EVALUATION OF IN VITRO ANTICANCER ACTIVITY OF METHANOL EXTRACTS OF EVOLUVLUS ALSINOIDES LINN.

N. Vijayalakshmi[1] and P.T. Pratheesh[2]
[1 & 2]Assistant Professor, Department of Biochemistry with Nanotechnology, Nehru Arts and Science College, Coimbatore -641105

ABSTRACT

The study was designed to evaluate *in vitro* cytotoxicity of methanol extract of *Evolvulus alsinoides* Linn. (Convolvulaceae) leaves against human cervical cancer cell line (HeLa), human laryngeal epithelial carcinoma cells (HEp-2) and mouse embryonic fibroblasts (NIH 3T3). The cytotoxicity was assessed using the MTT assay. The methanol extract displayed remarkable cytotoxic activity against the cell lines with IC_{50} values of 31.22 ± 0.04 and 60.12 ± 0.20 and 32.42 ± 0.02µg/ml against HeLa, HEp-2 and NIH 3T3 cell lines, respectively. The results obtained from this study suggest that the methanol extract tested may be useful chemotherapeutic agents to inhibit the growth of carcinoma cells and this study would bring light to carry out further experiments and to prevent the cancer in human.

Key Words: *Evoluvlus alsinoides*, cytotoxicity, cervical, laryngeal epithelial carcinoma, mouse embryonic fibroblast.

INTRODUCTION

Cancer is the major cause of death worldwide, claiming over 6 million lives every year. In the recent years alternative therapies have gained importance over conventional cancer therapies for the treatment of cancer [1]. Chemotherapy is restricted by both intrinsic and acquired cell resistance to drugs. This has necessitated the use of natural products for the treatment of cancer. There are compelling evidences for experimental investigations on the efficacy of plant drugs against cancer [2].

Evolvulus alsinoides L., belonging to Convolvulaceae family, is known as morning glory and *Sankhapushpi*, grows wildly in open grassy places throughout India. Plant extracts have been used in traditional medicine for treatment of bronchitis, asthma and brain disorders [3]. *E. alsinoides* is well known for its memory enhancing property in traditional Indian system of medicine and extensively commercialized as nervin tonic in Asian countries. It was reported to possess antibacterial and anthelmintic, anti-ulcer and anti-catatonic activity, and immunomodulatory activity. The leaves exhibited significant scavenging activity against DPPH and superoxide radicals and inhibited acetylcholinesterase enzyme. The ethanol extract of *E. alsinoides* showed potent antioxidant activity [4,5]. Antioxidant compounds scopoletin, umbelliferone, scopolin and 2- methyl-1, 2, 3, 4-butanetetrol were isolated from *E. alsinoides*. Anti-stress compounds were obtained from *E. alsinoides*. However, to the best our knowledge, the in vitro anticancer activity of this plant has not been investigated. In the present

investigation, we therefore determined *in vitro* cytotoxic activity of methanol extract of *E. alsinoides* leaves. The cytotoxic activity was evaluated against human cervical cancer cell line (HeLa), Human laryngeal epithelial carcinoma cells (HEp-2) and mouse embryonic fibroblasts (NIH 3T3).

MATERIALS AND METHODS

Plant material and extraction

Fifty grams of plant leaf powder was exhaustively extracted in soxhlet apparatus with methanol (250 ml). The extract, thus collected, were evaporated to dryness using rotary flash evaporator (Buchi type, Switzerland) under reduced pressure at less than 40˚C. The resultant extract was used for assessing the *in vitro* cytotoxic activity [6].

Cell line and culture

The cells lines were obtained from National Centre for Cell Science (NCCS), Pune. The HeLa and HEp-2 cells were grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The NIH 3T3 fibroblasts were grown in Dulbeccos Modified Eagles Medium (DMEM) containing with 10% FBS [7].

In vitro cytotoxicity activity assay

The MTT assay was used to assess cytotoxicity towards the human cervical cancer cell line (HeLa), human laryngeal epithelial carcinoma cells (HEp-2) and mouse embryonic fibroblasts (NIH 3T3). Briefly stated, for screening experiment, the cells were seeded into 96-well plates in 100 µl of respective medium containing 10% FBS, at plating density of 10,000 cells/well and incubated at 37^0C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of extracts. The extracts were solubilized in dimethylsulfoxide (DMSO) and diluted in respective medium containing 1% FBS. After 24 h, the medium was replaced with respective medium with 1% FBS containing the extracts at various concentration (25, 50, 100, 200, 300, 400 µg/ml) and incubated at 37^0C, 5% CO_2, 95% air and 100% relative humidity for 48 h. Triplicate was maintained and the medium containing without extracts were served as control. After 48 h, 10 µl of MTT (5 mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and formazan crystals formed were solubilized in 100 µl of DMSO and the absorbance was measured at 570 nm using micro plate reader [8].

The percentage of cell inhibition was measured according to the following formula

Cell Inhibition (%) = 100- Abs (drug)/Abs (control) x 100.

The IC_{50} was expressed as the extract concentration in µg/ml that caused a 50% inhibition of growth of cell lines.

Statistical analysis

The experimental data were mean ± standard deviation of three measurements (n = 3). Linear regression analysis was used to calculate the efficient concentration (IC_{50}) values.

RESULTS

Effects of extract from *E. alsinoides* on human tumor cell lines

In vitro cytotoxic effect of methanol extract from *E. alsinoides* leaves was investigated at 25, 50, 100, 200, 300, 400 µg/ml against two human cancer cell lines and a mouse embryonic fibroblast. Fig. 1 illustrates the effects of different concentrations of *E. alsinoides* extract on cell proliferation of different cell lines. The inhibition of growth of the cell lines by the extract was concentration dependent. At 400 µg/ml, the extract showed 99%, 98% and 99% growth inhibition against HeLa cells, HEp-2 cells and NIH 3T3 cells respectively. The findings obtained in this study show that the IC_{50} of methanol extract from *E. alsinoides* was 31.22 ± 0.04 µg/ml in the HeLa cells, 60.12± 0.20 µg/ml in the HEp-2 cells and 32.42 ± 0.02 µg/ml in the NIH 3T3 cells. Maximum cytotoxicity by methanol extract was observed against HeLa human cancer cell line.

