Anti-Cancer Activity Of *Allium Ascalonicum* Ethanol Extract Against Human Hepatocellular Carcinoma Cell Line (HepG-2)

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Abstract

Chemotherapy is a widely used medicine for the treatment of cancer globally. However, these agents are highly toxic and very little effective in cancer. Determining the anti-cancer medicine from plant sources with selective toxicity and high potency is essential. In this study, we evaluated the anti-cancer potential of ethanol extract of *Allium ascalonicum*. Solvent-solvent extraction methods were used to collect the extract from the plant. The anti-cancer study was carried out in HepG-2 cells. The results showed the anti-cancer potential of Allium ascalonicum ethanol extract on HepG-2 cells, determining dose-dependent apoptosis and selective cytotoxicity. The extract might modulate the mitochondrial membrane potential and ROS levels to induce apoptosis of cancer cells. In conclusion, *Allium ascalonicum* might be useful for treating Hepatocellular Carcinoma.

Keywords: HepG-2 cells, Vero cells, MTT assay, DAPI staining, DCFDA staining.

Significance | Investigating plant-derived treatments like *Allium ascalonicum* offers safer, effective alternatives to toxic chemotherapy for combating cancer with targeted potency.

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Introduction

Cancer is a significant global health challenge affecting both developed and developing countries (Bruijnincx & Sadler, 2008; Jiao et al., 2013; Solowey et al., 2014; Evidente et al., 2015; Grigalius & Petrikaite, 2017). The demand for cancer treatments is high, leading researchers to seek medications with fewer side effects, often turning to natural sources due to limitations in current pharmaceutical manufacturing processes. As a result, there's been a recent shift towards investigating anti-cancer compounds derived from plants. Plants have a long history of being used in cancer treatment, and plant-based diets are known to play a crucial role in preventing various diseases, including cancer (Bhalla et al., 2013). Plants contain numerous biologically active compounds that have proven effective in fighting cancer (Lichota & Gwozdzinski, 2018; Jiao et al., 2016; Sawicka et al., 2012; Colic & Pavelic, 2000). Substances like Vinblastin, Camptothecin, Topotecan, Paclitaxl, and Vincristine have been derived from plants and used as anti-cancer agents. New drugs like flavopiridol and combretastatin A4 phosphate also show promise in targeting specific molecular pathways in cancer cells. However, some of these agents have faced challenges in clinical trials (Evidente et al., 2014; Holla et al., 2004; Bayal et al., 2014; Jungwirth et al., 2011; Liu et al., 2014).

Shallots (*Allium ascalonicum*), belonging to the Liliaceae genus, have attracted attention beyond their culinary use due to their diverse health benefits. Studies have shown that shallots possess antifungal, antibacterial, and antioxidant properties, and they can also combat infections like Helicobacter pylori (Mohammadi-Motlagh et al., 2011; Răduică & Popescu, 2010; Raeisi et al., 2016). Research into the chemical composition of shallots has identified

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potent components such as mannose, antifungal peptides, furostanols, selenium, sulfur, and various flavonol glucosides, all of which have been found to inhibit tumor cell growth in laboratory studies on tumor cell lines such as HeLa and MCF-7 (Pobłocka-Olech et al., 2016). Moreover, studies have demonstrated the cytotoxic effects of shallots on leukemia and cervical carcinoma cells, indicating their potential to stop cancer cell growth and inducing cell death (Irfan, 2013). These findings suggest that natural compounds found in shallots, particularly in ethanol extracts, may hold promise as prospective cancer treatments. Given the urgent need for effective and targeted cancer therapies, further exploration of these natural compounds is warranted.

Materials and Methods

Chemicals and reagents

DMEM, foetal serum of bovines and penicillins G and streptomycin, is obtained from Himedia. Bought from Eagles modified essential medium (EMEM) and Delbecco modified eagles media F12 (DMEM/F12), Ribon nuclease (RNase), and propidium iodide (PI), MTT, 2', 7'- dichlorofluorescéin diacetate (DCFDA). Cells stored with 10 percent foetal calf serum in EMEM and DMEM mediums and 5 percent CO2 with antibiotics in humidified environment at 37^{0}C. The NCCS (Pune), India, developed hepG-2 (human hepatocellular carcinoma) lines and the Vero cells (Kidney epithelial cells of African green monkey).

Collection and identification of *Allium ascalonicum*

Allium ascalonicum were purchased from Koyambedu vegetable market and were authentically identified by Dr. P. Jayarama, PARC, West Tambaram, Chennai, Tamil Nadu.

Extraction of *Allium ascalonicum*

Allium ascalonicum was extracted using ethanol, according to Ashok and Babu's method (2020). Fresh leaves of Vigna radiata were collected from in vitro cultured and field-grown micropropagated plants. These leaves were air-dried in a closed, dark environment at 40°C for 7 days. Once dried, they were powdered using a mixture grinder and stored at 4°C for later use. The powdered plant material was then extracted using two different solvents (methanol and acetone) for 12 hours using a Soxlet apparatus. After extraction, the solvent extracts were air-dried for 24 hours and stored at 4°C for future use.

