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AN *INVITRO* EVALUATION OF CYTOTOXIC ACTIVITY OF *SOPHORA INTERRUPTA*

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ABSTRACT

In this study we evaluated the cytotoxicity activity of Sophora interrupta. For evaluation HeLa and HePG2 cell lines were treated with the various concentrations of Methanolic extract. The result showed an IC_{50} value of 211.5µg/ml and158.2µg/ml respectively in HeLa and HePG2 cell lines. The MTT assay results confirmed the cytotoxicity of the plant. Further, molecular characterisation studies and in vivo studies recommended to confirm still the anticancer activity of this plant.

KEYWORDS

Sophora interrupta, MTT assay, HeLa, HePG2, Cytotoxicity

1. INTRODUCTION

Cancer is one of the leading causes of mortality worldwide. On a yearly basis in US, 0.5% of the population is diagnosed with cancer. Despite improved imaging and molecular diagnostic techniques, cancer continues to affect millions of people globally. An efficient molecule to treat cancer is inevitable and explorations to develop new entities are going on. However, Nature has long been shown an excellent and reliable source of new drugs, including anticancer drugs. Plants are playing an important role as a source of anticancer drugs and the mechanism of interaction between many phytochemicals and cancer cells has been studied extensively[1, 2, 3].

Sophora interrupta belongs to the family Fabaceae (Leguminaceae, Papilonaceae) which is commonly called as Edwariamadarasapatna. There are more than hundreds of species belongs to this family which have various pharmacological activities such as anti-cancer, anti-inflammatory, antispasmodic, antibacterial. From the preliminary phytochemical studies it was identified that it has constituents like alkaloids, flavonoids, glycosides, phenols, carbohydrates and proteins[4] The aim of this study is to evaluate the cytotoxic activity of *Sophora interrupta* against HeLa, HePG2 cell lines. HeLa cell line is an immortal cell line used most commonly in medical research and HePG2 cell lines are also employed now-a-days in medical research[5].

2. MATERIAL AND METHOD

2.1. Plant Materials

The whole plant of Sophora interrupta was collected from Tirupathi, Andhra Pradesh in Feb 2011 and shade dried.

2.2. Preparation of Extract

The whole plant of *Sophora interrupta* was dried, powdered and exhaustively methanol extracted by soxhlet apparatus.

2.3. Cell lines used

The human cervical cancer cell lines (HeLa) and human hepatic carcinoma cell line (HePG2) were obtained from National Centre for Cell Science (NCCS), pune.

The HeLa and HepG2 were grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS)

2.4. MTT assay[6, 7]

This assay measures the metabolism of 3-(4,5-dimethylthiazol-2-yl)-2-5-biphenyl tetrazolium bromide to form an insoluble formazan precipitated by mitochondrial dehydrogenase only present in viable cells.

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 370C, 5% CO2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the extracts and fractions. They were initially dissolved in neat dimethylsulfoxide (DMSO) and further diluted in serum free medium to produce five concentrations. One hundred microlitres per well of each concentration was added to plates to obtain final concentrations of 31.25 µg, 62.5 µg, 125 µg, 250 µgand 500 µg/ml. The final volume in each well was 200 µl and the plates were incubated at 370C, 5% CO2, 95% air and 100% relative humidity for 48h. The medium containing without samples were served as control. Triplicate was maintained for all concentrations.

After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added

to each well and incubated at 370C 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.

% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log10 concentration and IC_{50} was determined using GraphPad Prism software.

3. RESULTS AND DISCUSSION

The effect of methanolic extract of *Sophora interrupta* plant on the growth of the two cell lines were examined by MTT assay.

3.1. Cytotoxic activity against HeLa cell lines

In the experiment the IC_{50} value in HeLa cell line found to be 211.5µg/ml and the results were provided in the **Table 1, Table2 and Fig 1.**The photographs of the cytotoxicity were provided under.**Fig 2.**

Table 1
Concentration VS. Absorbance in HeLa cell line

Concentration(µg)	31.25	62.5	125	250	500	Control
Absorbance	0.475	0.469	0.431	0.171	0.005	0.464
	0.483	0.452	0.405	0.154	0.003	0.488
	0.491	0.461	0.422	0.18	0	0.481
Average	0.483	0.460667	0.419333	0.168333	0.002667	0.477667

