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Anticancerous Activity of Methanolic Extract of *Piper nigrum* on Lung Cancer Cell Line (A549 Cell Line)

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ABSTRACT

Piper nigrum is a medicinally important plant of family Piperaceae. It can be used for the treatment of various ailments. It is used as stimulant and carminative. The extracts of the plant exhibit antidepressant activity also. In the present study, the anticancerous activity of methanolic extract of *Piper nigrum* fruit was determined by MTT assay on A549 cell line (Lung cancer cell line). The results revealed that the fruit of *Piper nigrum* possess anticancerous property and extract showed significant result with LD50 value of 85.9189 μ g/ml.



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INTRODUCTION

Cancer is a deadly disease that leads to the death of millions of people globally. In spite of the advancements in the medical field, it continues to be a threat. Causes of cancer can be environmental or genetic. Some important environmental factors can be infectious agents (like EBV, HPV etc.), smoking, alcohol consumption, unhealthy diet including obesity and some environmental carcinogens (like UV rays, lead or arsenic etc). Genetic causes can be those affecting proto-oncogenes, tumor suppressor genes, DNA repair gene etc, which are mainly due to different types of mutation. The carcinoma of the lung is the one which takes the most number of lives in comparison to other cancers around the world, out of which men being more than women. The primary cause is a preventable one that is smoking. These tumours are well known for their poor prognosis with a usual survival rate of 30% for 1year. Carcinomas from other organs can metastasize to lungs as well like breast carcinoma and osteogenic carcinoma.

Anticancerous compounds derived from plants are significant due to it's beneficial effects coupled with less side effects. Piperaceae family consists of plants that act as source of fruitful biologically active compounds that possess the potential of being employed as therapeutic agents. *Piper nigrum*, commonly called as black pepper, belongs to Piperaceae subfamily of class dicotyledons. It is well known for it's antimicrobial, analgesic, antipyretic, antioxidant properties. In the present study, the anticancerous activity of methanolic extract of *Piper nigrum* fruit on lung cancer cell line (A549 cell line) was evaluated.

MATERIALS AND METHODS

Preparation of plant extract

Piper nigrum fruits were collected from Trivandrum. It was washed, air dried under shade at room temperature. It was converted to coarse powder, then soxhlet extraction was carried out using methanol solvent. The methanolic extract of *Piper nigrum* was used for the evaluation of anticancerous activity.

Anticancerous activity of *Piper nigrum*

A549 (Lung cancer) cell line was initially obtained from National Centre for Cell Sciences (NCCS), Pune, India and was maintained in Dulbecos modified Eagles medium (Gibco, Invitrogen). The cell line was cultured in 25 cm² tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO₂ incubator (NBS Eppendorf, Germany). Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension (5x10⁴ cells/well) was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO₂ incubator. After 24 hours the growth medium was removed, freshly prepared compound in 5% DMEM was taken from this- 6.25µl, 12.5µl, 25µl, 50µl, 100µl were taken and made up to 250µl using 5% MEM and were added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO₂ incubator. MTT Method was used for the evaluation of anticancerous activity. Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO₂ incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (DMSO) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm. The percentage of growth inhibition was calculated using the formula:

$$\text{Percentage of viability} = \frac{\text{Mean OD Samples} \times 100}{\text{Mean OD of control}}$$

Percentage viability can be used to evaluate the anticancer property. (1,2) and the LD50 value was calculated using ED50 PLUS V1.0 Software.

RESULTS AND DISCUSSION

The anticancerous activity was measured using A549 cell line. It is a lung cancer cell line. The *in vitro* anticancerous activity of *Piper nigrum* was measured by MTT Method and Doxorubicin was used as standard. The *in vitro* antiproliferative activity of methanolic extract on the A549 cell line is depicted. The cell viability of A549 cell lines decreased considerably from 76.98 to 48.04 percent as the concentration of methanolic extract of *Piper nigrum* increased from 6.25 to 100µg/mL. (Table 1 & Figure 1). The magnitude of the anticancerous activity was measured by using LD 50 value, where LD stands for Lethal Dose. LD 50 is described as the concentration of drug that can kill or retard the growth of one half of cells in a population. So it can also be understood that lesser is the LD 50 value, more is the anticancerous activity. Here LD 50 value is 85.9189 µg/mL. It is clear that methanolic extract of fruit of *P.nigrum* has anticancerous activity. *Piper nigrum* exhibited cytotoxic activity against human leukemia cell line, murine melanoma cell line and human colon cell line (3). It also showed antiproliferative effect against human epithelial type 2 cell line (4).

