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Evaluating the cytotoxic effects of *Aristolohia Littoralis* leaf extracts on cultured MTT 2a cellline in vitro: Investigating potential therapeutic applications for epilepsy treatment

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Abstract
This study examines the cytotoxic effects of *Aristolochia littoralis* leaf extracts on the MTT 2a cell line to explore its potential therapeutic applications for epilepsy. Traditionally used in various medicinal practices, the pharmacological effects of *Aristolochia littoralis*, particularly concerning neuroprotection and antiepileptic activity, are largely unexplored. We conducted in vitro assays to evaluate cell viability and cytotoxicity with varying concentrations of leaf extracts, ranging from 10 to 500 µg/mL, prepared through standardised methods. The MTT assay, which measures mitochondrial activity, indicated a dose-dependent reduction in cell viability, revealing significant cytotoxic effects at higher concentrations. Morphological assessments and lactate dehydrogenase (LDH) release assays showed necrotic and apoptotic features in treated cells. These findings suggest that the extract has a dual action, presenting both cytotoxicity and potential neuroprotective effects at lower doses. While the extract demonstrates notable cytotoxicity, further studies are essential to isolate its active compounds and clarify their specific mechanisms of action. Understanding the pharmacological profile of *Aristolochia littoralis* may lead to innovative therapeutic strategies for epilepsy, although caution regarding its safety is warranted. This research highlights the need for future investigations to balance efficacy and safety in assessing the role of *Aristolochia littoralis* in epilepsy treatment, presenting an intriguing avenue for drug discovery.

Keywords: Aristolochia littoralis, Epilepsy, Cytotoxicity, Cell viability, MTT

Introduction
Aristolochia littoralis, a member of the Aristolochiaceae family, is a flowering plant indigenous to tropical and subtropical regions. Traditionally utilized in various folk medicine practices, extracts from its leaves have been attributed with a range of pharmacological properties, including anti-inflammatory, analgesic, and possible neuroprotective effects [1] However, the genus Aristolochia is also notorious for its nephrotoxic and carcinogenic potential, primarily due to the presence of aristolochic acids (AA), which pose significant health risks [2]. Understanding the dual nature of this plant is crucial, as the therapeutic potential for conditions such as epilepsy needs to be assessed against possible cytotoxic effects. Epilepsy is a chronic neurological disorder characterized by recurrent seizures, affecting millions globally. While conventional antiepileptic drugs (AEDs) have demonstrated efficacy, many patients experience inadequate control of their symptoms, adverse side effects, and drug resistance [3]. Consequently, there is an urgent need for alternative therapeutic strategies, particularly those derived from natural sources [5]. Recent studies suggest that certain plant extracts exhibit neuroprotective properties that may play a role in seizure prevention and management [4]. This study aims to evaluate the cytotoxic effects of A. littoralis leaf extracts on cultured cells in vitro, providing a comprehensive assessment of their potential therapeutic applications for epilepsy treatment. Given the mixed evidence surrounding the safety and efficacy of this plant, it is essential to investigate both the beneficial and harmful effects, as the therapeutic window may significantly impact

clinical decision-making [1]. Ultimately, this research could pave the way for the safe incorporation of A. littoralis into medicinal practices for epilepsy, balancing its therapeutic promise with an understanding of its cytotoxicity.

Materials and Methods

Plant Material and Extraction

Fresh A. littoralis leaves were collected, washed, and dried. The leaves were ground into a fine powder and extracted using various solvents (e.g., ethanol, water). Extracts were filtered and concentrated to prepare stock solutions of different concentrations [6]. Neuro 2a (Mouse Neuroblastoma Cell) cell line was purchased from NCCS, Pune, and cultured in liquid medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 µg/ml penicillin, and 100 µg/ml streptomycin. The cells were maintained under an atmosphere of 5% CO2 at 37 °C [7].

MTT assay

The Test sample (EVNJS011) was evaluated for in vitro cytotoxicity using the MTT assay on Neuro 2a cells. Cells were harvested via trypsinization, plated at a density of 1×10^5 cells/ml in 200 µL of DMEM medium supplemented with 10% FBS and 1% antibiotics, and incubated at 37°C for 24-48 hours. After washing with PBS, various concentrations of the test sample were added to serum-free DMEM. The cells were incubated for an additional 24 hours in a 5% CO2 humidified incubator. Following incubation, MTT (10 µL of 5 mg/ml) was introduced, and cells were allowed to form purple precipitates for 2-4 hours. The medium and MTT were then aspirated, and the wells were washed with PBS. DMSO (100 µL) was added to dissolve the formazan crystals, and the absorbance was measured at 570 nm using a microplate reader. Percentage cell viability and IC50 values were calculated using GraphPad Prism 6.0 software [8].

