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Anti- Proliferative Effect of Andrographis Elongataon Hela Cell Line

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ABSTRACT

Andrographiselongata (Vahl) T. Anderson or Mullurinjipachilla is an endemic medicinal plant. The plant is usually used against many ailments in old Travancore. The plant part is used in the treatment of snake bite, diabetes, cough, skin diseases. The main objective of this study is to identify the scientific basis of this plant used in traditional practices. In this study we analyze the anti-proliferative effect of the leaves of *A. elongata* on HeLa cell line. The cytotoxicity of the methanol leaf extract was evaluated *in vitro* by employing MTT assay. The extract showed dose dependent anti-proliferative activity with an IC_{50} of <100 µg/ml in HeLa cell line.

KEYWORDS: *Andrographis elongata*, HeLa cell line, MTT assay, cytotoxicity

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INTRODUCTION

Cervical cancer is both the fourth-most common cause of cancer and the fourth-most common cause of death from cancer in women in the world.It is the most common cause of cancer death in undeveloped countries. In India also, cervical cancer is the leading cancer among women. The main factors responsible for this disease were early age of marriage, multiple sexual partners, multiple pregnancies, smoking, use of oral contraceptives. Cervical cancer is caused by a virus called Human Papilloma Virus.[1] In the traditional system of medicine, plants and its products are used as a remedy for various ailments.[2]*A.elongata* is a medicinal plant used for the treatment of various ailments. The main objective of the present study is to evaluate the anti-proliferative effect of the plant.

MATERIALS AND METHODS

Plant Material

The leaves of *Andrographis elongata* (Vahl) T. Anderson were collected from Parassala of Thiruvananthapuram district. The plant was identified with the help of different standard texts and flora .[3]

Preparation of Methanol Leaf Extract

About 500gm of leaves of *A.elongata* was dried under the shade for 10 days. The dried leaves were powdered and extracted with methanol using soxhlet apparatus.[4]

Cell Line Used

In medical research, the most famous immortal cell line known as HeLa was developed from cervical cancer cells of a woman named Henrietta Lacks.HeLa cell line was obtained from National Centre for Cell Science(NCCS) Pune and maintained in Dulbecco's modified Eagle's media (HIMEDIA) supplemented with 10% FBS (Fetal Bovine Serum) and grown to confluence at 37°c in 5 % CO_2 in a humidified atmosphere in a CO_2 incubator(NBS, EPPENDORF, GERMANY). The cells were trypsinzed (500µl of 0.025% Trypsin in PBS/ 0.5mM EDTA solution (Himedia) for 2 minutes and passaged to T flasks in complete aseptic conditions.

Preparation of Working Extract

The methanol leaf extract was dissolved in DMSO giving a concentration of 1mg/ml. From this stock solution, Different concentrations of the extract (6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml and100µg/ml) were added to grownHeLa cells at a final concentration of 6.25 µg/ml, 12.5

µg/ml, 25 µg/ml, 50 µg/ml and100µg/mlfrom a stock of 1mg/ml and incubated for 24 hours. The % difference in viability was determined by standard MTT assay after 24 hours of incubation.

MTT Assay (Arunget al., 2009)

MTT is a colorimetric assay that measures the reduction of yellow 3-(4, 5dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent Dimethyl sulfoxide (Himedia) and the released, solubilized formazan product was measured at 540nm. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

The cells was washed with 1x PBS and then added 30 µl of MTT solution to the culture (MTT -5mg/ml dissolved in PBS). It was then incubated at 370C for 3 hours. MTT was removed by washing with 1x PBS and 200µlof DMSO was added to the culture. Incubation was done at room temperature for 30 minutes until the cell got lysed and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2minutes to precipitate cell debris. Optical density was read at 540 nm using DMSO as blank in a micro plate reader (ELISASCAN, ERBA).[5]The % cell viability and % cell inhibition were calculated with the following formula:

Calculation:

Percentage ofcell viability = (OD of Test / OD of Control) X 100

Percentage ofcell death = 100- (OD of sample / OD of Control) X 100

RESULT AND DISCUSSION

The anti-proliferative effect of *A.elongata* leaves on HeLa cell lines were showed in Table -1 and graphically represented in Figure -1.

