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Evaluation of the Cytotoxic Potential of Homeopathic Lycopodium Clavatum 200C Against Human Colorectal Carcinoma (HCT116) Cell Line Using MTT Assay

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ABSTRACT

Background: Ultra-diluted preparations have gained scientific attention in cancer biology, where established cell-line models enable objective evaluation of cellular responses. This study assessed the effect of Lycopodium Clavatum 200C on HCT116 colorectal carcinoma cells using a standardized MTT assay.

Methods: HCT116 cells were exposed to graded concentrations of Lycopodium Clavatum 200C for 24 hours under standard culture conditions, and cell viability was quantified by MTT reduction. IC_{50} values were obtained by non-linear regression, with cisplatin included as a reference standard.

Results: Lycopodium Clavatum 200C produced a dose-dependent reduction in HCT116 viability, with an IC_{50} of 17.83 µL under the assay conditions. Cisplatin showed an IC_{50} of 99.66 µg/mL; the differing units reflect distinct dosing schemes rather than direct potency equivalence. Morphological assessment confirmed reduced adherent cell density at higher concentrations.

Conclusion: Lycopodium Clavatum 200C elicited a measurable biological response in a colorectal cancer cell model, demonstrated by reduced cell viability and supportive morphological changes. These preliminary findings justify further mechanistic and comparative studies on potentized preparations in cancer cell systems, while recognizing that translational implications require additional investigation.

KEYWORDS: Lycopodium, Colorectal cancer, HCT116, MTT assay, cytotoxicity, Homeopathy.

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INTRODUCTION

Colorectal cancer (CRC) is a major public health challenge globally, ranking among the top three most commonly diagnosed malignancies. In 2020 alone, over 1.9 million new CRC cases and approximately 930,000 related deaths were reported worldwide [1]. In India, CRC incidence has steadily increased, with around 70,000 new cases and 41,000 deaths annually, making it the fourth most frequent cancer in the country [2]. Contributing factors include lifestyle changes, urbanization, and delayed diagnosis. While curative surgery is feasible in early-stage disease, many patients present with advanced or metastatic CRC, which significantly limits treatment success and survival rates [3].

Standard CRC management includes surgery followed by chemotherapy and/or radiotherapy. Though systemic treatments like fluorouracil, oxaliplatin, and irinotecan have improved survival in metastatic CRC, long-term outcomes remain modest and are often accompanied by severe side effects such as neuropathy, mucositis, and immunosuppression [4]. Moreover, tumor heterogeneity and acquired drug resistance further limit efficacy. Targeted therapies and immunotherapy offer promise but are applicable to only a subset of CRC patients, particularly those with microsatellite instability [5]. These limitations underscore the need for affordable, low-toxicity, complementary strategies that could enhance conventional therapy or mitigate its adverse effects.

Homeopathy, as a widely used medical system, has gained interest in oncology research due to its reported immunomodulatory, anti-inflammatory, and cytotoxic effects in preclinical models [6,7]. Several potentized preparations, including Ruta graveolens, Condurango, and Carcinosinum, have demonstrated cytotoxicity, apoptosis induction, and modulation of key pathways such as

NF-κB and p53 in cancer cell lines [8–10]. Lycopodium clavatum, a plant-based homeopathic remedy traditionally indicated for digestive and hepatobiliary complaints, has emerged as a candidate of interest in colorectal oncology. Studies using low potencies (5C and 15C) have shown anti-proliferative and pro-apoptotic activity in HeLa and MCF-7 cells, involving caspase-3 activation and Bax/Bcl-2 modulation [11,12]. Moreover, experimental studies on *Lycopodium* extracts have revealed anti-inflammatory effects and oxidative stress reduction in animal models of colitis, suggesting relevance in gastrointestinal and colorectal pathology [13].

The present study aims to evaluate the in vitro cytotoxic and apoptotic potential of *Lycopodium Clavatum* 200C against human colorectal carcinoma (HCT116) cells, using the MTT assay and cisplatin as a reference standard. This work contributes to the growing body of evidence assessing high-dilution homeopathic medicines through modern pharmacological tools. Given the global and regional burden of CRC, this study provides preliminary in-vitro data to support further laboratory exploration of *Lycopodium Clavatum* 200C in colorectal cancer research.

MATERIALS AND METHODS:

Test items detail

Sr. No	Name	Storage Conditions	Handling Precautions
1	Lycopodium Clavatum	Room Temperature	Standard Laboratory Precautions

Experimental procedures

Preparation of Test Material

All Test Samples were filter sterilized using 0.22µ filters and diluted by double dilution method in MEM with FBS.

