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Original Research Article

In-Vitro Evaluation of the Cellular Viability Response of Hydrastis canadensis 200C in A549 Human Lung Adenocarcinoma Cells Using the MTT Assay

Article History:

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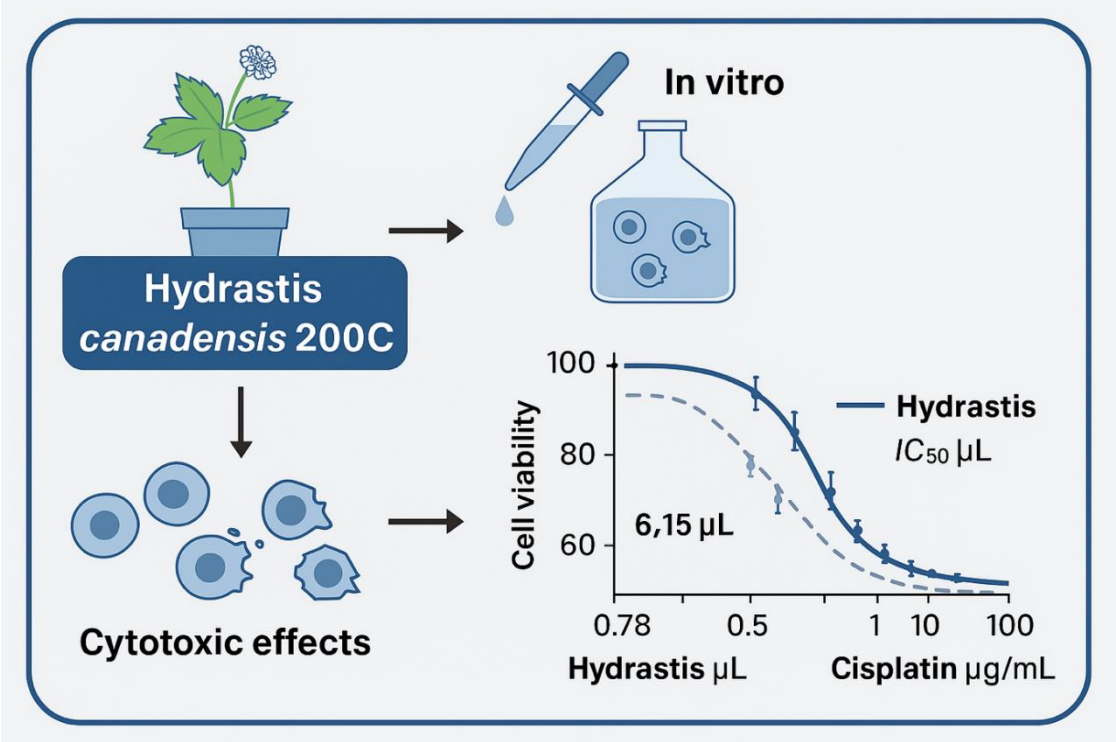
Abstract: ***Objectives:*** To assess the concentration-dependent cytotoxic effects of Hydrastis canadensis 200C on A549 human lung adenocarcinoma cells using the MTT assay and to determine its IC_{50} value under defined in-vitro conditions. ***Methods:*** A549 cells were cultured in MEM supplemented with 10% fetal bovine serum and exposed to graded concentrations of Hydrastis canadensis 200C (0.78–100 µL) for 24 hours. Cell viability was quantified through MTT reduction, and IC_{50} values were calculated by non-linear regression. Cisplatin (0.78–100 µg/mL) was included as a reference standard. Microscopic evaluation of cellular morphology was performed using an inverted phase-contrast microscope. ***Results:*** Hydrastis canadensis 200C produced a concentration-dependent decline in A549 cell viability, decreasing from 65.24% at 0.78 µL to 5.18% at 100 µL. The calculated IC_{50} was 6.15 µL. Cisplatin demonstrated an IC_{50} of 74.15 µg/mL under the same assay conditions. Because units differ, these IC_{50} values represent distinct response profiles and do not imply direct potency comparison. Morphological findings showed reduced adherent cell density at higher concentrations, supporting the cytotoxic trend. ***Conclusions:*** Hydrastis canadensis 200C demonstrated measurable in-vitro cytotoxic activity in A549 cells, reflected by reduced metabolic viability and supportive morphological changes. These findings provide preliminary evidence for further mechanistic studies exploring oxidative stress parameters, apoptosis markers, and tumour-microenvironmental pathways.

