

# ENHANCED ANTICANCER ACTIVITY OF *Barleria longiflora* L.f IN HUMAN CERVICAL CANCER (HeLa) CELLS

\*<sup>1</sup>A.Baskaran, <sup>2</sup>V.Karthikeyan, <sup>3</sup>C.Sebastian Rajasekaran

<sup>1</sup>P.G and Research Department of Botany, Bishop Heber College, Tiruchirappalli-620017, Tamil Nadu, India

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**Abstract:** The objective of this study was to examine the cytotoxic effect of *Barleria longiflora* L.f stem extract on cervical cancer (HeLa) cell lines. HeLa cells were cultured in EMEM medium and incubated with different concentrations (12.5, 25, 50, 100 and 200 µg/ml) of ethanol extract of *Barleria longiflora* L.f. The method used for this research to determine cell viability was an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. *Barleria longiflora* decreased cell viability in malignant cells in a concentration dependent manner. The IC<sub>50</sub> value of *Barleria longiflora* extract was found to be 184.3 µg/ml. These data showed ethanolic extract of *Barleria longiflora* reduces the viability of cervical cell lines and may have potential as anti-cancer compounds.

**Keywords:** Cervical Cancer cell, HeLa, *Barleria longiflora*.

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## 1. INTRODUCTION

Cancer is a major health problem in both developed and developing countries. Cancer is one of the leading causes of death worldwide and is characterized by proliferation of abnormal cells [1]. China, India, and Russia, which share rapidly rising cancer incidence, have cancer mortality rates that are nearly twice as high as in the UK or the USA. Vast geographies, growing economies, aging populations, increasingly westernized lifestyles, relatively disenfranchised subpopulations, serious contamination of the environment, and uncontrolled cancer causing communicable infections have all contributed to its rapid rise in incidence [2]. Under Indian circumstances cancer could lead to severe social and economic consequences, frequently causing family hardships and societal inequity. In a population of ~1.2 billion, nearly > 1 million new cases of cancer are diagnosed every year causing ~600,000e700,000 deaths in 2012 [3]. Surgery, chemotherapy, and radiotherapy methods are used in cancer treatment. However, these standard methods are expensive and have side effects with limitations of their use, so there is an urgent need for effective, inexpensive and non-toxic, treatments with minimal side effects that are acceptable by people [4].

Cervical cancer is a major cause of death. It is the second most frequent cancer in women worldwide. Human Papilloma virus (HPV) is considered as the etiologic agent of cervical cancer. Epidemiological and biological studies have shown close relationship between HPV infection and cervical cancer development. High risk HPV, such as HPV16 and HPV18, has been detected in 94 - 100% of cervical precancerous lesions and cancer [5]. Though the cervical cancer therapy is in advance, side effects due to the non-specific cytotoxicity of drugs and resistance to treatment represent a great problem in the cervical cancer management. Therefore, development and search of novel and effective anticancer agents, which in addition should overcome resistance, have become very important issues [6]. Natural compounds have provided many effective anticancer agents in current use. Currently, over 50% of drugs used in clinical trials for anticancer activity were isolated from natural sources or are related to them [7]. The use of plants or plants products, traditionally, as antiviral agents is relatively wider than their use in modern medicine. Some antiviral substances have so far been isolated from higher plants, algae and lichens [8]. The National Cancer Institute collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity [9]. Over 3000 species of plants with antitumour properties have been reported [10]. Natural products have been regarded as important sources of potential chemotherapeutic agents and many anticancer drugs have originated from natural sources [11].

## 2. MATERIALS AND METHODS

### 2.1. Preparation of Stem Extract

*Barleria longiflora* stem were collected from Rattamalai, Tiruchirappalli and were air dried and grounded to fine powder. Then the powdered material was extracted with ethanol by using soxhlet apparatus. The solvent was removed by evaporation and extract was concentrated by using vacuum rotator evaporator.

### 2.2. Cell Line

The human cervical cancer cell line (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37°C, 5% CO<sub>2</sub>, 95% air and 100% relative humidity. Maintenance cultures were passage weekly, and the culture medium was changed twice a week.

### 2.3. Cell Treatment Procedure

The monolayer cells were detached with trypsin-ethylenediaminetetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1x10<sup>5</sup> cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO<sub>2</sub>, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved or dispersed in dimethyl sulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37°C, 5% CO<sub>2</sub>, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

### 2.4. MTT Assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula: Percentage cell inhibition = 100 Abs (Sample) / Abs (Control) x 100.