Chemicals and Materials

Sr No.	Chemicals	Materials
1.	Cell Culture Plates	96 well microtiter plates (Himedia)
2.	Cell culture flasks	T25 Flasks (Himedia)
3.	Trypsin/EDTA	0.25%Trypsin and 0.02% EDTA in Dulbecco's Phosphate Buffered Saline (Himedia)
4.	DMSO	Dimethyl sulfoxide (Himedia)
5.	Cell culture Medium	Eagle's Minimum Essential Medium (EMEM)10% (v/v) Fetal Bovine Serum
6.	Cell Line	HCT116 (A human colorectal carcinoma cell line) from NCCS Pune.
7.	Culture Conditions	37ºC with 5% CO_2
8	Homeopathic Remedy	*Lycopodium Clavatum* 200C Schwabe India, procured from Om Pharmacy, Mumbai, Maharashtra Stored at room temperature

Préparation of Cells :

'HCT116 '(A human colorectal carcinoma cell line) cells were cultured in MEM with NEAA media supplemented with 10% (v/v) fetal bovine serum. Cells were cultured at 37ºC and 5% CO_2; complete medium was changed every 2 to 3 days.

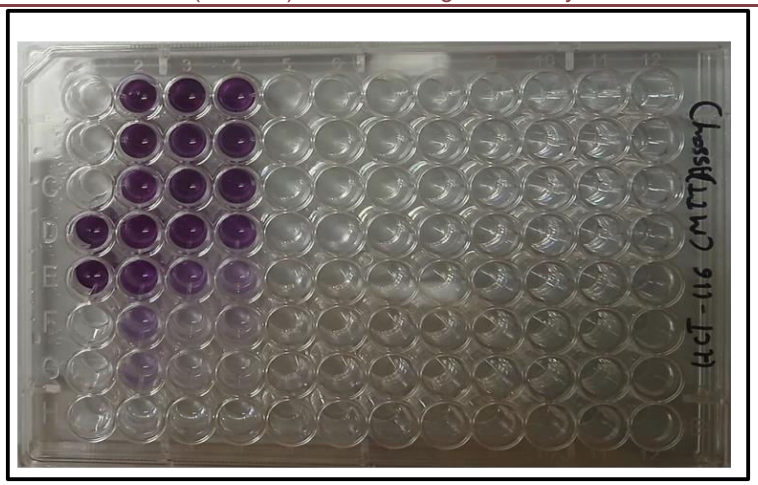

Figure 1

MTT Assay Procedure

Cells were seeded in 96 well plates at a concentration of 1,00,000 cells per well (100ul). The plates were incubated at 37^0C at 5% CO_2 for 24hrs.

After the incubation period cells were observed for half confluence monolayer.

Culture medium was removed and the cells were treated with different concentrations of test item.

Cells in cell culture medium without any test item incubated and under the same conditions served as control.

Plates were incubated 37^0C and 5% CO_2 for 24hrs.

After 24hrs, cells were observed under inverted microscope for changes in morphology or death if any.

After observation, culture medium was removed and 100ul fresh medium added with 10ul MTT reagent in each well.

Plates were incubated for 4hrs at 37^0C in 5% CO_2 incubator.

100ul solubilization solution (DMSO) added into each well.

Plates were allowed to stand for 1hr at 37^0C in 5% CO_2.

After checking for complete solubilization of the purple formazan crystal absorbance was measured at 570nm using microplate reader.

IC50 values were calculated by plotting a log graph for the concentration of the test items vs %cell survival.

Percentage of cell survival was calculated using the formula:

Percentage cell survival (%) = (Absorbance of test/ Absorbance of control) * 100

Statistical Analysis:

Data were expressed as mean ±SD from triplicates, and IC50 values were calculated using linear regression analysis with GraphPad Prism 5.0. Individual absorbance values from triplicates are reported; comprehensive statistical analysis with standard deviation would strengthen future validation.

RESULTS:

Dose-dependent cytotoxicity of *Lycopodium Clavatum* 200C was observed in HCT116 cells. Morphological changes included cell rounding, shrinkage, and detachment under higher concentrations, consistent with apoptosis. Microscopic examination revealed progressive morphological alterations in HCT116 cells with increasing concentrations of *Lycopodium Clavatum* 200C. At lower concentrations (0.78-6.25 µL), cells showed mild rounding and reduced confluence. At moderate to high concentrations (12.5-100 µL), marked cell shrinkage, membrane blebbing, and detachment from the culture surface were observed, consistent with cytotoxic stress. Similar morphological changes were observed with Cisplatin treatment.