Keywords: Lung neoplasms; Adenocarcinoma; Cell viability; Apoptosis; Oxidative stress

Graphical Abstract



INTRODUCTION

Lung cancer continues to represent one of the most serious global health burdens, consistently ranking among the leading causes of cancer-related mortality worldwide.[1] Adenocarcinoma forms the predominant subtype, contributing significantly to morbidity, mortality, and diagnostic complexity.[2] In India, reported incidence patterns indicate progressive rise, particularly in urban and industrial regions, where delayed detection and access disparities add to the clinical impact.[3] The economic implications are substantial, with conventional treatment strategies-systemic chemotherapy, targeted agents, immune-based therapies, advanced imaging, and long-term care-imposing considerable financial strain on patients and healthcare systems.[4–5] Globally, lung cancer therapy remains among the cost-intensive domains of oncology, prompting interest in preclinical laboratory models that offer low-cost platforms to study cellular responses and biological mechanisms.[6–7]

A549 human lung adenocarcinoma cells are widely utilised in this context because they display stable proliferative behaviour, defined metabolic patterns, and secretion of mediators such as VEGF and IL-8 that participate in tumour–vascular signalling.[8–9] These characteristics make the A549 line an appropriate in-vitro system to evaluate cytotoxicity, metabolic inhibition, oxidative responses, and apoptotic triggers under controlled assay conditions. *Hydrastis canadensis* (Goldenseal), traditionally recognised for mucosal and inflammatory applications, contains the phytochemical berberine, which has been extensively investigated in experimental oncology. Pharmacological studies demonstrate that berberine can induce mitochondrial-dependent apoptosis, alter ROS generation, and influence central signalling pathways including PI3K/AKT, MAPK, AMPK, and NF-κB.[10–13] These intersections are relevant to tumour progression, microvascular remodelling, endothelial activation, and angiogenic switching.[14–15]

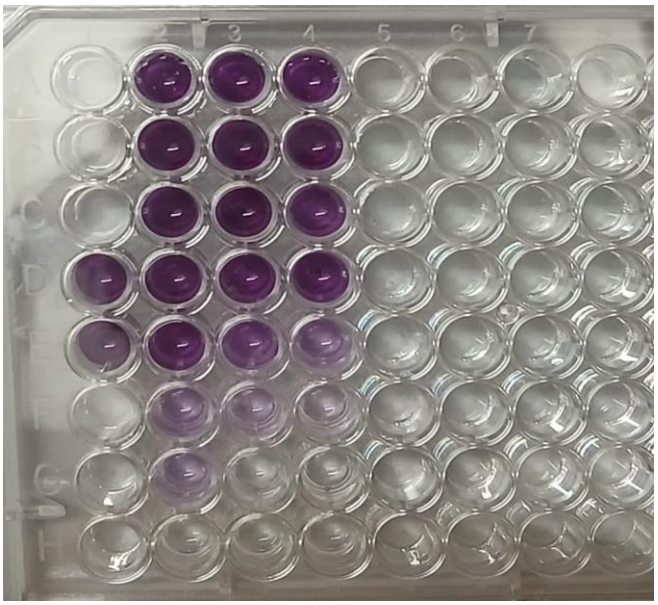
Alongside phytochemical investigations, experimental studies involving potentised botanical preparations have reported apoptosis-linked morphological changes, oxidative stress modulation, and reduction in metabolic activity in certain tumour cell models.[16–18] While variability in methods and reproducibility remain acknowledged limitations, such reports support systematic, assay-based evaluation of potentised preparations under reproducible experimental environments.

Within this framework, the aim of the present study was to determine whether *Hydrastis canadensis* 200C elicits a measurable biological response in A549 lung adenocarcinoma cells when tested through the MTT assay. Specifically, the study sought to evaluate concentration-dependent changes in cell viability and

derive an IC_{50} value under standardised in-vitro conditions, thereby generating preliminary data to inform future mechanistic research involving oxidative stress markers, apoptotic pathways, or tumour–microenvironment interactions.

