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Abhrak Bhasma (Biotite mica nanoparticles) Induces Cytotoxicity in Adenocarcinoma Human Alveolar Basal Epithelial Cells (A549)

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Abstract:- Mica has been proven to have anticancer effects, however, there is not much data available on its efficacy and treating mechanism. In Ayurveda- an Indian system of medicine, a medicine named 'Abhrak bhasma', is incinerated mica-based nano-material being used for centuries to treat various respiratory conditions and lung-related diseases. Nanoparticles are proven to have advantages in cancer treatment. The purpose of this study was to examine ' Abhrak Bhasma' induced cytotoxicity on the A549 cell line.
Method - Cytotoxicity was measured by MTT assay on A549 cell line
Result - Mica nanoparticles at up to 200µ/mL for 24 hours showed concentration dependant cytotoxic activity. The calculated IC50 value was 168.60µg/mL
Conclusion - This is the first report showing that Abhrak bhasma or mica nanoparticles induce cytotoxicity in A459 cells.

I. INTRODUCTION

Mica is known to have anti-tumor as well as immunostimulatory effects. Recent studies have shown that mica has chemoprotective power against colorectal cancer. (1) Some studies have shown that the immunostimulatory effect can be used effectively to suppress tumor growth, many studies using mica have investigated anti-tumor as well as immunostimulatory effects. (2) One more study detects the potential involvement of mica nanoparticles in suppressing MCF-7 cell growth by regulating the interaction between tumor cells and anti-tumor immune cells. (3) There is not much data available on the anticancer activity of mica nanoparticles.

Ayurveda is an Indian system of medicine where mica is known as Abhraka and incinerated mica is called Abhrak bhasma. Abhraka is identified as biotite mica. The process of incineration follows multiple steps, First, the mica went an elaborate process of purification called shodhana and this process is followed by the incineration phase, which involves the trituration of some other minerals and/or herbal extract and putting it under high temperature to obtain small particle-sized ash. Repeated incineration is performed to attain nano size. (4)

This abhrak bhasma when characterized using FEG-SEM was said to have a particle size ranging from 19nm to 80nm making it a nanoparticle. (5) Abhrak bhasma has various therapeutic effects and is being successfully used to treat various respiratory conditions and lung-related diseases. This was the reason why the A489 cell line was selected for the study. A549 cells were chosen to model the alveolar Type II pulmonary epithelium.

The cells are used as a lung cancer study model and the development of various drug therapies against it. The MTT assay in the anticancer study model is one of the most commonly used tests to test your cancer-fighting function in both natural extracts and natural products and natural product extracts. It is highly reliable, and color-based testing is easily performed on a variety of cell lines. The present study's aim was to identify the cytotoxic potential of abhrak bhasma against A549

II. MATERIALS AND METHOD

- *Abhrak Bhasma Sample*

A market sample of Dhootapapeshwar Abhraka Bhasma (Sahasraputi) was procured. The sample was sealed and sent to the Averin Biotech laboratory in Hyderabad for MTT assay. Following procedures were followed during the MTT assay study.

- *Maintenance of cell line*

The A549 cell line which stands for adenocarcinoma human alveolar basal epithelial cells were purchased from NCCS, Pune, India. Maintenance of cells was done in DMEM high glucose media which was supplemented with 10 % FBS

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along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO2, 18-20% O2 concentration at 37 degrees Celsius temperature in the Carbon dioxide incubator and it was sub-cultured for every 2 days.