MTT assay

The viability of cells HepG-2 and Vero was analysed as defined by Mosmann, 1983. Following the method described by as described by Mosmann (1983) and Ashok and Sivakumari (2020), the anti-cancer activity was assessed using the MTT assay. HepG-2 and Vero cells were seeded onto 96-well plates at a density of 1 x 10^5 cells/ml (100 µl per well), with sterile phosphate buffer saline (PBS) added to the edge as a blank control. After incubating at 37 °C with 5% CO2 for one day to allow cell attachment, the medium was removed, and the monolayer cells were washed twice with 1 ml of trypsin (0.25%)/EDTA (0.05%) solution when reaching full confluence. The sample extract was diluted in DMEM medium (containing 2% serum), with 0.1 ml of each dilution pipetted into the wells, while the control wells contained only DMEM medium. After incubation at 37 °C, the plates were examined for signs of toxicity. Subsequently, 20 µl of MTT (5 mg/ml PBS) was added to each well and incubated for 1-5 h at 37 °C in 5% CO2. To dissolve the formazan (MTT metabolic product), 200 µl of DMSO was added to each well and stirred. The absorbance at 560 nm was then recorded using a microplate reader (MR-96A, Mindray, China). The survival cell percentage was calculated using Equation 1.

$$\%\text{Cell viability} = \frac{\text{A treated cell}}{\text{A control cells}} \times 100 \qquad \text{----------(1)}$$

DAPI staining

A nuclear staining for HepG-2 has been performed by using DAPI stain, which has been treated with *Allium ascalonicum* ethanol extarct, and by using filtering suitable for Spector *et al.*(2001) process to monitor the apoptotic morphology of cells below a fluorescence microscope.

DCFDA staining

Fluorescent microscope and DCFDA stain were used to monitor reactive oxygen (ROS) generation. Briefly, cells were seeded in six-well plates and were allowed to bind 5-FU and *Allium ascalonicum* ethanol extract concentrations to IC_{50} for 24 hours, along with control for the night. The cells were stained by fluorescent microscopy for 30 minutes with the 5 mM/L DCFDA at 37^{0}C Figueroa et al (2018).

Results and Discussion

The MTT test determined the viability of cells, a colorimetric test to measure the behaviour of the tetrazolium dye MTT, which is an insoluble formzan with a violet hue. Cell viability has been measured. *Allium Ascalonicum* ethanol extarct was administered at various doses (20-100 µg/ml) for 24 hours to assess cell viability inhibition of cancer cells. The dosage rises and 50 per cent of cells (HepG-2 cells) were lower at approximately 80 µg/ml extract compared to the average (Vero cells) of treatment cells as shown in Figure 1. By measuring the O.D of the blank treatment, the percentage of cell viability was calculated.

The present research showed that the DAPI nuclear staining test established that *Allium ascalonic* ethanol extract induced dose-dependent apoptosis in hep G-2 cells (Figure 2). Extract-induced DNA strand cleavage in HepG-2 cells was observed that 80µg/ml of *Allium ascalonicum* ethanol extract therapy could induce apoptosis in HepG-2 cells. But, plant-based medicines with powerful anti-cancer benefit were found to work toward finding an inexpensive medicine with the least therapeutic side-effects in the human cervical cancer row. Therefore we have tested the cytotoxicity of

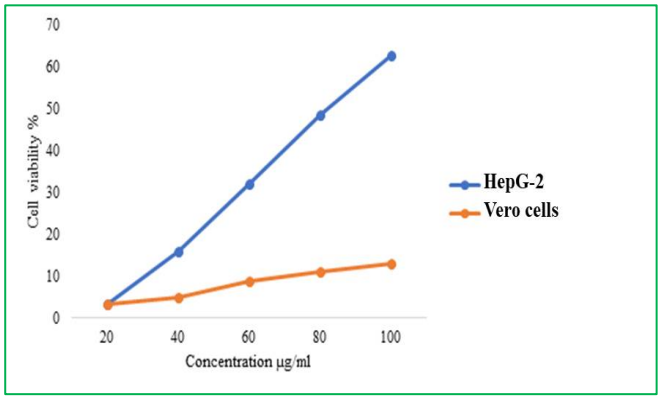

Figure 1. The drug dosage indicates percent HepG-2 and Vero cell inhibition after treatment with Allium ascalonicum ethanol extract. The outcome of the MTT test can be observed that the IC_{50} dosage of *Allium ascalonicum* ethanol extract is 80µg/ml, whereas *Allium ascalonicum ethanol* extract does not substantially inhibit the growth of Vero cells. If the dosage increases, the viabilty of HepG-2 cells decreases, whereas such control cell inhibition (Vero cells) was not seen with *Allium ascalonicum* ethanol extract.