The extract of *E. alsinoides* at 400 µg/ml induced about 95% of cell death in HeLa and HEp-2 cancer cells. The adherent tumor HeLa cells were detached from the culture plate and become floated resulting in rounding up of cellular shape (Fig. 2B). The HEp-2 cancer cells treated with methanolic extract of *E. alsinoides* significantly increased the number of apoptotic cells compared to control (Fig. 2C).

Table 1: Cytotoxity of methanol extract from *E. alsinoides* leaves against human cancer cell lines

	Cytotoxicity (IC_{50}) (µg/ml)		
Sample	HeLa	HEp-2	NIH 3T3
Methanol extract of *E. alsinoides*	31.22 ± 0.04	60.12 ± 0.20	32.42 ± 0.02

Each value in the table was obtained by calculating the average of three experiments ± standard deviation (n = 3).

Fig 1: Effect of *E. alsinoides* methanol extract against HeLa, Hep2 and NIH 3T3 cell lines.

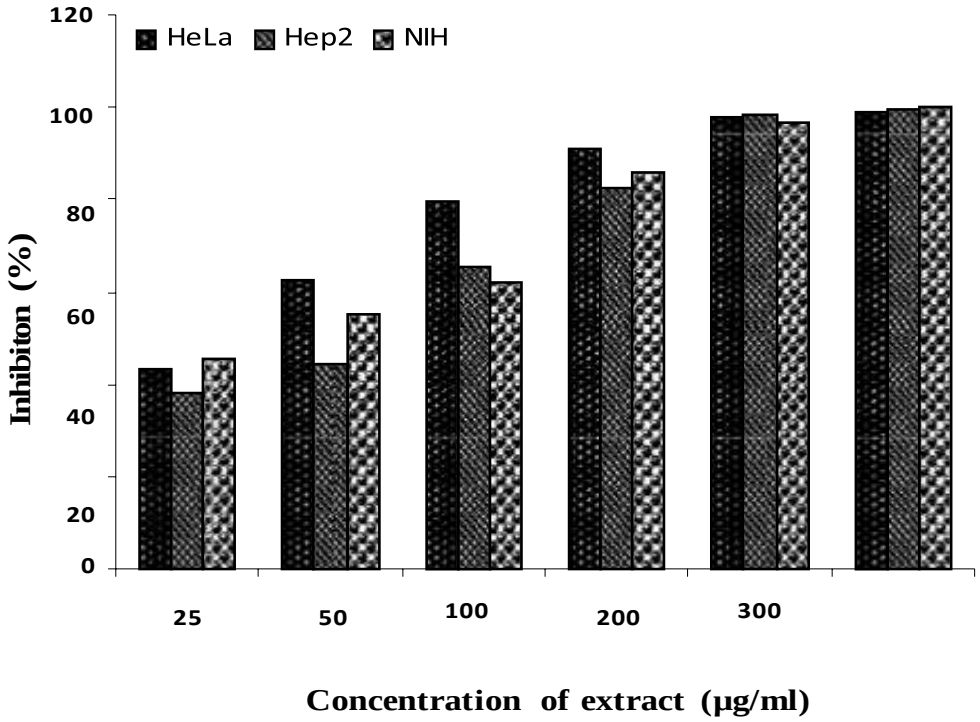

Fig. 2(a). Normal HeLa cell line **(b)** HeLa cell line treated with methanol extract of *E. alsinoides* at (400 µg/ml) **(c)** Normal Hep 2 cell line

DISCUSSION

The search for novel anticancer drugs from plants has been successful worldwide. Many anti- cancer components have been isolated and are nowadays used to treat human cancer. Our present study observed that the methanol extract of *E. alsinoides* inhibited the proliferation of human cancer cell lines such as human cervical cancer cell line (HeLa), and human laryngeal epithelial carcinoma cells (HEp - 2), and a mouse embryonic fibroblast (NIH 3T3). The extract displayed dose dependent and cell specific antiproliferative cytotoxicity against the cell lines tested. From the results obtained, it is apparent that extract from *E. alsinoides* was active in inhibiting *in vitro* cell proliferation. Maximum cytotoxicity by methanol extract was observed against HeLa human cancer cell line. Significant morphological changes were observed compared to control in the plant extract treated HeLa cells. This degenerative morphological change ultimately led the cells to death. The cells undergo shrinkage, rounding and membrane blobbing when exposed to high concentration of plant extract. The number of colonies decreased significantly in a concentration-dependent manner, suggesting that *E. alsinoides* effectively reduced the malignancy and suppressed the regeneration potential of cancer cells. The results of this study showed a remarkable activity of plant extract against the tested cell lines though the IC_{50} values are not lower than the dose recommended by the protocols of the national Cancer Institute of USA. Thus, further *in vivo* study is warranted to confirm its cytotoxicity.

CONCLUSION

Overall, from the results obtained it was observed that the methanol extract of *E. alsinoides* was active against the tested human cancer cell lines. The induction of apoptosis in tumor cells is considered a valuable way to treat cancer. A wide variety of natural substances have been recognized to have the ability to induce apoptosis in various tumor cells. It is thus considered important to screen apoptotic inducers from plants, either in the form of crude extracts or as components isolated from them. Hence it is proposed for further studies on possible anticancer activity *in vivo*.

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