Original Article

Potential Anti-Cancer Activity of Grewia Asiatica Ethanolic Extract on Sf767 Cell Line

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ABSTRACT

Objective: This study aims to assess the anti-cancer potential of the Grewia asiatica plant extract against SF767 cell lines, exploring its efficacy as a cost-effective and low side-effect alternative in cancer treatment.
Study Design: In Vitro Experimental Study.
Place and Duration of Study: This study was conducted at the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, from July 2021 to Dec 2021.
Methods: SF767 cell lines are treated with ethanolic Grewia asiatica plant extracts, and their viability is systematically assessed through MTT, crystal violet, trypan blue, and scratch assays. These methods collectively provide insights into the growth rate, metabolic activity, and overall viability of SF767 cells exposed to the plant extract.
Results: SF767 cells treated with ethanolic Grewia asiatica plant extracts exhibit a significantly reduced proliferation rate, as indicated by the MTT assay. Both crystal violet and trypan blue assays confirm a substantial inhibition of cell viability. The scratch assay further supports these findings, emphasizing the efficacy of Grewia asiatica in impeding cancer cell proliferation.
Conclusion: In conclusion, the Grewia asiatica plant extract demonstrates robust in vitro anti-cancer properties against SF767 cell lines. The observed reduction in tumor cell growth, inhibited metabolic activity, and compromised cell viability underscore the potential therapeutic significance of the plant extract in anti-cancer treatments. Further research, including animal and human trials, is imperative to validate and translate these in vitro findings into practical applications for cancer therapy.
Key Words: normal cells, cancer, apoptosis, anti-proliferation, angiogenesis

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INTRODUCTION

Gliomas are obtained from glial cells, which are neoplasms heterogeneous group and account for 40%–45% of all intracranial tumors [(1)]. These neuroepithelial tumors include anaplastic astrocytomas, oligodendrogliomas, and glioblastoma multiform astrocytomas[(2)]. Astrocytoma, glioblastoma, and anaplastic astrocytoma are different types of gliomas, whereas types of Ependymomas include subependymoma, anaplastic ependymoma, and myxopapillary ependymoma. Moreover, oligodendroglia, anaplastic oligodendroglioma, and anaplastic oligoastrocytoma are included in the types of oligodendrogliomas [(3).]

So, it is an uncommon condition that is undeniably challenging to treat among any remaining kinds of disease. Over 90% of patients inhale the dust because of this condition, and the people who make it live with other clinical diseases. The kind of glioma decides your treatment and your guess. Generally, glioma therapy choices incorporate medical procedures, radiation treatment, chemotherapy, designated treatment, and exploratory clinical preliminaries [(4)].

Grewia asiatica is a colorful shrubbery plant thought of horticulturally as a little natural product crop yet utilized as a people's medication. The ready phalsa natural products are devoured new, in pastries, or handled into reviving foods grown from the ground drinks delighted in during warm late spring a very long time in INDIA [(5)]. Be that as it may, phalsa natural product has a short usability period and is considered reasonable just for nearby promotion. This plant can be locally found in the regions of India and Southeast Asia yet is developed on a business scale for the most part in

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India's northern and western territories It was introduced in the East Indies and the Philippines before the turn of the 20th century, and it was accepted gradually in parched regions of Luzon Island.

Grewia asiatica is a plant native to South Asia, known for its edible leafy foods with therapeutic properties. It is a rich source of nutrients and bioactive compounds. Various parts of the plant have pharmacological effects, such as anti-cancer, anti-microbial, anti-emetic, and pain-relieving properties. [6].

Natural products have been perceived as significant for human health since the beginning of civilization. They play an important role in many countries' social and health systems and are believed to have health benefits due to their antioxidants, bio-functional and chemo-preventive compounds. Fruits, in particular, are viewed as a source of essential nutrients for a healthy lifestyle.[7].

"Grewia asiatica" is named after Nehemiah Grew, an inventor of plant physiology, and its Asian origin. It is known for its nutritional and healing properties. However, due to the lack of writing on this plant, it has been dismissed.

METHODS

SF767 cell line was obtained from the University of Lahore. Dried Grewia asiatica plant was processed into powder and added to a hydro-ethanoic solvent for 3 days. The extract was filtered and evaporated to obtain a thick concentrate. The extract was tested on SF767 cells and compared to a negative control. MTT Analysis using 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide

MTT analysis was applied to determine cell feasibility (Sigma Aldrich, USA). The experiment was performed in triplicates. SF767 and normal cells were used for the assessment, and an MTT assay was performed utilizing a 96-well plate. The cells were rinsed using PBS (phosphate buffer saline) and cultured for 2 hours in 100µl of serum-free DMEM medium and 25 µl of MTT solution (with concentration 5mg/ml). After that, the purple-colored formazan crystals were solubilized using 10% sodium dodecyl sulfate (SDS), and the absorbance at 570 nm was measured.