Table No1: MTT result- Cisplatin

Sr. No.	Concentration (ug/mL)	Absorbance	%Cell Survival	% Inhibition	IC 50
1.	7.81	1.297	82.61	17.39	99.66
2.	15.63	1.186	75.54	24.46	
3.	31.25	1.042	66.37	33.63	
4.	62.5	0.816	51.97	48.03	
5.	125	0.638	40.64	59.36	
6.	250	0.448	28.54	71.46	
7.	500	0.264	16.82	83.18	
8.	1000	0.107	6.82	93.18	

Table No 2: MTT result - *Lycopodium Clavatum* 200C

Sample name	Concentration (µL)	Absorbance	% cell survival	% Inhibition	IC 50
Blank	-	1.570	100	-	-

Lyco 200	0.78	1.083	68.98	31.02	17.83
	1.56	0.921	58.66	41.34	
	3.12	0.875	55.73	44.27	
	6.25	0.76	48.41	51.59	
	12.5	0.542	34.52	65.48	
	25	0.387	24.65	75.35	
	50	0.239	15.22	84.78	
	100	0.102	6.50	93.50	

IC_{50} (Lycopodium *Clavatum* 200C): 17.83 µL
IC_{50} (Cisplatin): 99.66 µg/mL
IC_{50} values for Lycopodium (µL) and cisplatin (µg/mL) represent independent dosing formats and are not directly comparable.

DISCUSSION

This study shows that *Lycopodium Clavatum* 200C produces a measurable, dose-dependent reduction in the viability of HCT116 colorectal carcinoma cells. The decline in metabolic activity and visible reduction in adherent cells indicate a consistent cytotoxic response under *in-vitro* conditions. These findings align with earlier reports in which potentized plant-based preparations, including Condurango, Ruta graveolens, and other *Lycopodium* potencies, demonstrated apoptosis-related or metabolic inhibitory effects in tumour cell models. Although the mechanistic basis of these effects remains unclear, previous studies suggest potential involvement of oxidative stress, mitochondrial imbalance, or caspase-linked pathways. The present results offer preliminary support for further examination of these possibilities. HCT116 cells are known to influence endothelial behaviour through secretion of VEGF and pro-inflammatory cytokines; therefore, changes in tumour viability may indirectly affect tumour–vascular signalling, although this was not directly assessed in the present study. Given that colorectal cancer cells, including HCT116, influence angiogenic signalling, reductions in tumour cell viability may have downstream implications for tumour–vascular interactions, which merit systematic evaluation. The study is limited by reliance on a single assay and absence of mechanistic markers. Future work should include apoptosis assays, oxidative stress measurements, and angiogenesis-related endpoints.

CONCLUSION

The present study shows that *Lycopodium Clavatum* 200C produced a measurable, dose-dependent reduction in HCT116 colorectal carcinoma cell viability under controlled in-vitro conditions. The observed cytotoxicity is consistent with earlier experimental findings in which potentized preparations—such as Ruta graveolens, Condurango, and Carcinosin,Chelidonium combinations—elicited biological responses across diverse cancer cell models. Although the mechanisms underlying these effects remain unclear, the reproducibility of the present observations suggests that specific ultra-diluted preparations can influence cellular metabolism in vitro.

Because HCT116 cells contribute to angiogenic signalling through factors such as VEGF and IL-8, reduced tumour cell viability may have downstream implications for tumour–vascular interactions; however, this study did not assess endothelial or angiogenic markers, and such interpretations remain speculative. Given these limitations, including the use of a single assay and absence of mechanistic profiling, the findings should be regarded as preliminary.

Future investigations employing apoptosis markers, oxidative-stress assays, gene-expression analysis, and endothelial co-culture models will be essential to determine the biological specificity and translational relevance of these effects.

In summary, *Lycopodium Clavatum* 200C demonstrated reproducible cytotoxic activity in HCT116 cells, supporting cautious, systematic follow-up within both preclinical oncology and integrative research frameworks.

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Conflicts of Interest
The authors have no conflict of interest and certify hereby that this work has never been published. All the authors have fully consented to submit this manuscript in this journal. The test medicine was purchased commercially at standard retail price. The manufacturer (Schwabe India) had no role in study design, data collection, analysis, or manuscript preparation.

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LEGENDS -FIGURE 1

Representative 96-well microplate showing MTT assay results for HCT116 colorectal carcinoma cells treated with serial dilutions of Lycopodium Clavatum 200C for 24 hours. Purple coloration indicates viable cells with active mitochondrial dehydrogenase activity; intensity decreases with increasing drug concentration, indicating dose-dependent cytotoxicity.