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Evaluation of antiproliferative properties of *Lavatera cachemeriana* roots

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Abstract
Brest cancer is the leading cause of deaths across the world and plant derived bioactive molecules are considered as potent and safer agents to tackle and prevent therapeutically challenged breast cancer incidences. The current study investigated *In vitro* anti-proliferative activity of *Lavatera cachemiriana* methanol root extracts against breast cancer cell line (MCF-7) through MTT assay, L-6 cell line acted as a negative control. Results have demonstrated that extracts showed insignificant levels of anti-proliferative activities against tested cell lines (IC_{50}> 1000 µg/ml). The morphology of both treated and untreated cells was same with no changes reported. In conclusion, the methanol root extract possesses no marked anti-proliferative activities against breast cancer cell line at the tested extract concentrations.

Keywords: Anticancer, anti-proliferative, MCF-7, L-6, MTT assay

Introduction
Plant derived medicines are considered potential agents for the treatment of many diseases (Parekh and Sumitra, 2007) [1] as they possess many important biological activities such as antioxidant, preservation, anti-inflammatory, antimicrobial, anticancer, antidiabetic etc. (Fernandez, 2006) [2]. Around 60% of anti-cancer drugs that are in the market or under clinical trials are of natural origin (Rates, 2001) [3]. There are mounting evidences that natural products which are currently used in medicine possesses a wider range of chemical diversity; due to which they possess potential to be the source for modern drug discovery. Screening of more number of natural sources including medicinal and aromatic plants seems a promising strategy to identify source of lead bioactive molecules against different cancers. Breast cancer, being a leading cause of death and an important health issue across the world (Hany, 2013) [4], the treatment options for advanced breast cancer patients are lesser due to relapse or more toxicity of currently used drug regimens.
Lavatera cachemeriana (Family-*Malvaceae*) is used as a folklore medicine in Kashmir Himalaya against different clinical conditions such as mumps, common cold, laxative, renal colic, anti-dandruff, throat problems (Kaul, 2010; Jeelani *et al*., 2013; Malik *et al*., 2011) [5-7]. There is a growing interest within the scientific community to screen more and more number of plants so as to identify potential plant based molecules, which could treat or prevent breast cancer incidences (Anupam *et al*., 2011) [8]. Therefore, the current study was attempted to evaluate anti-proliferative activity of *Lavatera cachemeriana* roots.

Materials and Methods
Collection, authentication and extraction
The root samples of *L. cachemeriana* were collected from Gulmargh region of Jammu and Kashmir (10,020 feet above sea level), cleaned and a part was deposited at the University of Kashmir herbarium (KASH-1726). Root samples were air dried at room temperature (25^0C) for 3 days and subjected to grinding into fine powder. The crude extract was filtered using Whatman No. 1 filter paper and the extract so obtained was dissolved in DMSO to prepare a concentration range (62.5-1000 µg/ml) of crude extract. (Tiwari *et al*., 2011) [9].

Solvents and chemicals
The media components for animal cell culture (Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin. EDTA, Glucose, MHA medium, PDB medium and antibiotics) were procured from Hi-Media Laboratories Ltd., Mumbai.

Biological materials and culture medium

Two animal cell lines i.e. Rat Muscle (L-6) and human Breast carcinoma (MCF-7) were procured from National Centre for Cell Sciences (NCCS), Pune, India. The stock cells were cultured under Dulbecco's Modified Eagle's Medium i.e. 10% inactivated FBS, amphotericin B (5 µg/ml), penicillin (100 IU/ml) and streptomycin (100 µg/ml), 5% CO2 at 37 °C until confluent. The dissociation of these cells was under TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS) and using 25 cm^2 culture flasks, the stock cultures were transferred in them.

***In vitro* antiproliferative activity by MTT assay**

The method used for determination of cytotoxicity studies of sample extracts was same as described by Francis and Rita, 1986 [10]. The percentage growth inhibition was calculated using the following formula and the concentration of test sample needed to inhibit cell growth by 50% (IC_{50}) values was generated from the dose-response curves for both the cell lines.

$$\% \text{ Growth inhibition} = \frac{\text{Mean O.D of individual test group}}{\text{Mean O.D of control group}} \text{X } 100$$

Results and Discussion

The morphological changes in two selected cell lines (L6-normal rat muscle and MCF7-human breast carcinoma) were observed by microscopic examination which revealed no marked cytotoxicity in the given extract concentration range against tested cell lines (Fig.1). The IC_{50} values against both the cell lines were found to be >1000 µg/ml (Table-1). The capacity of cells to resist toxic shock has remained the foundation of most cytotoxicity assays and MTT assay is based on the principle that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present in the medium and the mitochondrial enzyme succinate dehydrogenase activity per cell. This enzyme brings cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan). It is found that number of cells present is directly proportional to the extent of formazan produced by the cells (Francis and Rita, 1986) [10].