Figure 1
HeLa cell line

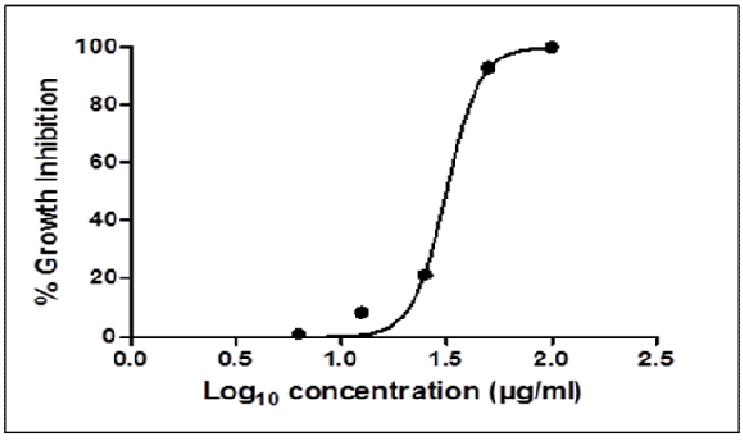
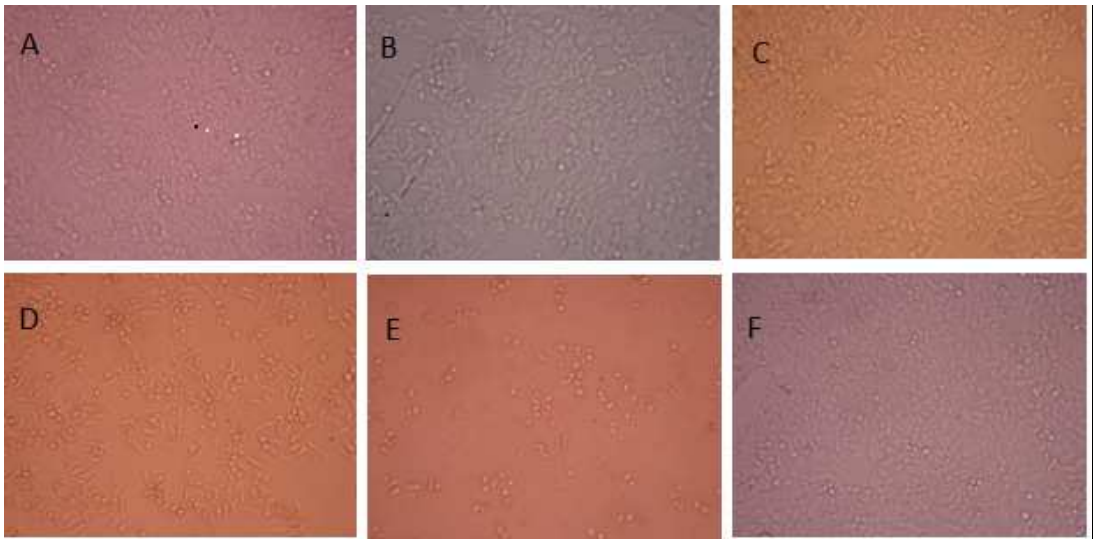


Table 2
Concentration VS % inhibition in HeLa cell line

Concentration (µg)	% Cell Inhibition
31.25	-1.11654
62.5	3.558967
125	12.21214
250	64.75925
500	99.44173

Figure 2
HeLa cell lines treated with the concentrations of
A) 31.25µg/ml, B) 62.5µg/ml, C) 125µg/ml, D) 2 50µg/ml, E) 500µg/ml, F) Control



3.2. Cytotoxic activity against HePG2 cell lines

In the experiment the IC_{50} value in HePG2 cell line found to be 158.2µg/ml and the results were provided in the **Table3, Table4 and Fig 3.** The photographs of the cytotoxicity were provided under **Fig 4.**

Table 3
Concentration VS Absorbance in HePG2 cell line

Concentration(µg)	31.25	62.5	125	250	500	Control
Absorbance	0.476	0.457	0.299	0.105	0.003	0.478
	0.475	0.416	0.305	0.117	0.003	0.453
	0.461	0.42	0.292	0.108	0.002	0.455
Average	**0.470667**	**0.431**	**0.298667**	**0.11**	**0.002667**	**0.462**