MATERIAL AND METHODS

A549 (A human lung adenocarcinoma cell line)



Test Item

The test preparation used in this study was *Hydrastis canadensis* 200C (Sample A), obtained from a GMP-certified manufacturer. The remedy was supplied in sterile glass vials and handled under aseptic conditions. For experimental use, the preparation was diluted in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS) to achieve working concentrations ranging from 0.78 µL to 100 µL per well. Immediately before each assay, the test solution was passed through a 0.22 µm sterile syringe filter to ensure particulate-free preparation.

2. Cell Line and Culture Conditions

A549 (human lung adenocarcinoma) cells were obtained from a certified cell repository and maintained as per standard culture guidelines. Cells were grown in MEM containing 10% FBS, 1% L-glutamine, and 1% penicillin–streptomycin. Cultures were maintained at 37 °C in a humidified incubator with 5% CO_2. Only cells within the logarithmic growth phase and passage numbers below 25 were used for experimentation to ensure uniformity.

3. Experimental Design

All experiments were performed in 96-well flat-bottom tissue-culture plates. A549 cells were seeded at a density of 1×10^4 cells per well and allowed to adhere for 24 hours. After stabilization, culture medium was replaced with fresh medium containing varying concentrations of *Hydrastis canadensis* 200C (0.78, 1.56, 3.12, 6.25, 12.5, 25, 50, and 100 µL). Untreated wells served as negative controls.

3.1 Positive Control

Cisplatin (reference chemotherapeutic drug) was used as the positive control and prepared at standard laboratory concentrations (0.78–100 µg/mL) under sterile conditions.

4. MTT Cytotoxicity Assay

Cytotoxicity was evaluated using the MTT assay, which measures mitochondrial dehydrogenase activity as an indicator of cell viability. After 24 hours of exposure to the test preparation, 10 µL of MTT reagent (5 mg/mL) was added to each well, followed by 3 hours of incubation in the dark at 37 °C. Formazan crystals formed in viable cells were dissolved with 100 µL DMSO, and absorbance was measured at 570 nm using a microplate reader.

5. Calculation of Cell Viability and IC_{50}

Cell viability (%) was calculated relative to untreated control wells. Dose–response curves were generated using non-linear regression analysis. The half-maximal inhibitory concentration (IC_{50}) was calculated using GraphPad Prism software. All measurements were performed in triplicate, and mean ± standard deviation (SD) values were reported.

6. Morphological Assessment

Cellular morphology was documented using an inverted phase-contrast microscope. Observations included cell rounding, shrinkage, detachment, and membrane irregularities, which were considered indicators of cytotoxic or apoptosis-related effects.

7. Statistical Analysis

Data were analyzed using one-way ANOVA followed by post-hoc multiple comparison tests where applicable. A p-value < 0.05 was considered statistically significant.

RESULT

Morphological Observations

Treatment with *Hydrastis canadensis* 200C produced a dose-dependent decline in cell viability. At the lowest concentration (0.78 µL), survival decreased to 65.24%, while the highest concentration (100 µL) reduced viability to 5.18%.

Microscopic inspection showed a progressive reduction in the number of adherent A549 cells with increasing concentrations of *Hydrastis canadensis* 200C. Higher doses produced visibly fewer intact cells and a thinning of the monolayer compared with the untreated control. No additional structural details were inferred beyond the visible decrease in cell density.

Table No1: MTT result- Cisplatin

Sr. No.	Concentration (ug/mL)	Absorbance	%Cell Survival	% Inhibition	IC 50
1.	7.81	1.226	69.80	30.20	74.15
2.	15.63	1.13	64.33	35.67	
3.	31.25	1.073	61.09	38.91	
4.	62.5	0.785	44.69	55.31	
5.	125	0.573	32.62	67.38	
6.	250	0.398	22.66	77.34	
7.	500	0.298	16.97	83.03	
8.	1000	0.118	6.72	93.28	

Cytotoxicity of Cisplatin on A549 Cells

Cisplatin demonstrated a clear, concentration-dependent reduction in metabolic activity in A549 cells. Increasing concentrations from 7.81 to 1000 µg/mL progressively decreased cell survival from 69.80% to 6.72%.