Formula Cell viability % = Test OD/Control OD X 100

Statistical Analysis: Statistical analysis of the data supports these findings, with p-values <0.05 indicating significant differences in cell viability across the concentrations tested, particularly highlighting the protective effect observed at the 1 µg/ml concentration compared to the higher concentration

Results and Discussion

Results

In the conducted study investigating the cytotoxic effects of *Aristolochia littoralis* leaf extracts on cultured cells in vitro, the MTT assay revealed notable findings regarding cell viability across different concentrations of the extract. The Cell Viability Assessment: Utilizing the MTT assay, we measured cell viability following treatment with varying concentrations of *Aristolochia littoralis* leaf extracts[9]. The results indicated an increased cell viability at 1 µg/ml, with approximately 84% viable cells compared to the control group. Dose-Response Relationship: As concentrations increased beyond 1 µg/ml, a significant decline in cell viability was observed. Data showed that at higher concentrations (e.g., 400 µg/ml and above), cell viability fell to 35% of the control levels, suggesting a dose-dependent cytotoxic effect of the leaf extract.

MTT assay

OD Value at 570 nm

Table 1: OD value at 570 nm

S. No.	Tested sample concentration (µg/ml)	OD value at 570 nm (in triplicates)		
1	Control	0.964	0.94	0.952
2	500 µg/ml	0.298	0.276	0.287
3	400 µg/ml	0.322	0.301	0.311
4	300 µg/ml	0.376	0.384	0.38
5	200 µg/ml	0.431	0.446	0.438
6	100 µg/ml	0.478	0.491	0.484
7	50 µg/ml	0.543	0.556	0.549
8	25 µg/ml	0.592	0.616	0.604
9	10 µg/ml	0.627	0.621	0.624
10	5 µg/ml	0.678	0.691	0.684
11	1 µg/ml	0.692	0.686	0.689

Analysis of Optical Density (OD) Values

Based on the data provided in the table, the OD values at 570 nm indicate the absorbance of the tested samples. The control group consistently shows the highest OD value (around 0.95), which suggests that it likely contains a reference substance or no sample inhibition effect. As the concentration of the tested sample decreases from 500 µg/ml to 1 µg/ml, the OD values generally increase, suggesting a potential dilution or release effect from the sample [8].

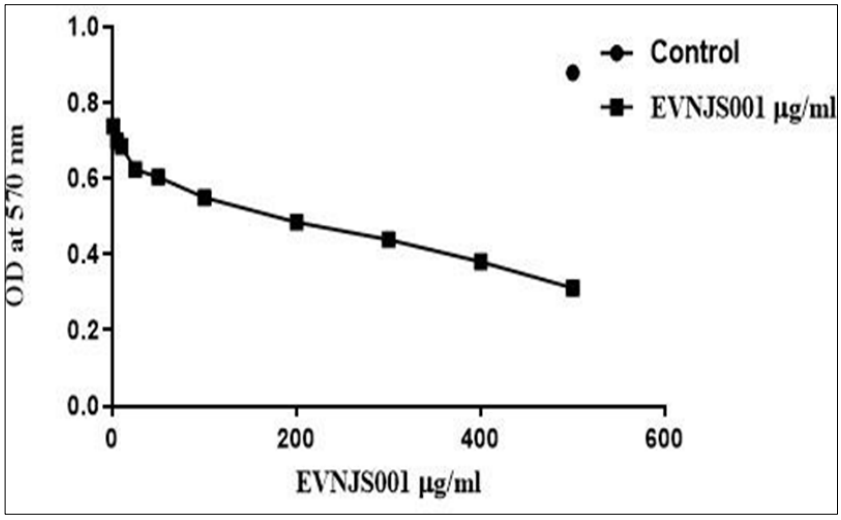

Fig 1: Analysis of Optical Density

Cell Viability (%)

Table 2: Cell Viability

S. No.	Tested sample concentration (µg/ml)	Cell viability (%) (in triplicate)			Mean Value (%)
1	Control	100	100	100	100
2	500 µg/ml	37.268	33.668	35.438	35.458
3	400 µg/ml	43.518	42.953	43.230	43.234
4	300 µg/ml	49.884	49.888	49.886	49.886
5	200 µg/ml	55.324	54.921	55.119	55.121
6	100 µg/ml	62.847	62.192	62.514	62.517
7	50 µg/ml	68.518	68.903	68.714	68.712
8	25 µg/ml	72.569	69.463	70.989	71.007
9	10 µg/ml	78.472	77.291	77.872	77.879
10	5 µg/ml	80.092	78.970	79.522	79.528
11	1 µg/ml	84.606	83.221	83.902	83.910

According to the Dose-Response Relationship, there is a clear inverse relationship between increasing sample concentration and cell viability [10]. As the concentration of the tested sample increases, cell viability decreases, which is typical in cytotoxicity assays. Moreover, at the highest concentration of 500 µg/ml, cell viability drops dramatically to around 35.5%. This indicates that this concentration is significantly toxic to the cells [11]. Whereas Lower concentrations (below 100 µg/ml) retain higher cell viabilities (62.5% and above), suggesting that these may be more suitable for exploratory studies on effects or mechanisms without causing excessive toxicity [12].