Crystal Violet Analysis

Crystal violet reagent was used to assess cell viability of SF767 cells. The cells were treated with 0.1% crystal violet mixed with 2% ethanol, hatched with color for 15 minutes at room temperature, and then washed. Next, 1% SDS was used to solubilize the crystal violet stain, and the absorbance of cell suspensions was recorded at 595 nm using a spectrophotometer on a miniature titer plate. Live dead measure

Trypan blue was used to identify alive and deceased cells, and the percentage of dead cells was calculated. The cells from numerous experimental collections, treated and untreated, were washed multiple times with PBS for at least 5 minutes before incubating with trypan blue (Invitrogen Inc., USA). After that, these cells were rinsed multiple times using PBS and examined via the magnifying instrument. Trypan blue-stained cells will be regarded as diseased cells.

Scratch Assay

Scratch measure was performed in 6 well cell culture plates using the IC50 upsides of concentrates following Liang et al. convention. Pictures were caught at 0, 48 and 72 hours.

RESULTS

Cellular Metabolic Activity by MTT Assay: MTT assay determined that the conversion of tetrazolium reagent into a formazan reagent occurred in metabolic active cells. In the SF767 treated cell line, more tetrazolium was found than the purple formazan dye, which concluded that cell growth and proliferation are inhibited. According to the data in Figure 1, the metabolic activity of the cells is reduced by increasing the drug doses from 20µg/ml to 1000µg/ml, and increasing the drug dose further causes cell inhibition and growth.

Table No. 1: The Cell Viability Values of Untreated and Treated Hepg2 Cells

Groups & Doses	Values(±SEM)
Untreated	0.295 ± 0.0348
G. asiatica ethanolic extract treatment (20µg/ml)	0.411 ± 0.126
G. asiatica ethanolic extract treatment (50µg /ml)	0.419 ± 0.144
G. asiatica ethanolic extract treatment (100µg /ml)	0.234 ± 0.0631
G. asiatica ethanolic extract treatment (500µg /ml)	0.191 ± 0.0432
G. asiatica ethanolic extract treatment (1000µg /ml)	0.0527±0.0100

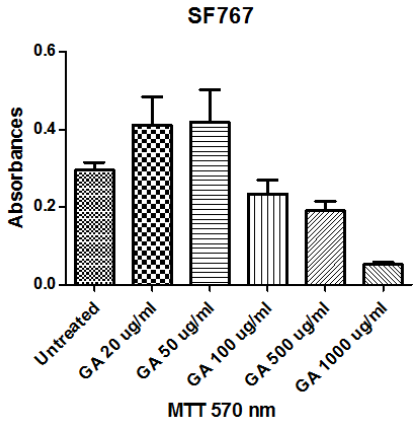

Figure 1: Plant extract inhibited SF767 proliferation. The cells were treated with different concentrations of plant extract (20µg/ml to 1000µg/ml) for 72 hr.

The absorbances of viable cells were determined using MTT analysis. The growth of SF767 cells was inhibited in a dose-dependent manner.

Ic50 Evaluation: After 72 hours of experimenting, the MTT analysis was applied using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to determine the IC50. Subsequently washing the monolayer of cells with PBS (phosphate buffer saline), the cells were treated for 2 hours in 100 µl complete medium comprising 25 µl MTT solution. MTT was transformed to a purple-colored formazan in active cells, which was formerly solubilized with DMSO (dimethyl sulfoxide) and was measured at the absorbance of 570 nm.

MTT Assay was performed to evaluate the IC50 dose and applied to cancer cells; there were reduced viable cells. MTT assay determined that the conversion of tetrazolium reagent to a formazan reagent occurred in metabolic active cells. In the SF767 treated cell line, more tetrazolium was found than the purple formazan dye, which concluded that the cell growth and proliferation were inhibited. According to the data in Figure 2, the metabolic activity of the cells was reduced by increasing the drug doses from 20µg/ml to 1000µg/ml, which caused cell inhibition and growth, and IC_{50} was observed at dose 643.1µg/ml.

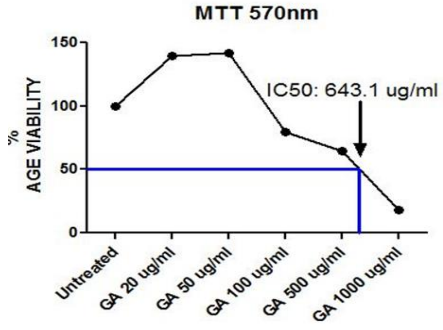

Figure No. 2: Plant extract inhibited SF767 proliferation.