Table No 2: MTT result-Hydrastis Canadensis

Sample name	Concentration (µL)	Absorbance	% cell survival	% Inhibition	IC 50
Blank	-	1.757	100	-	-
Hydrastis	0.78	1.503	65.24	34.76	6.15
	1.56	1.409	62.28	37.72	
	3.12	1.239	53.12	46.88	
	6.25	0.82	37.97	62.03	
	12.5	0.324	25.05	74.95	
	25	0.211	12.81	87.19	
	50	0.131	8.26	91.74	
	100	0.109	5.18	94.82	

Cytotoxicity of *Hydrastis canadensis* 200C on A549 Cells

Morphological Observations

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DISCUSSION

The present in-vitro study evaluated the cellular response of A549 human lung adenocarcinoma cells to *Hydrastis canadensis* 200C using a standard MTT-based viability assay. The findings demonstrated a reproducible, concentration-dependent reduction in cell viability, supported by corresponding morphological changes, indicating that the potentized preparation elicited a measurable biological response under controlled experimental conditions.

The MTT assay reflects mitochondrial metabolic activity and is widely used to assess changes in cellular viability. The progressive decline in metabolic activity observed with increasing concentrations of *Hydrastis canadensis* 200C suggests an alteration in cellular metabolic regulation rather than random variability. The consistency of this pattern across experimental replicates supports the robustness of the observed response.

A549 cells are a well-established in-vitro model for lung adenocarcinoma and are frequently used to investigate

tumour biology and cellular stress responses. Although angiogenic or inflammatory markers were not evaluated in the present study, reductions in tumour cell viability may have relevance for tumour–microenvironment interactions, warranting further targeted investigation.

The observations of this study are interpreted within the framework of high-dilution research, which aims to examine biological responses to potentized preparations using contemporary experimental tools. The ability to detect reproducible changes in cell viability using a standardised in-vitro assay supports the view that homoeopathic medicines can be systematically investigated within modern biological research paradigms.
Morphological assessment provided qualitative support for the quantitative MTT findings, with treated cultures exhibiting reduced adherence and altered cell density at higher concentrations. These features are consistent with metabolic stress and reduced cellular viability.

While the observed effects are experimentally demonstrable, the study is limited by reliance on a single viability assay and the absence of mechanistic endpoints. Further investigations incorporating complementary cellular and molecular assays will be necessary to better characterise the biological processes associated with the observed responses.

CONCLUSION

Hydrastis canadensis 200C was associated with a measurable, concentration-dependent reduction in the viability of A549 human lung adenocarcinoma cells, with an IC_{50} value of 6.15 µL under the experimental conditions. The observed reduction in cell viability, supported by corresponding morphological changes, indicates that the potentized preparation produced a reproducible biological response in this in-vitro model. Although downstream signalling or vascular-related endpoints were not examined in the present study, the observed cellular responses warrant further investigation to determine whether high-dilution preparations may influence biological pathways relevant to tumour–microenvironment interactions. Future studies incorporating complementary assays, including evaluation of angiogenic mediators, oxidative stress parameters, mitochondrial function, and endothelial–tumour cross-talk, will be necessary to further characterise the biological context of the observed effects.

Author Contributions

Contribution Roles	Dr. Avishkar Zagday	Dr. Maneesha Soni	Dr. Vinay Upadhyay	Dr. Madhuri Sathe	Dr. Usha Garje	Dr. Samreen Kavare	Dr. Pulin K. S
Concepts	✓	✓	✓				
Design	✓	✓	✓				
Definition of Intellectual Content	✓	✓	✓				
Literature Search	✓	✓	✓				✓
Experimental Studies	✓	✓	✓	✓	✓		
Data Acquisition	✓	✓	✓	✓	✓	✓	
Data Analysis	✓	✓	✓				
Statistical Analysis	✓	✓	✓				
Manuscript Preparation	✓						
Manuscript Editing	✓	✓	✓				✓
Manuscript Review	✓	✓	✓	✓		✓	
Guarantor of Study	✓						

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funding agencies in the public, commercial, or not-for-profit sectors. All experimental work was supported by institutional funds and existing laboratory infrastructure

Conflict of interest

The authors declare that there are no conflicts of interest regarding the conduct, analysis, or reporting of this study

Ethical statement

This study was limited to in-vitro cell culture experiments and did not involve human participants or animal subjects; therefore, formal ethics committee approval was not required.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209–49. doi:10.3322/caac.21660.

2. Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JHM, Beasley MB, et al. The 2015 World Health Organization classification of lung tumors. J Thorac Oncol. 2015;10(9):1243–60. doi:10.1097/JTO.0000000000000630.

3. Mallath MK, Taylor DG, Badwe RA, Rath GK, Shanta V, Pramesh CS, et al. The growing burden of cancer in India: epidemiology and social context. Lancet Oncol. 2014;15(6):e205–12. doi:10.1016/S1470-2045(14)70115-9.

4. Sullivan R, Peppercorn J, Sikora K, Zalcberg J, Meropol NJ, Amir E, et al. Delivering affordable cancer care in high-income countries. Lancet Oncol. 2011;12(10):933–80. doi:10.1016/S1470-2045(11)70141-3.

5. Dandekar RH, Jain RA, Dash R. Economic burden of lung cancer and financial toxicity in Indian oncology settings. Indian J Cancer. 2020;57(3):299–305. doi:10.4103/ijc.IJC_845_19.

6. Meropol NJ, Schulman KA. Cost of cancer care: issues and implications. J Clin Oncol. 2007;25(2):180–6. doi:10.1200/JCO.2006.09.6081.

7. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: an evolving paradigm. Nat Rev Cancer. 2013;13(10):714–26. doi:10.1038/nrc3599.

8. Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. Nature. 2018;553(7689):446–54. doi:10.1038/nature25183.

9. Korrodi-Gregório L, Soto-Cerrato V, Vitorino R, Fardilha M, Pérez-Tomás R. Functional characterization of A549 and H1299 lung cancer cell lines. PLoS One. 2016;11(3):e0150648. doi:10.1371/journal.pone.0150648.

10. Yen YT, Chen YA, King CC, Brossay L, Kuo CJ, Chen NT. IL-8 promotes cell migration through extracellular signal-regulated kinase pathway in lung cancer. Br J Cancer. 2012;106(12):1997–2005. doi:10.1038/bjc.2012.184.

11. Wang H, Liu Y, Wei S, Yan M, Liu J, Yang J, et al. Berberine induces apoptosis via ROS generation in human pancreatic cancer cells. Cancer Lett. 2014;349(1):66–75. doi:10.1016/j.canlet.2014.03.001.

12. Sun Y, Xia M, Yan H, Han L, Zheng H, Liu Y, et al. Apoptosis induction as a core anticancer mechanism of berberine. Front Pharmacol. 2022;13:803717. doi:10.3389/fphar.2022.803717.

13. Zhang Y, Li X, Zou D, Liu W, Yang J, Zhu N. Berberine and cancer: mechanisms, obstacles and strategies. Molecules. 2014;19(8):12349–75. doi:10.3390/molecules190812349.

14. Kumar A, Bhandari M. Anti-cancer mechanisms of berberine: a review. Cancer Manag Res. 2017;9:267–72. doi:10.2147/CMAR.S134244.

15. Preethi K, Ellanghiyil S, Kuttan G, Kuttan R. Induction of apoptosis of tumor cells by some potentiated homeopathic drugs: implications on mechanism of action. Integr Cancer Ther. 2012;11(2):172–82. doi:10.1177/1534735411400310.

16. Arora S, Tandon S. DNA fragmentation and cell cycle arrest as hallmarks of apoptosis induced by Ruta graveolens in human colon cancer cells. Homeopathy. 2015;104(1):31–42. doi:10.1016/j.homp.2014.08.001.

17. Khan S, Nayak D, Khurana A, Manchanda RK, Tandon C, Tandon S. In vitro assessment of potentised Hydrastis canadensis on breast cancer cell lines. Homeopathy. 2020;109(4):198–206. doi:10.1055/s-0040-1709668.

18. Das J, Biswas SJ. Modulation of oxidative and apoptotic pathways by potentized preparations in tumour models. Indian J Res Homoeopathy. 2011;5(4):42–8.