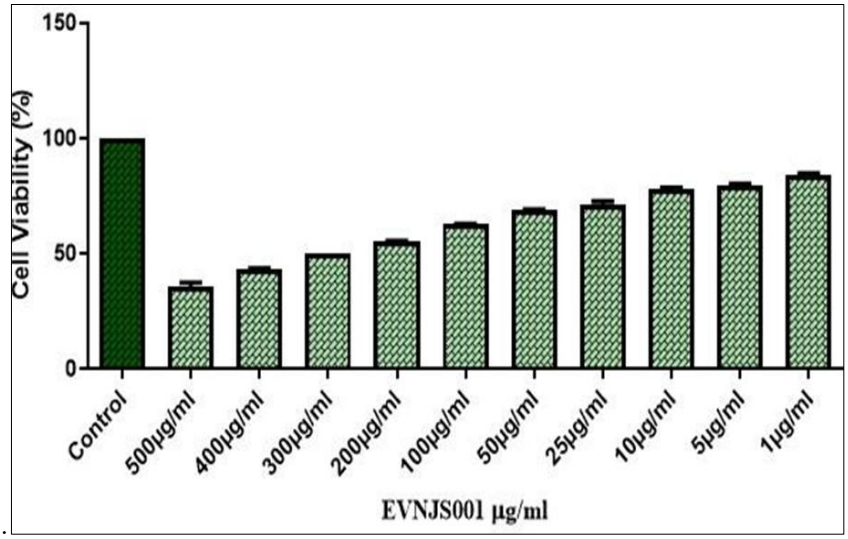

Fig 2: Cell Viability

IC50 Value of tested sample: 100 µg/ml

log(inhibitor) vs. normalized response --Variable slope	
Best-fit values	
LogIC50	2.000
HillSlope	-1.005
IC50	100.0
95% CI (profile likelihood)	
LogIC50	1.923 to 2.074
HillSlope	-1.191 to -0.8511
IC50	83.80 to 118.5
Goodness of Fit	
Degrees of Freedom	28
R squared	0.9511
Sum of Squares	1508
Sy.x	7.338
Number of points	
# of X values	30
# Y values analyzed	30