Figure 3
HePG2 cell line

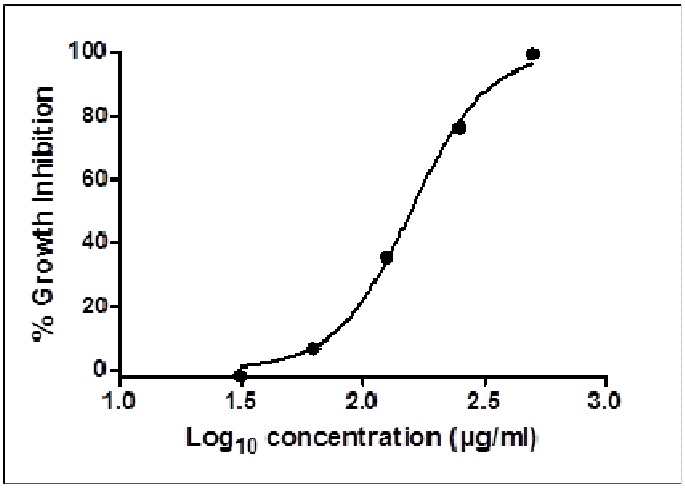
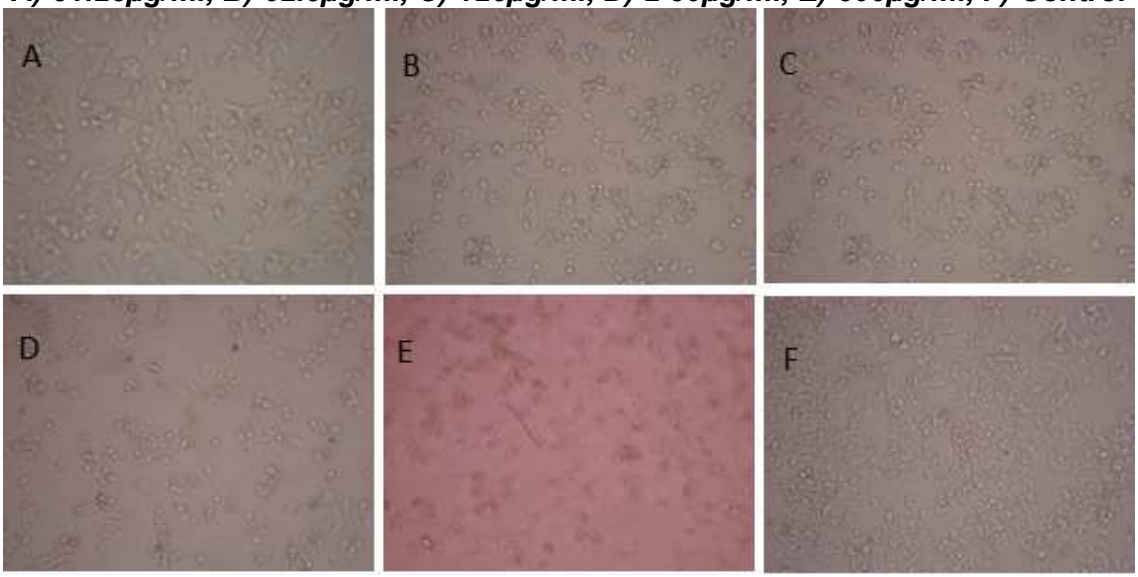


Table 4
Concentration VS % inhibition in HePG2cell line

Concentration (µg)	% Cell Inhibition
31.25	-1.8759
62.5	6.709957
125	35.35354
250	76.19048
500	99.4228

Figure 4
HePG2 cell lines treated with the concentrations of
A) 31.25µg/ml, B) 62.5µg/ml, C) 125µg/ml, D) 2 50µg/ml, E) 500µg/ml, F) Control



The above result affirms that the cytotoxicity of Sophora interrupta extract substantially increased with increase in concentration. The IC_{50} value of the extract on HeLa cell line was found to be 211.5µg/ml while the IC_{50} value on HePG2 cell line was found to be 158.2µg/ml. In both cell lines the plant showed a moderate cytotoxic activity.

4.CONCLUSION

From the study, it was evaluated that the whole plant of *Sophora interrupta* had cytotoxic activity against HeLa and HePG2 cell lines.

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