➢ *Materials*

Cell line: A549-Human lung adenocarcinoma cell line (From NCCS, Pune))Cell culture medium: DMEM-High glucose media - (Cat No:2120785, Gibco) ,Adjustable multichannel pipettes and a pipettor (Benchtop, USA) ,Fetal Bovine Serum (#RM10432, Himedia) ,MTT Reagent (5 mg/ml) (# 4060 Himedia) ,DMSO (#PHR1309, Sigma) ,Cisplatin (#PHR1624, Sigma), D-PBS (#TL1006, Himedia) ,96-well plate for culturing the cells (From Corning,USA) ,T25 flask (# 12556009, Biolite - Thermo) ,50 ml centrifuge tubes (# 546043 TORSON) ,1.5 ml centrifuge tubes (TORSON) ,10 ml serological pipettes (TORSON) ,10 to 1000 ul tips (TORSON)

➢ *Equipment*

Centrifuge (Remi: R-8oC), Pipettes: 2-10µl, 10-100µl, and 100-1000µl ,Inverted biological microscope (Biolinkz) ,96 well plate ELISA reader (ELX-800, BioTeK, USA) ,37°C incubator with humidified atmosphere of 5% CO2 (Healforce, China)

i) Medium control (medium without cells)
(ii) Negative control (medium with cells but without the experimental drug/compound)
(iv) Positive control- (medium with cells treated with Cisplatin with 7uM/ml)-For A549 cells

200µl cell suspension was seeded in a 96-well plate at the required cell density (20,000 cells per well) without the test agent. The cells were allowed to grow for about 24 hours. Appropriate concentrations of the test agent were added (Mentioned in the results - Excel sheet) The plate was incubated for 24hrs at 37°C in a 5% CO2 atmosphere. After the incubation period, The plates from the incubator were pulled out and the spent media was removed whereas the MTT reagent was added to a final concentration of 0.5mg/mL of total volume. The plate was wrapped with aluminum foil to avoid exposure to light. The plates were returned to the incubator and incubated for 3 hours. The MTT reagent was removed and then 100µl of solubilization solution (DMSO) was added. Gentle stirring in a gyratory sieve shaker enhanced dissolution. Occasionally, pipetting up and down completely dissolved the MTT formazan crystals, especially in dense cultures. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm wavelength.% cell viability is calculated using the below formula:(see Table 2 for reference)

% cell viability=Abs of treated cells/Abs of Untreated cells x 100

1. The IC50 value was determined by using a linear regression equation i.e. Y =Mx+C.

Here, Y = 50, M, and C values were derived from the viability graph.

MTT Assay result(Table 1)

S. NO	Sample code	IC50 conc (ug/ml)
		A549
1	ABNP	168.60

Table 2

Concentration Unit: µg/ml		Incubation:24hrs						
Concentration	BLANK	UNTREATED	STD	12.5	25	50	100	200
Abs Reading 1	0.035	0.906	0.472	0.876	0.828	0.801	0.649	0.406
Abs Reading 2	0.047	0.928	0.454	0.857	0.834	0.787	0.658	0.387
Mean Abs	0.041	0.917	0.463	0.8665	0.831	0.794	0.6535	0.3965
Mean Abs (Sample-Blank)		0.876	0.422	0.8255	0.79	0.753	0.6125	0.3555
STANDARD DEVIATION		0.015556349186104	0.0127279220613579	0.0134350288425444	0.00424264068711928	0.00989949493661166	0.00636396103067893	0.0134350288425444
STANDARD ERROR		0.011	0.0090000000000000	0.0095	0.003	0.007	0.0045	0.0095
Cell Viability %		100	48.1735159817352	94.2351598173516	90.1826484018265	85.958904109589	69.9200913242009	40.5821917808219

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Chart 1. showing the IC50 value of the Test Compound ABNP treated A549 cell lines after the incubation period of 24hrs.