Table No. 1: Percentage viability of Anticancerous property of *Piper nigrum*

Sample – *Piper nigrum*

| Sample Concentration (µg/mL) | OD value I | OD value II | OD value III | Average OD at 540nm | Percentage Viability |
|------------------------------|------------|-------------|--------------|---------------------|----------------------|
| Control | 0.5192 | 0.4866 | 0.5318 | 0.5125 | 100 |
| 6.25 | 0.4639 | 0.3938 | 0.4436 | 0.3945 | 76.98 |
| 12.5 | 0.443 | 0.3569 | 0.4103 | 0.3714 | 72.47 |
| 25 | 0.4255 | 0.3482 | 0.3433 | 0.3276 | 63.93 |
| 50 | 0.4056 | 0.3474 | 0.3426 | 0.2940 | 57.37 |
| 100 | 0.2975 | 0.3177 | 0.3083 | 0.2462 | 48.04 |

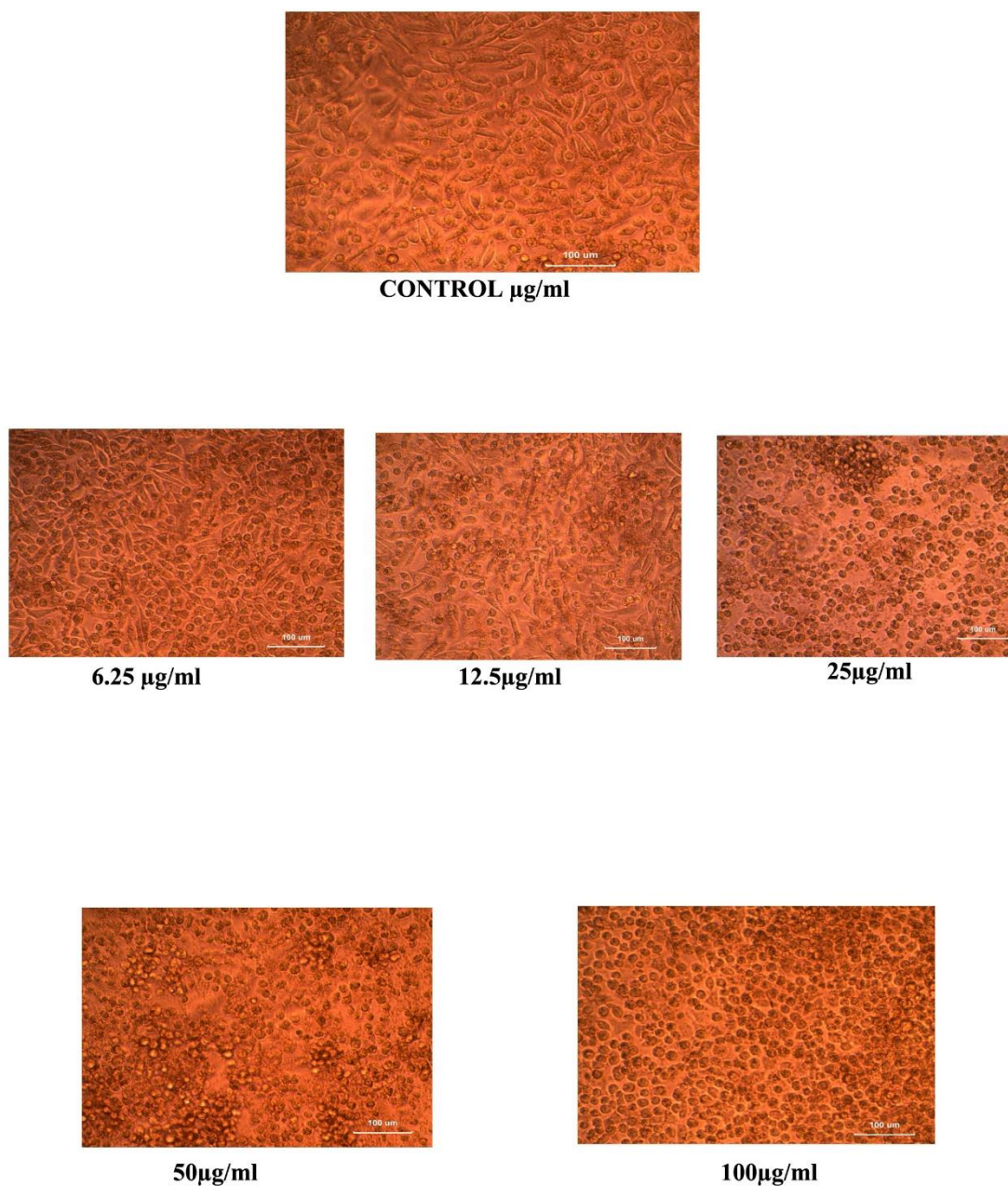


Figure No. 1: Anticancerous activity of methanolic extract of *Piper nigrum*

CONCLUSION

The anticancerous activity of methanolic extract of fruit of *Piper nigrum* has been studied. The LD 50 value of *Piper nigrum* is found to be 85.9189 $\mu\text{g/ml}$, which shows that the extract has anticancerous property. It shows highest anticancerous activity against A549 cell line at 100 $\mu\text{g/ml}$ concentration. This data suggests that it can be used in pharmaceutical industry for the fight against cancer.

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