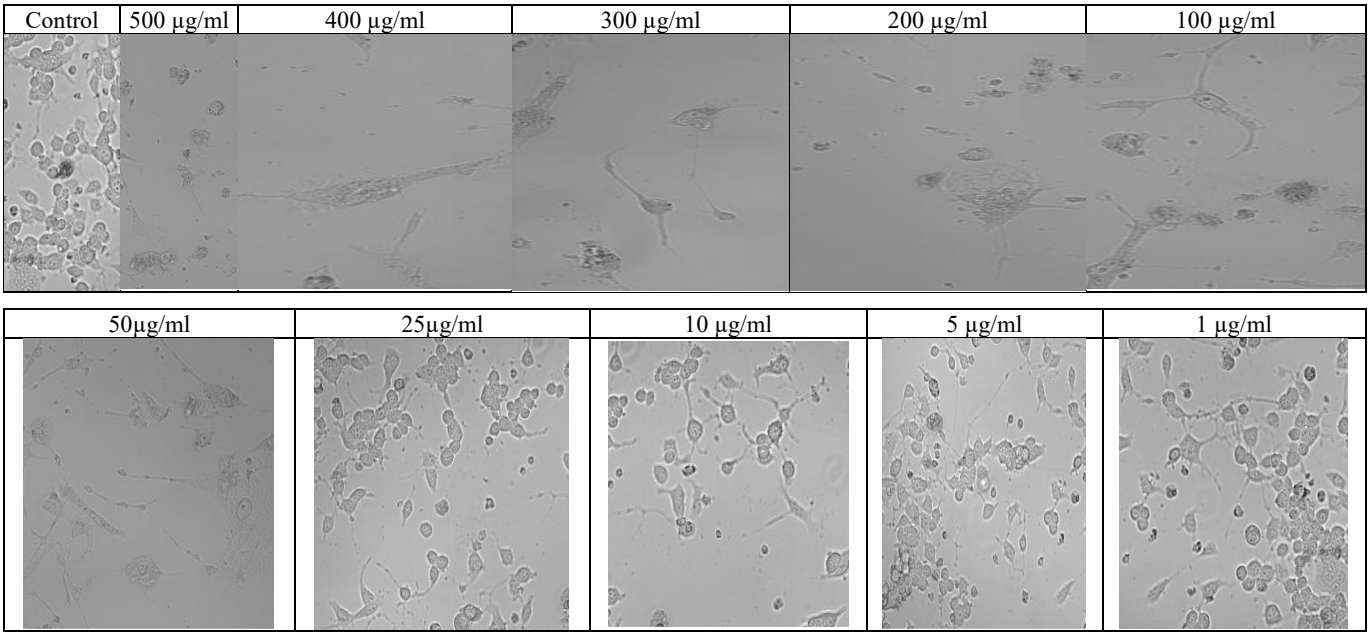

Fig 3: Images of control cells and treated cells

Discussion

The present study demonstrates that the leaf extracts of *Aristolochia littoralis* exhibit a clear dose-dependent cytotoxic effect on cultured cells, as assessed by the MTT assay. At the lowest concentration tested (1 µg/ml), cell viability was reduced to approximately 84% relative to the untreated control, with progressive declines observed as concentrations increased. Notably, viability dropped to around 35% at 500 µg/ml, underscoring a potent inhibitory response at higher doses. The calculated IC_{50} value of 100 µg/ml further quantifies this cytotoxicity, indicating the concentration at which half-maximal inhibition occurs. These findings align with the morphological changes evident in the treated cells, where higher extract concentrations likely induced cellular stress, membrane disruption, or apoptotic features, as suggested by the accompanying images.

This dose-response pattern is consistent with the known pharmacological profile of the *Aristolochia* genus, which is rich in secondary metabolites such as aristolochic acids, flavonoids, and alkaloids. These compounds are implicated in generating reactive oxygen species (ROS), leading to DNA damage, cell cycle arrest (particularly in the G2/M phase), and ultimately apoptosis via intrinsic pathways. For instance, low doses of aristolochic acids have been shown to elevate ROS levels and compromise cellular integrity, mirroring the gradual viability reduction observed here. The relatively high viability at 1 µg/ml may reflect a threshold below which the extract's bioactive components exert minimal disruption, potentially allowing for adaptive cellular responses, while escalating concentrations overwhelm these defenses.

Comparative analyses with prior research on *Aristolochia* species reveal similar cytotoxic trends. A study on chloroform extracts from *A. littoralis* seeds reported an IC_{50} of 81.02 µg/ml against A431 human epidermoid carcinoma cells [13], slightly lower than the value obtained here, possibly due to differences in extraction solvents or plant parts used. This suggests that leaf extracts, as investigated in this work, retain substantial bioactivity, though seeds may concentrate certain cytotoxic agents more effectively. Broader genus-wide evaluations indicate that dichloromethane extracts from *A. foetida* stems and leaves yield IC_{50} values of 45.9 µg/ml and 47.3 µg/ml,

respectively, against MCF-7 breast cancer cells, with selectivity for malignant over non-malignant cells. Such selectivity hints at therapeutic potential, as the extracts in that case triggered late apoptosis without significant membrane damage or calcium flux alterations. Similarly, a comprehensive review of *Aristolochia* extracts documented IC_{50} ranges below 100 µg/ml across multiple species and cell lines, attributing efficacy to phenolic and terpenoid constituents [14].

The cytotoxic effects observed may hold implications for anticancer applications, given the genus's historical ethnomedicinal use against tumours and inflammatory conditions [15]. However, the presence of aristolochic acids raises concerns, as these are well-documented nephrotoxins and carcinogens in chronic exposures. Thus, while the in vitro data suggest promise for targeted cytotoxicity, careful phytochemical fractionation is warranted to isolate beneficial compounds while mitigating risks. Limitations of this study include its reliance on an in vitro model, which may not fully recapitulate in vivo pharmacokinetics or organ-specific toxicities.

Conclusion

In conclusion, the study presents promising evidence that *Aristolochia littoralis* leaf extracts exhibit a protective effect on cultured cells at a low concentration of 1 µg/ml, enhancing cell viability and suggesting potential therapeutic applications in the context of epilepsy treatment. However, the cytotoxic responses observed at higher concentrations highlight the necessity for cautious exploration of this natural extract for clinical use. Continual research will be essential to decipher its mechanisms of action, assess its safety profile, and establish effective dosages for therapeutic applications in neurological disorders.

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Conflict of Interest

The author declared no conflict of interest**.**

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