As per review article of Stoner *et al*., 2008 [11] specifies that inhibition of cell proliferation depends on various factors such as the type of the extract, cell line being used, stability of extract components in different media, length of treatment time, differential uptake of phenolics etc. The absence of cytotoxicity against MCF-7 cell line could be because we used only single cancerous cell line and it will be possible that cytotoxicity activity will be present against other cancerous cells, this is because this herb has shown significant antioxidant activity and total phenolic and flavonoid content; which enhances further chances to possess anticancer properties. Also, there are few previous studies that have reported promising cytotoxicity activity of *L. cachemeriana* seeds against prostate (PC-3), breast (MCF-7) cell lines, THP-1 (leukemia), NCIH322 (lung) and Colon205 cell lines (Rakashanda, *et al*., 2013) [12] which is attributed to presence of protease inhibitors. Furthermore, Dar *et al;* 2004 has reported isolation of two diterpene compounds {ent-pimmaran 8(14),15-diene-19-oic acid and ent-pimmarane 7(8), 9(11),15-diene-19 oic acid} from *L. cachemeriana* which have showed promising *in vitro* cytotoxicity against five human cancer cell lines I.e. SK-N-MC (CNS), HT-29 (colon), A-549 (Lung), Hep-2 (liver), OVCAR-5 (Ovary) and PC-3 (Prostate). The cytotoxicity activity of extracts against any specific cancerous cell depends upon the type of phytoconstituents present in those extracts such as phenolic acids (hydroxycinnamic and hydroxybenzoic acids), flavonoids (anthocyanins, flavanols, flavonols), condensed tannins (proanthocyanins), hydrolysable tannins (ellagitannins and gallotannins), stilbenoids,lignans, triterpenes and sterols (Dhanukar *et al*., 2000) [14]. Therefore, it is recommended for future studies to include more number of cell lines to verify the anti-proliferative activities of different extracts of *L. cachemeriana* along with proper isolation and spectral characterization of lead bioactive molecules.

Table 1: *In vitro* cytotoxicity effect of methanolic extract of *Lavatera cachemeriana* against L6 (Normal rat muscle) and MCF-7 (Human breast carcinoma) Cell lines

Sl. No	Name of cell line	Test Conc. (µg/ml)	% Cytotoxicity	IC_{50} (µg/ml)
1	L6	1000	23.09±1.2	>1000
		500	21.55±4.8	
		250	19.41±4.4	
		125	19.15±3.1	
		62.5	16.24±5.4	
2	MCF7	1000	22.97±5.5	>1000
		500	21.14±1.2	
		250	21.01±1.9	
		125	18.90±0.3	
		62.5	18.75±2.0	

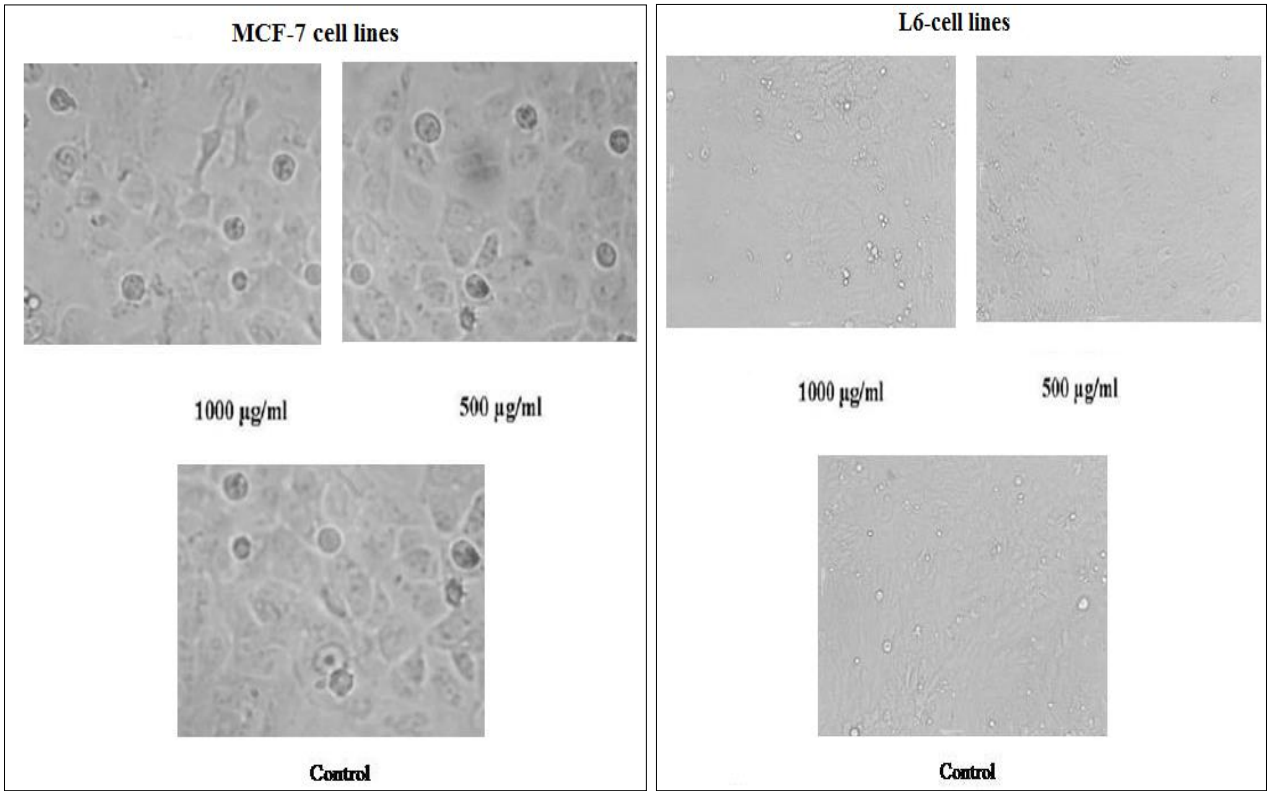

Fig 1: Microscopic examination of L6 (Normal rat muscle) and MCF-7 (Human breast carcinoma) cell lines after exposed to methanolic extract of *Lavatera cachemeriana***.**

Conclusion

The present work was aimed to test the cytotoxicity activities of methanolic root extracts of *L. cachemeriana.* Lower insignificant levels of cytotoxic properties were observed with IC_{50} value of >1000 µg/ml.

Conflict Of Interest

All the authors confirm that there is no conflict of interest.

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