### 2.5. STATISTICAL ANALYSIS

Non linear regression graph was plotted between % cell inhibition and log<sub>10</sub> concentration and IC<sub>50</sub> was determined using GraphPad Prism software.

## 3. RESULTS AND DISCUSSION

The results for cell growth inhibition by the extract against HeLa cell lines for various concentrations is shown in table 1. In the present study HeLa cells showed growth inhibition in a dose dependent manner when treated with *Barleria longiflora* extract at concentrations ranging from 12.5µg to 200µg. The percentage of dead cells for each concentration was found to be 0.24, 0.06, 3.06, 20.4, and 54.4. The 50% cytotoxic effect (IC<sub>50</sub>) of *Barleria longiflora* extract was found to be 184.3µg / ml (Table I and Figs. 1 & 2). The utility of cell lines acquired from tumors allows the investigation of tumor cells in a simplified and controlled environment [12]. There are specific advantages and disadvantages to exploit cancer cell lines over animal models. These then dictate the nature of the experiment that can be organized. In the last few decades, studies with cell lines can serve as an initial screen for agents that might regulate drug resistance. *Barleria longiflora* has been widely studied for its antioxidant activity. Now-a-days, after this antioxidant was found to offer protection against the occurrence of cancer activity [11]. In the present study the HeLa cell lines are used as a model for studying cervical cancer. Several mechanisms of action were detected in HeLa cells. The IC<sub>50</sub> of extract on cell line less than 100 µg / ml is categorized as a potential cytotoxic substance [13]. In the present study, ethanol extract of *Barleria longiflora* was found to be moderately cytotoxic towards human HeLa in MTT assay and the concentration required for

50% cell death was found to be 184.3  $\mu\text{g} / \text{ml}$ . Hence present study shows the efficacy of *Barleria longiflora* for the antiproliferation of HeLa cells thus suggesting protection against cervical cancer.

In summary, the present study demonstrated that ethanol extract of *Barleria longiflora* is a potent anti-cancer compound with an  $\text{IC}_{50}$  of 184.3  $\mu\text{g} / \text{ml}$  inducing growth inhibition in the human cervical cancer cells.

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#### APPENDICES - 1

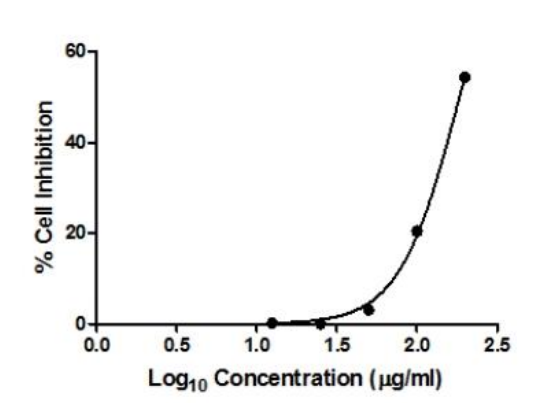
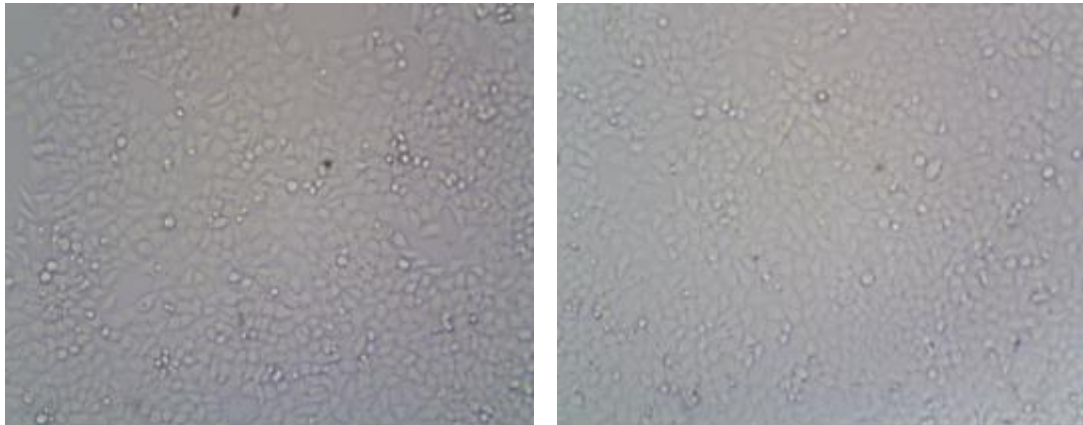