Altered concentrations of plant extract (20µg/ml to 1000µg/ml) were used to treat the cells for 72 hr. IC50 was calculated as 643.1µg/ml.

Normal Cell Line:

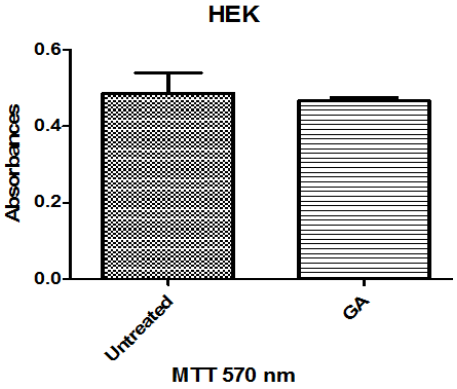

Figure No. 3: Figure shows the activity of plant extract on HEK cell line

Cell Adhesion Assay By Crystal Violet Staining: The CV dye binds to only adherent cells and not to dead floating cells; based on this, the relative adherence of cells can be calculated under different concentrations of the drug.

According to the data in Figure 4, adherence was reduced with an IC50 dose of plant extract as compared to untreated cells, which means the viable and adherent cell count has been reduced by plant extract.

Table No. 2: The Cell Viability Values of Untreated and Treated Hepg2 Cells

Groups & Doses	Values (±SEM)
Untreated	0.413 ± 0.0345
G. asiatica ethanolic extract treatment (643.1µg/ml)	0.246± 0.0157

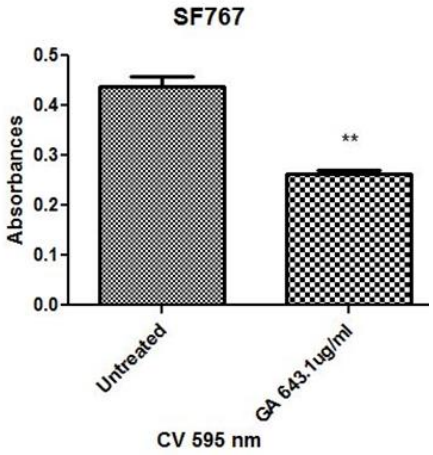

Figure 4: The graphical representation illustrates that plant extract significantly interfered with the primary adhesion of SF767 cells when treated for 72 hours of experiment, and absorbance was taken at 595nm of optical density. Asterisk shows significant values between treated and untreated groups.

Trypan Blue Assay: The trypan blue dye binds to only dead floating cells based on it; calculation was done by counting live and dead cells under IC50 concentration of plant extract. According to the data in Figure 5, adherence is reduced with the IC50 dose compared to the control, in which the activity of plant extract has reduced the viable and adherent cell contact.

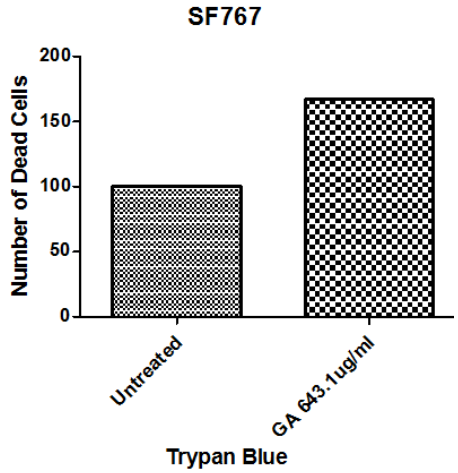

Figure 5: The graphical representation illustrates that plant extract significantly interfered with the primary adhesion of SF767 cells when treated for 72 hours of the experiment.

Scratch Assay Discloses The Migration Ability Of Sf767 Cell Line: The Scratch assay evaluated the effect of plant extract on the migration of the SF767 cell line. The images were captured using phase contrast microscopy.

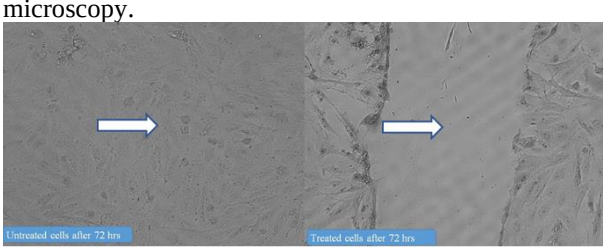

Figure 6: Phase contrast imaging of untreated and plant extract-treated SF767 cells.

The untreated cells had almost filled the whole scratch gap, depicting the metastatic ability of cancer cells. The SF767 cells treated with plant extract in 643.1µg/ml contraptions for 72 hrs. have significant anti-metastatic effects.

The images of cells after 72 hours in Figure 6 show cells along with linear scratch. After 72 hours, the scratch gap is filled in the untreated group. Plant extract efficiently reduced the migratory potential of SF767 cells, visible from the unfilled gap area.