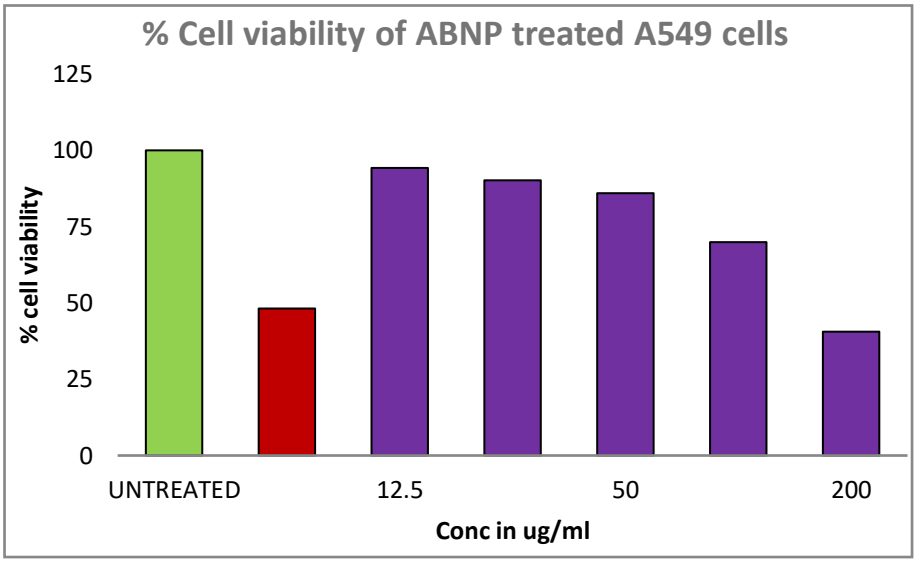


Table 1

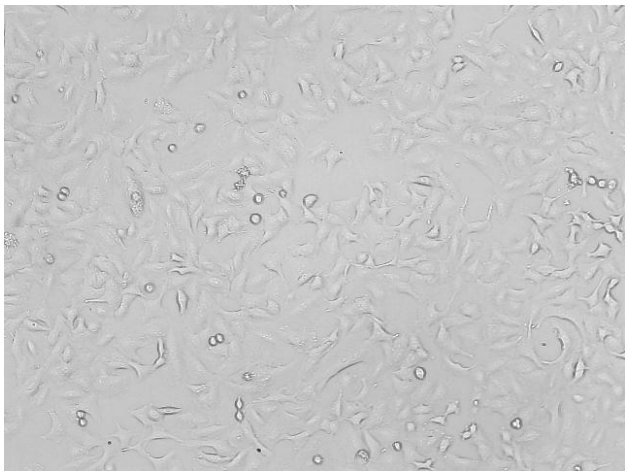
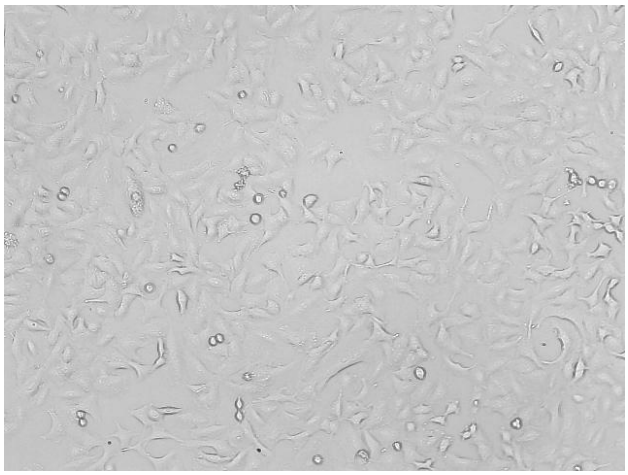
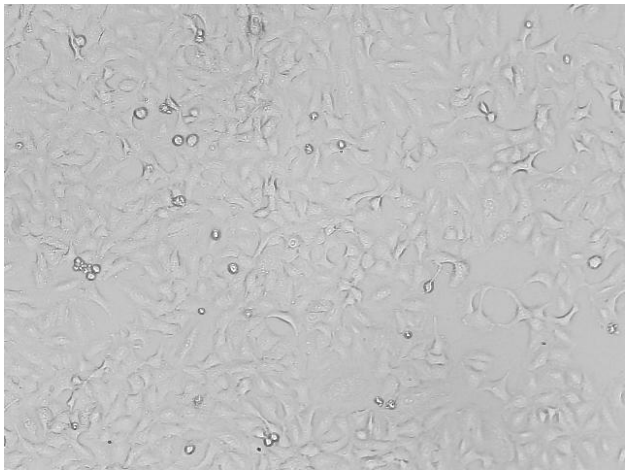
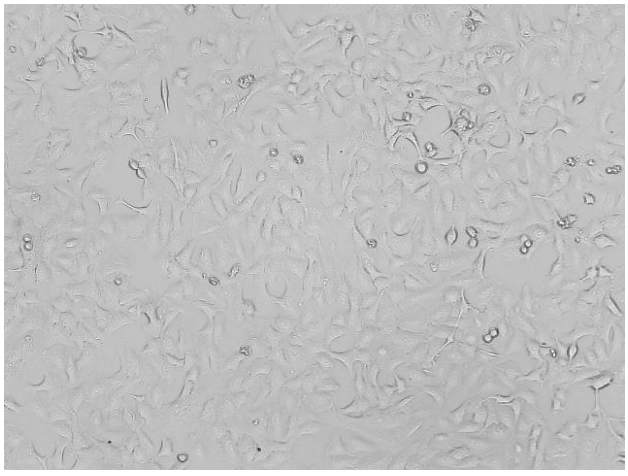
 12.5uG/dl	 12.5uG/dl
 25uG/dl	 25uG/dl