Fig.1 Percentage growth inhibition of *Barleria longiflora* against HeLa cell line

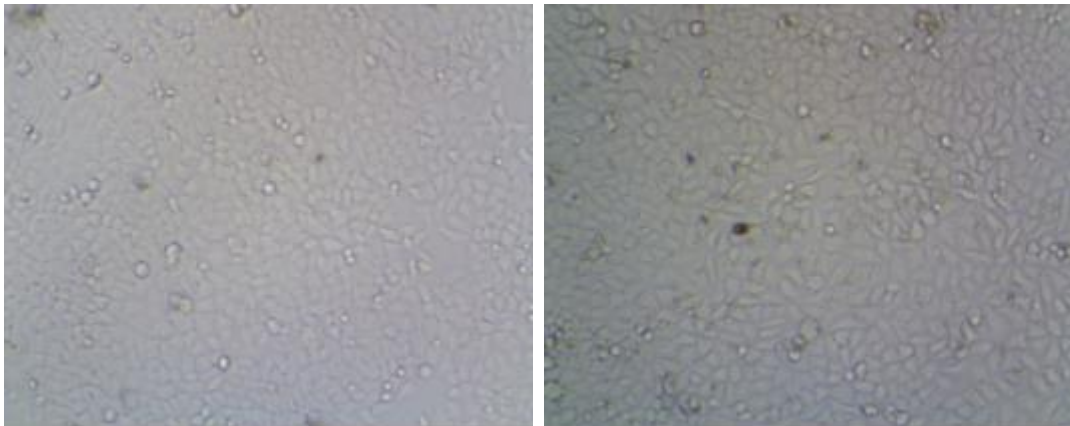
Table.1 In vitro Cytotoxicity of *Barleria longiflora* on HeLa cell lines

Plant Extract	Concentration ( $\mu\text{g} / \text{ml}$ )	% inhibition	IC50 $\mu\text{g} / \text{ml}$	R2
B.1 Stem	12.5	0.249843848	184.3 $\mu\text{g}/\text{ml}$	0.998
	25	0.062460962		
	50	3.060587133		
	100	20.42473454		
	200	54.40349781		



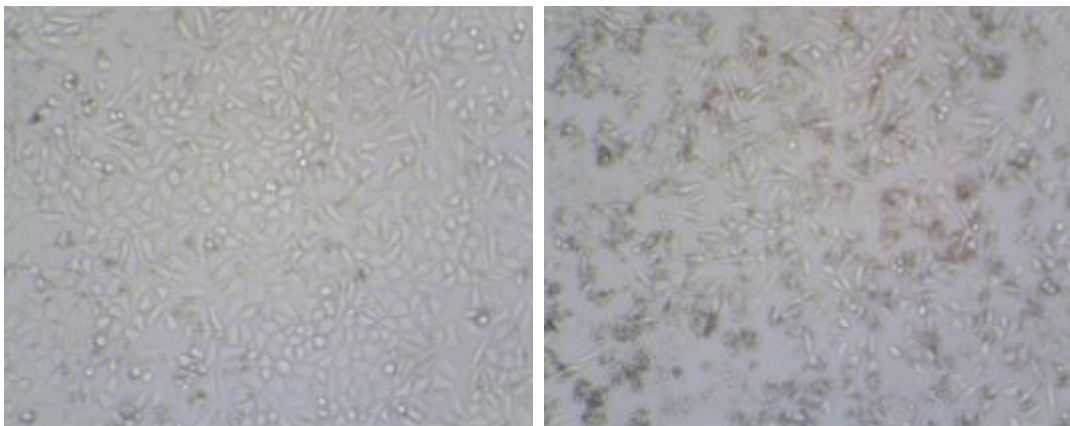
A

B



C

D



E

F

Fig.2 Proliferation of HeLa cells treated with *Barleria longiflora*

A - Control ; B - 12.5 g ; C - 25 g ; D - 50 g ; E - 100 g ; F - 200 g