DISCUSSION

Cancer is a lethal disease that spreads to different body parts and has various treatment choices with side effects. It results from uncontrolled cellular proliferation with genetic variability.[8].

Various chemotherapeutic medications are now accessible for treating and regulating cancer, thanks to the noteworthy improvements in cancer treatments. Nevertheless, these drugs have been proven beneficial in cancer treatment, yet they exhibited several drawbacks, including drug resistance and non-specific treatment[9]. Curative and therapeutic plants are widely employed in practice worldwide due to their remedial properties for various ailments [10]. WHO (World Health Organization) has proclaimed that these therapeutic plants are valuable in storing and reserving essential phytochemicals that may contain numerous pharmacological effects. Furthermore, relating to synthetically prepared medicines, the therapeutic compounds acquired from these medicinal plants are comparatively harmless and cost-effective. Consequently, it is imperative to concentrate on the pharmaceutical drugs prepared from natural plants, proving to be reasonable, harmless, and operative [11]. Herbal plants are largely employed in traditional medicine due to their diverse healing characteristics, increasing researchers' curiosity to discover more about them [12].

Plant-derived natural chemicals are expected to be crucial in developing prospective medications for lethal syndromes. It's significant to look for novel anti-cancer drugs extracted from natural plants with extraordinary antioxidant properties, as nutritional inconsistencies can be the main reasons for several cancer types worldwide[13]. The study's major purpose was to explore the anti-proliferative properties of Grewia asiatica against the Glioblastoma SF767 cell line. For this purpose, ethanolic extract from the plant was prepared. Our findings suggested that SF767 cells inspected using ethanolic plant extracts proliferated at a considerably lower rate.

Some plant extracts showed anti-cancer effects when evaluated with MTT assay [14]. Marya et al. found that Grewia asiatica extract showed significant activity against cancer cells in an MTT assay. The extract inhibited cell growth and proliferation in a dose-dependent manner, with an IC50 dose of 643.1µg/ml.

Crystal violet discoloration is a very versatile and quick method designed to monitor cell feasibility even in varied stimulation environments [15]. However, this theoretically collaborates using proliferative reactions arising simultaneously with cell death reactions. Further, advanced studies can be performed later to understand specific cell death mechanisms. The stages of molecular studies can be performed to specifically state the nature of deceased cells [16]

In this study, the anti-cancer potential of plant extract was assessed through the crystal violet technique, which exhibited the declined tendency of cell viability. The declined feasible cells suggested that this plant

extract action proved to be very operative in contradiction of glioblastoma cells because a reduced amount of feasible cells were observed in treated cell groups when matched with untreated groups.

Furthermore, a trypan blue exclusion assessment identifies how many deceased cells exist in the cell solution. It implies that living cells possess complete cell membranes that act as discharging specific dyes, like propidium, eosin, or trypan blue. However, deceased cells own nothing like that. In this test, a cell suspension was mixed with dye and visually examined to determine whether cells take up or exclude dye. The present study assessed the cell feasibility of Grewia asiatica through the trypan blue technique, which displayed dead cells when IC_{50} of plant extract was used. The enhanced quantity of deceased cells suggested disruption in the cell membranes of glioblastoma carcinoma cells.

Cell migration is an important feature of tissue repair, development, and regeneration [17]. In cancer cells, the migration assay depicts the metastatic potential of the cells. The biological ability of cancer cells to colonize, migrate, and seed to distant sites in the body makes it an aggressive death agent [18]. After treatment with plant extract, the cells were poorly migrated, the scratch width was still visible, and the cells could be seen under stress. Plant extract significantly altered the cell migration and metastatic ability of SF767 cells, and a big gap area can be seen. However, untreated cells showed migration and filled the gap within 3 days.

Our findings suggested that SF767 cells inspected using ethanolic plant extracts proliferated at a considerably lower rate.

CONCLUSION

Grewia asiatica extract has shown effective in vitro anti-cancer ability, as confirmed by MTT, crystal violet, trypan blue, and scratch assays. Its concentration was found to be inversely proportional to cell viability. This study suggests that natural plant extracts like Grewia asiatica can be a cost-effective and promising alternative for cancer treatment, but further research is needed to test it on other cell lines, animals, and humans.

Author's Contribution:

Concept & Design of Study:	Rubab Aftab
Drafting:	Ahmad Farooq Butt, Qurrat-Ul-Ain Leghari
Data Analysis:	Nooria Naeem, Sara Mukhtar, Maimoona Nasreen
Revisiting Critically:	Rubab Aftab, Ahmad Farooq Butt
Final Approval of version:	Rubab Aftab

Conflict of Interest: The study has no conflict of interest to declare by any author.

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Ethical Approval: No.UOL/IMBB/189-9-21 dated 11.07.2021

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