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Table 1

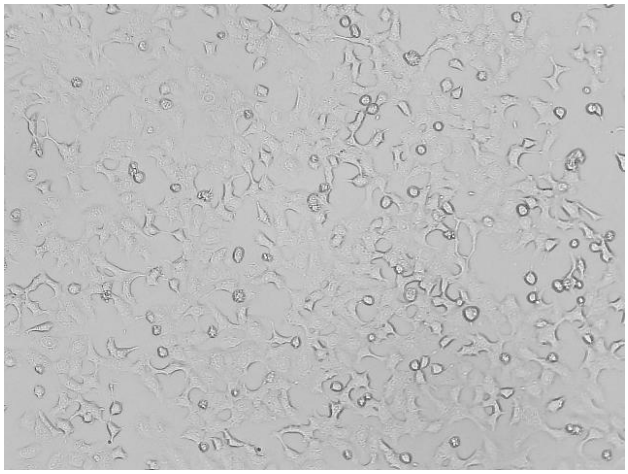
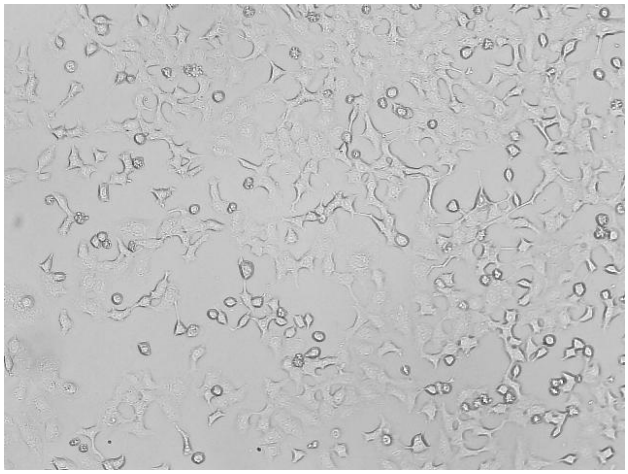
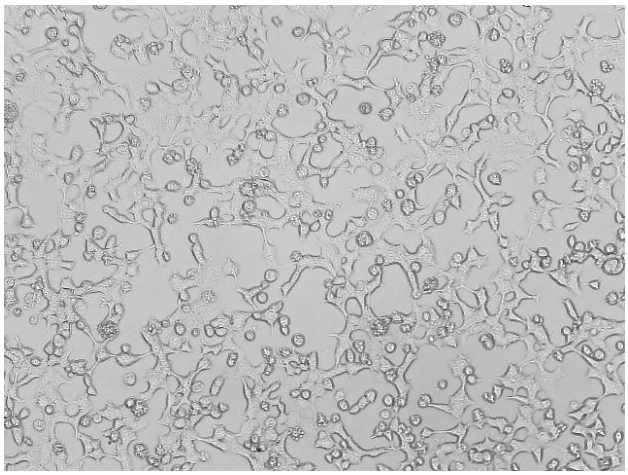
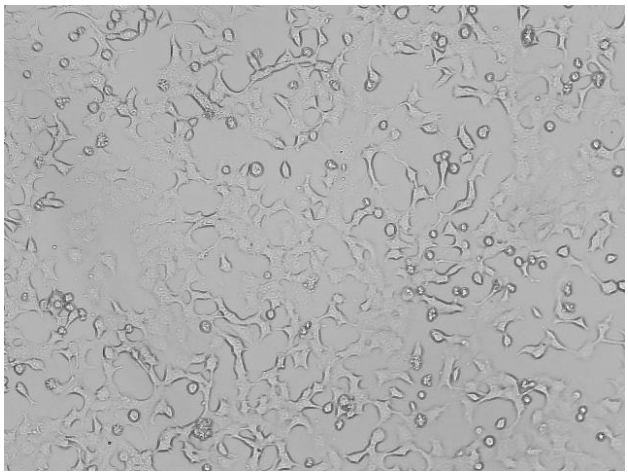
