of plants that have been widely consumed in human diet, the use of vegetable extracts provides an interesting approach because these extracts contain several bioactive molecules.[20,21]

A. ascalonicum is one of the important *Allium* species commonly used in the diet of Asians and in traditional medicine since ancient times. A lot of evidence also suggested that *Allium* possesses anticancer properties as shown by their ability to suppress tumor proliferation in vivo and in vitro.[22,23] Despite wide consumption of *A. ascalonicum*, reports regarding its biological effects are rarely compared to other *Allium* species such as garlic and onion. *A. ascalonicum* is usually known for hypocholesterolemic, antimicrobial, antidermatophytic, and anti-angiogenic properties.[4,7,24,25]

A. ascalonicum has been also reported to exhibit antioxidative and free-radical scavenging capacities. These properties appear to be related to the high contents of flavone, sulfur-containing compounds, and polyphenolic derivatives in the bulb of *A. ascalonicum*. Furthermore, it is shown that the antioxidant potential of *A. ascalonicum* comparing several onion varieties and some garlic preparations is more noticeable.[6,26] Previous literature review revealed that, shallot like other *Allium* species contains organosulfur compounds including allicin-decomposition products (diallyl disulfide, diallyltrisulfide, and ajoene), *S*-allyl cysteine was shown to have potential antitumor activities. Because of the abundance of organosulfur compounds of *Allium* plants, these compounds may be responsible for some beneficial properties of these plants. Thus, the biological properties of *Allium* plants, such as antiangiogenesis and anticancer effects, could be more possibly attributable to organosulfur ingredients.[27]

The present study was performed to examine the possible potential of *A. ascalonicum* extracts. For this purpose, we examined in vitro cytotoxic effect of ethanolic extract of dry and fresh *A. ascalonicum* (shallot) against HepG2 cell line (liver cell line) at different concentrations in MTT assay. The finding showed that *A. ascalonicum,* which contains many beneficial phytochemicals, significantly affects the viability of cancer cells indicated by IC_{50} value.

Table 2: HepG2 cell viability with different concentration of fresh Allium ascalonicum **ethanolic extract**

Concentration (μg/ml)	0 (control)	5	10	25	50	100	200
Row-1	4.211	4.97	3.671	4.19	2.09	1.024	0.304
Row-2	4.718	3.823	4.09	3.342	2.922	0.109	0.131
Row-3	4.354	4.231	4.373	4.056	2.009	0.292	0.092
Average	4.4645	4.341333	4.044667	3.862667	2.340333	0.475	0.175667

Conclusion

In conclusion, this study has provided evidence of anticancer or cytotoxic activity of ethanolic extract from shallot bulbs in vitro. The ethanolic extracts of both fresh and dry shallots were tested for cytotoxic activity in HepG2 (liver cancer) cell line by MTT assay. The result shows that both dry and fresh shallot extracts has anticancer potential than with IC_{50} of 50 μg/mL. These findings emphasize promoting increased consumption of shallot plant as an essential means to prevent or even to treat cancer. However, further research is essential for exploration of the molecular mechanisms on anticancer activity of active compounds from shallot.

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