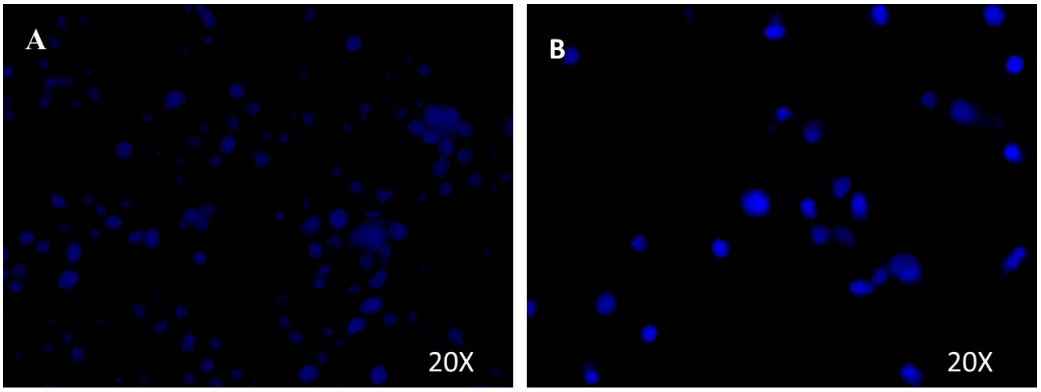

Figure 2. As a result of DAPI staining Fig. 2 B in comparison with (A), Control-A and treated B the genotoxic action of the *Allium ascalonicum* ethanol extract. DAPI staining is a reliable apoptotic test used in fluorescent microscopy to detect the nuclear morphology of cells. The luminous, fluorescent cells showing the apoptotic cells can be detected from (B). After 24 hours of administration to HepG-2, the assay was performed with Allium ascalonic ethanol (80 µg/ml *i.e.* IC_{50}).

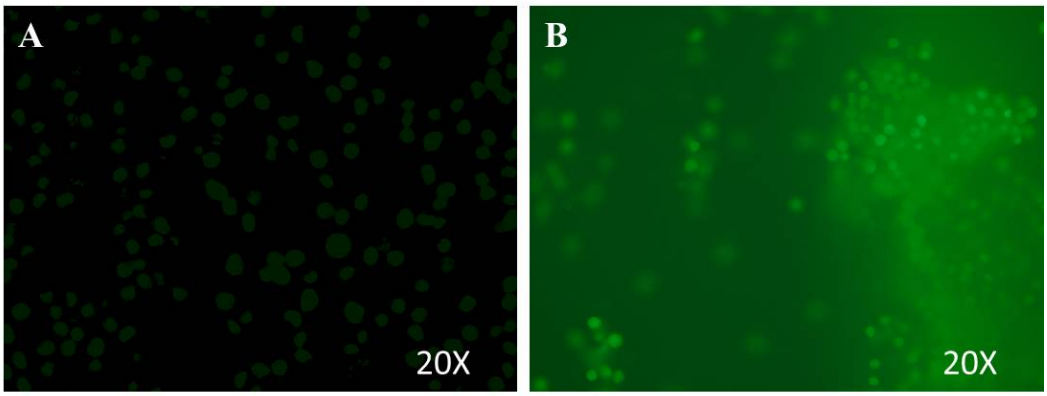

Figure 3. After treatment with *Allium ascalonicum* ethanol extract (B) HepG-2) higher ROS was found in cancer cells by DCFDA staining compared with cancer management (A) HepG-2 Control). There has been a limited focusing of *Allium ascalonicum* ethanol extract since only hepG-2 cells released ROS.

Allium ascalonicum's ethanol extract shows cytotoxic effects on HepG-2 cells, whereas the *Allium ascalonicum* ethanol extract has shown its non-toxicity to human (Vero) cells, as shown by the MTT trial. As it was found with many staining effects, the extract of *Allium ascalonic* ethanol discriminates against cancer and the normal cells, and then the ROS-mediated apoptosis in cancer and normal cells has been stained with DCFDA (Figure 3). The effect of DCFDA staining was noted that ROS showing green fluorescent cells were more than controlling cells, while after treatment of the IC_{50} concentration of *allium ascalonic* ethanol extract in HepG-2 cells, fluorescent cells did not increase significantly relative to control cells. The manipulation of ROS levels by redox modulation thus is a way of killing cancer cells selectively without inducing major cell toxicity Rutley & Miller (2020) and Ezaki et al (2000). This finding conflicts with previously published literature in which the resistance to chemical agents relative to those generated on two-dimensional surfaces was decreased by cells grown in three-dimensional culture systems Kumar et al (2018), Chen et al (2019), Goud et al (2019) and Donthiboina et al (2019).

Conclusion

The effectiveness of *Allium ascalonicum* ethanol extract against cancer cells, particularly in HepG-2 monolayer culture, is noteworthy. This extract has inhibited cancer cell growth, including monolayers and spheroids, without affecting normal cell function (Vero cells). Our study also investigated the anti-cancer effects of *Allium ascalonicum* ethanol extract, focusing on its impact on p53 and selectivity. Results revealed improvements in mitochondrial membrane potential and increased ROS signals in HepG-2 cells compared to Vero cells, leading to apoptosis induction through molecular pathways.

Author contribution

G.N.B., C.V., S.M., M.S. conceptualized, reviewed the literature, and wrote the article.

Acknowledgment

None declared

Competing financial interests

The authors have no conflict of interest.

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