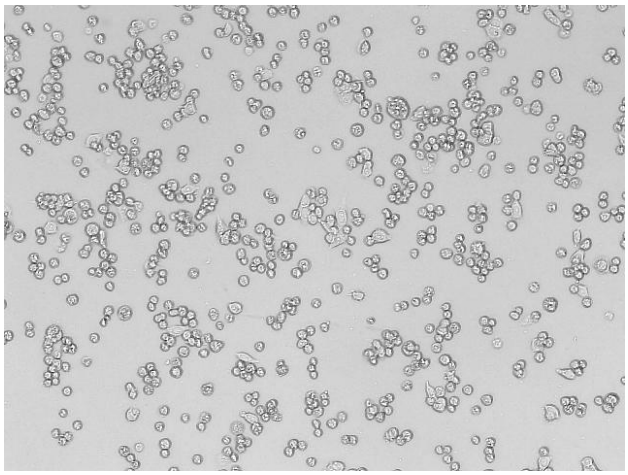
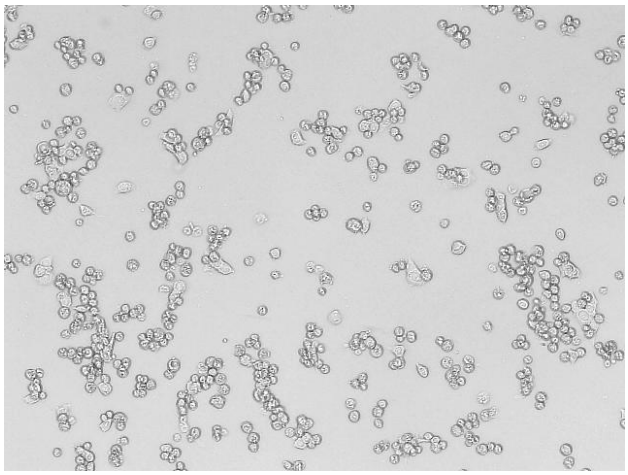
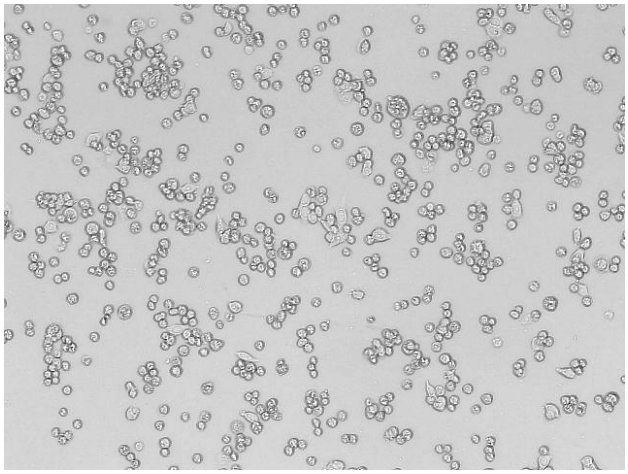
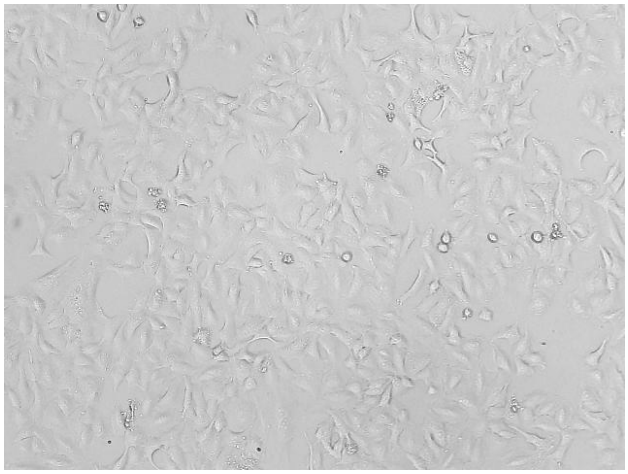
 50uG/dl	 50uG/dl
 100uG/dl	 100uG/dl
 200uG/dl	 200uG/dl

