



INVITRO CYTOTOXIC EFFECT OF ETHANOLIC EXTRACT OF PSEUDARTHRIA VISCIDA LINN

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Received: 11 Feb 2010, Revised and Accepted: 04 March 2010

ABSTRACT

Cancer prevention and treatment using traditional medicines have attracted increasing interest. The present study is designed to evaluate the cytotoxicity of ethanolic extract of *Pseudarthria viscida* (EEPV) Linn by MTT assay on HT29 (human colorectal cancer cell line), C<sub>2</sub>C<sub>12</sub> (mouse muscle cell line) and 3T3 L1 (mouse, embryo fibro blast) cell lines. Out of these three cell lines the extract shows potent cytotoxicity against HT29 cell line at 1000 µg/ml with CTC<sub>50</sub> 625.25±2.69 µg/ml compared to that of the other two cell lines.

**Key words:** *Pseudarthria viscida*, MTT assay, HT29, C<sub>2</sub>C<sub>12</sub>, 3T3 L1.

INTRODUCTION

Cancer is the disease characterized by excessive, uncontrolled growth of abnormal cells, which invade and destroy other tissues. Cancer develops in almost any organ or tissue of the body, but certain types of cancer are more life-threatening than others. In US and Canada cancer ranks as the second leading cause of death, exceeded only by heart disease. Each year, about 1.7 million Americans and more than 150,000 Canadians are diagnosed with cancer, and more than half a million Americans and about 70,000 Canadians die of the disease. Among various diseases attributed to mortality in humans all over the world, cancer is a leading cause.

Since ancient times, plants, herbs and spices have been important resources in traditional medicine<sup>1</sup>. The use of plants in the treatment of a variety of diseases, including cancer, has played a significant role in nearly every culture on earth and is the basis of modern medicine. Natural products are considered powerful sources of novel drug discovery and development. Their dominant role in anticancer chemotherapeutics is evident with approximately 74% being either natural products or natural product-derived<sup>2</sup>.

The plant *Pseudarthria viscida* (Fam: Fabaceae) is semi erect diffuse under shrub, distributed throughout all districts of South India, also reported from Srilanka and Timor. Traditionally, the plant is used for the treatment of intermittent fever, urinary diseases, tumors, oedema, burning sensation, difficult breathing and toxic conditions<sup>3</sup>.

EXPERIMENTAL

Plant Material

The plant *Pseudarthria viscida* Linn. was collected from Kolli hills, Tamil Nadu, India. The plant material was taxonomically identified by the Botanical Survey of India, Southern Circle, TNAU campus, Coimbatore (No. BSI/SC/5/23/06-07/Tech-116)

Extraction

The whole plant of *Pseudarthria viscida* Linn. was dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve No. 42 and stored in an airtight container for further use.

The dried powder material of whole plant (500 g) was defatted with petroleum ether (60-80 °C) by hot continuous extraction method in a soxhlet apparatus for 48-72 h. The defatted powder material was further extracted with ethanol (95 %v/v) for 72 h in soxhlet apparatus. The extract was made solvent free by distillation process and the resulting semisolid mass was vacuum dried to yield a solid residue i.e. Pet. ether extract (1 %w/w) ethanolic extract (5 % w/w).

Reagents

3-(4, 5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA., Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM), EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl sulfoxide (DMSO) and propanol were purchased from EMerck Ltd., Mumbai, India.

Cell lines and culture medium

HT-29 (Human, colorectal cancer), C<sub>2</sub>C<sub>12</sub> (Mouse, Muscle cell line) and 3T3-L1 (Mouse, embryo fibroblast) cell cultures were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells of HT-29, C<sub>2</sub>C<sub>12</sub> and 3T3-L1 were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in a humidified atmosphere of 5% CO<sub>2</sub> at 37 °C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm<sup>2</sup> culture flasks and all experiments were carried out in either 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Determination of cell viability by MTT assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10<sup>5</sup> cells/ml using DMEM medium containing 10% FBS/NBCS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37° C for 3 days in 5% CO<sub>2</sub> atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the extract solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37° C in 5% CO<sub>2</sub> atmosphere<sup>4</sup>. The supernatant was removed and 100 µl of Propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a micro plate reader at a wavelength of 540 nm<sup>5</sup>. The % growth inhibition was calculated using the following formula and concentration of drug or test extract needed to inhibit cell growth by 50% (CTC<sub>50</sub>) values were generated from the dose-response curves for each cell line<sup>6</sup>.