When HeLa cells were treated with the methanol extract of the leaves of *Andrographiselongata*, the result showed a concentration dependent cytotoxic effect. As the concentration increased from 6.25 -100 µg/ml, percentage of inhibition increases from 12.13 % - 46.99 %.

Table 1: Cytotoxicity on HeLa cells by methanol leaves extract of *A.elongata*

Sl.No	Concentration (µg/ml) Control	% cell viability (Extract)	% cell inhibition (Extract)	% cell viability (doxorubicin)	% cell inhibition (doxorubicin)
1	6.25	87.86	12.13	90.9	9.1
2	12.5	82.82	17.17	64.86	35.14
3	25	70.96	29.03	50.97	49.03
4	50	67.45	32.54	44.74	55.26
5	100	53.00	46.99	34.16	65.84

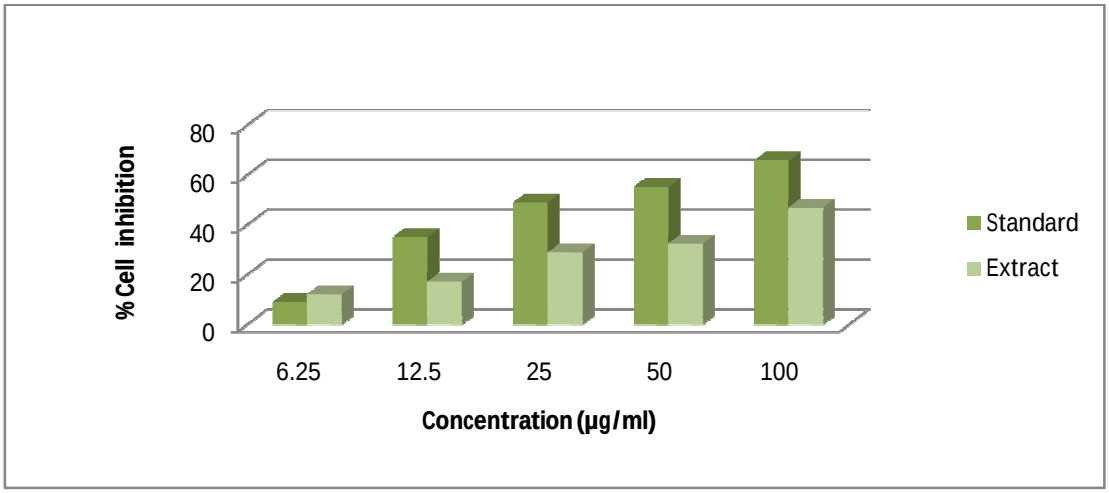

Figure 1: Effect of methanol extract of*A.elongata* leaves on HeLa cell inhibition

From the earlier findings it was clear that, flavonoids possessed a significant effect on cancer chemoprevention and chemotherapy.[6]The earlier studies documented the anticancer effect of *Andrographissps.*[7-9]Andrographolide is the main principal compound in *Andrographissps.*used for cancer prevention.[10]

From this study, the results confirmed the cytotoxic activity of *Androgrphiselongata* against cervical cancer. So it will helps to isolates and identify the lead molecules having cytotoxicity against cancer cells and non-toxic to normal cells. Discovery of such a herbal drug reduces the side effects of chemotherapy.

CONCLUSION

The results obtained from this study showed that, the methanolic leaves extract of *Andrographiselongata* possess moderate anticancer activity against HeLa cell line. When the sample concentration was increased, the percentage of cell inhibition also increased. The IC_{50} value was < 100 µg/ml. This study provides immense pleasure in the field of drug discovery against cancer

therapy. The anti-proliferative effect of *Andrographiselongata*providesvaluable information in the treatment of cancer.

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