Table 1

 A549-STD CONTROL(1)	 Untreated cell line A549

III. CONCLUSION

Abhrak bhasma nanoparticles were tested against A549-Human lung adenocarcinoma cells for detecting anticancer activity at concentration 0µg/mL,12.5µg/mL, 25µg/mL, 50µg/mL, 100µg/mL and 200 µg/mL for 24 h cytotoxicity was determined using MTT assays, MTT results have showed that as the concentration of nanoparticle increased to 12.5µg/mL, 25µg/mL, 50µg/mL, 100µg/mL and 200 µg/mL, cytotoxicity was seen in dose-dependent manner. In MTT assay cell viability was reduced to 94%, 90%, 85%, 69%, 40% FOR THE CONCENTRATIONS OF 12.5µg/mL, 25µg/mL, 50µg/mL, 100µg/mL, 200µg/mL respectively

IV. DISCUSSION

Nanotechnology is being studied for cancer therapeutics since nanoparticle play a major role in targeted drug delivery. Nanoparticle-based drug delivery has specific advantages such as biocompatibility, stability, enhanced permeability and retention effect, and precise targeting.

In indian system of medicine called Ayurveda, Bhasma is incinerated metal-mineral ash and it is having size in nanometers. These bhasma are being used successfully in clinical practice from many years. The motive behind the study was to find the anticancer activity i.e. cyrotoxic potential of abhrak bhasma using MTT assay.

Abhrak bhasma is a popular preparation in Ayurveda as a single drug or as an ingredient of other formulation. Abhraka is nothing but biotite type of mica. Abhrak bhasma is one of the important formulation of Ayurveda where mica is incinerated multiple times to attain nanoparticles and used in therapeutics to treat various conditions. Out of all clinical applications of mica, use in respiratory diseases treatment is the most common one hence it was thought to test abhrak bhasma on A549 cell which is a Human lung adenocarcinoma cell line.

A549-Human lung adenocarcinoma cells were exposed to Abhrak bhasma nanoparticles at the concentration of 0µg/mL,12.5µg/mL, 25µg/mL, 50µg/mL, 100µg/mL, and at last 200 µg/mL for 24 hours and cytotoxic action was determined using MTT assays, MTT assay has indicated that as the concentration of nanoparticle increased to 12.5µg/mL, 25µg/mL, 50µg/mL, 100µg/mL and 200µg/mL, cytotoxicity was observed in a dose-dependent fashion. In MTT assay cell viability was significantly reduced to 94%, 90%, 85%, 69%, 40% FOR THE CONCENTRATIONS OF 12.5µg/mL, 25µg/mL, 50µg/mL, 100µg/mL, 200µg/mL respectively.

Abhrak bhasma showed the IC50 value of 168.60ug/ml. Further studies like Cell Cycle Study by PI staining, Apoptosis study by Annexin V/PI staining, Apoptotic Protein expressions like Caspase 3, 7, 9, Bcl2, p53 and ROS study to evaluate the mechanism of action of test compounds.

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