% Growth Inhibition = 100 - [Mean OD of test group / Mean OD of control group] x 100

RESULTS AND DISCUSSION

The present cytotoxic study demonstrated that EEPV showed 61.25% of cytotoxicity against HT-29 cancer cell line induced cancer in *in vitro* anticancer model at the concentration of 1000 µg/ml (Table 1).

**Table 1: Cytotoxic properties of EEPV on different cell lines**

S. No	Cell lines	Test Concentration. (µg/ml)	% Cytotoxicity	CTC <sub>50</sub> (µg/ml)*
1	HT-29	1000	61.25	625.25±2.69
		500	48.10	
		250	33.50	
		125	27.39	
		62.5	22.85	
2	C <sub>2</sub> C <sub>12</sub>	31.25	16.72	754±1.82
		1000	58.75	
		500	44.50	
		250	29.35	
		125	11.65	
3	3T3 L1	62.5	3.45	>1000
		31.25	0.00	
		1000	35.46	
		500	21.09	
		250	14.75	
		125	7.85	
		62.5	0.00	
		31.25	0.00	

\*P<0.001 Values were Mean ± S.E.M (Tukey-Kramer equation)

On the other hand EEPV in the concentration of 1000 µg/ml produced a cytotoxicity of 58.75% when another model C<sub>2</sub>C<sub>12</sub> was used as *in vitro* anticancer model. Similarly, EEPV in the concentration of 1000 µg/ml produced 35.46% of cytotoxicity only against 3T3 L1 cancer cell line induced cancer. The results indicate that EEPV showed a potent activity against the *in-vitro* HT-29 cell line when compared to that of C<sub>2</sub>C<sub>12</sub> and 3T3 L1.

The percentage growth inhibition was calculated and concentration of drug or test extract needed to inhibit cell growth by 50% (CTC<sub>50</sub>) values is generated from the dose-response curves for the cell line. EEPV produced a CTC<sub>50</sub> value of 625.25 µg/ml in the case of HT-29 and 754 µg/ml in the case of C<sub>2</sub>C<sub>12</sub>. It produced CTC<sub>50</sub> value above 1000µg/ml in the case of 3T3 L1 model. Relatively less value of CTC<sub>50</sub> indicates the sample is more Cytotoxic and possesses anticancer activity. Hence it may be concluded that the EEPV is more potent anticancer agent in HT-29 model and moderately potent in C<sub>2</sub>C<sub>12</sub> model. While the EEPV extract failed to exhibit anticancer activity against 3T3 L1 model.

The phospholipids-rich lysosomal membrane is a potential site of free radical attack. Leakage of lysosomal enzymes into cell and the surrounding extracellular space has been implicated in the pathogenesis of cell injury. Irrespective of the inducing mechanism, a moderate degree of lysosomal rupture seems to result in cell damage of the apoptotic type, with pyknotic nuclei and shrunken cytoplasm, while more extensive lysosomal damage results in reparative autophagocytotic activity and only limited cell death<sup>7</sup>.

Although normal cells possess antioxidant defense systems against ROS, the continuous accumulation of damage to the cells induces diseases such as cancer. The antioxidants play a preventive role against these diseases by removing the ROS.

Anticancer agents on the other hand, are mainly related to their curative role in a damaged system. Under normal conditions, the cells in which the DNA or other components are irreversibly damaged by various causes undergo apoptotic cell death, which is a self-destructive metabolism according to the genetically encoded

cell death-signal<sup>8</sup>. However cancer cells, which are already irreversibly developed, obtain the capability to evade apoptosis by various ways. The aim of anticancer agents is to trigger the apoptosis signaling system in these cancer cells whilst disturbing their proliferation<sup>9</sup>.

The vast number of chemical constituents which are present in ethanolic extract of *Pseudarthria viscida* such as phenolic compounds, flavonoids, terpenoids, tannins, etc may be responsible for cytotoxic effect.

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