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STUDIES ON CYTOTOXIC EFFECT OF *ALBIZIA LEBBECK* METHANOLIC BARK EXTRACT ON HUMAN CELL LINES

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ABSTRACT

To investigate cytotoxic effects and the mechanism of cell death of *Albizia lebbeck* bark extract HeLa, Miapaca-2, HepG2, AGS, U373MG, A431, NIH3T3 and MCF7 cell lines was examined. MTT (3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were performed in cell line as well as human endothelial cells to analyse the cytotoxic activity of the extracts of *Albizia lebbeck*. The apoptosis inducing action of the extracts was determined by TUNEL test and cell death assay. The results showed that the methanol extract significantly inhibited cell growth and viability in dose dependent manner without inducing damage of non- cancerous cell lines indicated generation of methanol extract of *Albizia lebbeck* in MCF-7cells. This study may be containing of bark extract isolated in bioactive compounds for the treatment of cancer.

KEYWORDS: *Albizia lebbeck* bark, Human cell line.

INTRODUCTION

In recent years, there has been increasing importance in another therapy and the therapeutic natural products, in particular those derived from plants. This attention in drugs of plant source is due to some reasons, namely, the frequent inefficiency of conventional medicine, possible enhancement of region particular effects of synthetic drugs. In addition, the long history of use of folk medicine suggests that "natural" products are generally harmless.

A. lebbeck is a tree well known in the Indian subcontinent for its range of uses. *A. lebbeck* is used in Indian traditional system and folk medicine as well to treat several inflammatory

pathologies such as asthma, arthritis and burns.[1] As a part of the efforts to investigate therapeutic agents from local plants, the current phytochemical and biological studies on some extracts and isolated compounds from *A. lebbeck* bark extract was carried out. The bark has sharp taste and its extract showed antimicrobial activity. The active constituents of the bark extract were anthraquinone glycosides that cause the leakage of the cytoplasmic constituents.[2] The methanolic extract of the stem bark of *Albizia lebbeck*, a new cytotoxic saponin was isolated compound exhibited potent cytotoxic activity against human aqueous cell carcinoma (HSC-2 and HSC-3).[3] The bark and leaves of *Albizia procera* were extensively used for the treatment of variety of wounds and considered useful in pregnancy and stomachache. Lipophilic extracts of *Albizia gummifera* revealed very promising anti-trypanosomal activity.[4] In contrast to other cytotoxic echinocystic acid glycosides with N-acetyl glucosamine unit, the new glycosides were found inactive when assayed by MTT method for their cytotoxicities against the HEPG2, A549, HT29 and MCF7 cell lines.[5]

Literature reviews revealed that no study has been carried out on cytotoxicity *Albizia lebbeck* bark extracts on Hela and MCF7 cell line. In this study was conducted to evaluate the cytotoxic effect of bark extract of fore mentioned plant on this cell line.

MATERIAL AND METHODS

Collection of Plant Material

Bark samples of *Albizia lebbeck* was collected from the Eastern Ghats of South India.

Preparation of extract

Powdered bark measuring 200g each was extracted in sterile distilled water (3 l) on shaker (Stuart Scientific Orbital Shaker, UK) for 48 h. The extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate was quickly frozen at -40 °C and dried for 48 h using a freeze dryer to give a yield of 30 g of dry extract. The resulting extract was reconstituted with sterile distilled water to desirable concentration and used throughout study.

Chemicals

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

Cell lines and Culture medium

HeLa, NIH3T3 and MCF7cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells 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 an humidified atmosphere of 5% CO_2 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^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test Solutions

For Cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out Cytotoxic studies.

Determination of cell viability by MTT Assay

The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used (Francis and Rita, 1986).

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM containing 10% FBS. 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 of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37^o C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug 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^o C in 5% CO_2 atmosphere. 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 microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed

to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

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

Statistical Analysis

All the values determined Mean ± SD**.** The statistical analysis was performed Graph pad prism software.

RESULT AND DISCUSSION

The extract and fractions were tested *in vitro* for its potential human cancer cell growth inhibitory effect on HeLa, HepG2, Miapaca-2, AGS, A431 and U373MG cancer cell lines using both MTT assay y, a non radioactive, fast and economical assay wisely used to quantify cell viability and proliferation.

The IC50 values of the extract and fraction for the cell lines HeLa, HepG2, Miapaca-2, AGS, A431 and U373MG determined by both MTT assay and SRB assay was summarised and presented in fig 1, 2, 3, 4, 5 & 6. The cell lines HepG2 and Miapaca-2 were more sensitive among the cell lines used and demonstrated low IC50 values when compared to others. The bark extract and its fractions were active against all the six cell lines tested and showed the IC50 values of 38.35 to 79.8 µg/ml for HeLa, 28.55 to 60.45 µg/ml for HepG2, 30.65 to 63.35 µg/ml for Miapaca-2, 40.95 to 90.05 µg/ml for AGS, 42.05 to 86.4 for A431 and 36.95 to 82.6 µg/ml for U373MG.

Among the all extract and fractions the BEA showed higher cytotoxicity to all six cell lines and the IC50 value was 38.35, 28.55, 30.65, 40.95, 42.05 and 36.95 µg/ml for the cell lines HeLa, HepG2, Miapaca-2, AGS, A431 and U373MG respectively. The cytotoxicmeffect of the bark fractions may be due to the presence of high content of polyphenols.

Pereda-Miranda and co-workers in their study showed that cytotoxic effect of hexane extract of *I. pes-caprae* is related to some lipophilic glycoside compounds.[8] The aqueous extract of *P.betle* leaves exhibits cytotoxicity in Hep-2 cells in microculture tetrazolium assays and sulforhodamine B (SRB) assays.[9] The cytotoxic effect of studied proteins toward HeLa cell line cells has been evident and dependent on increasing dose of the protein. Therefore, the bark extracts present in *Albizia lebbeck* may be among bioactive component which contributed to the apoptosis pathways in MCF-7 cells.

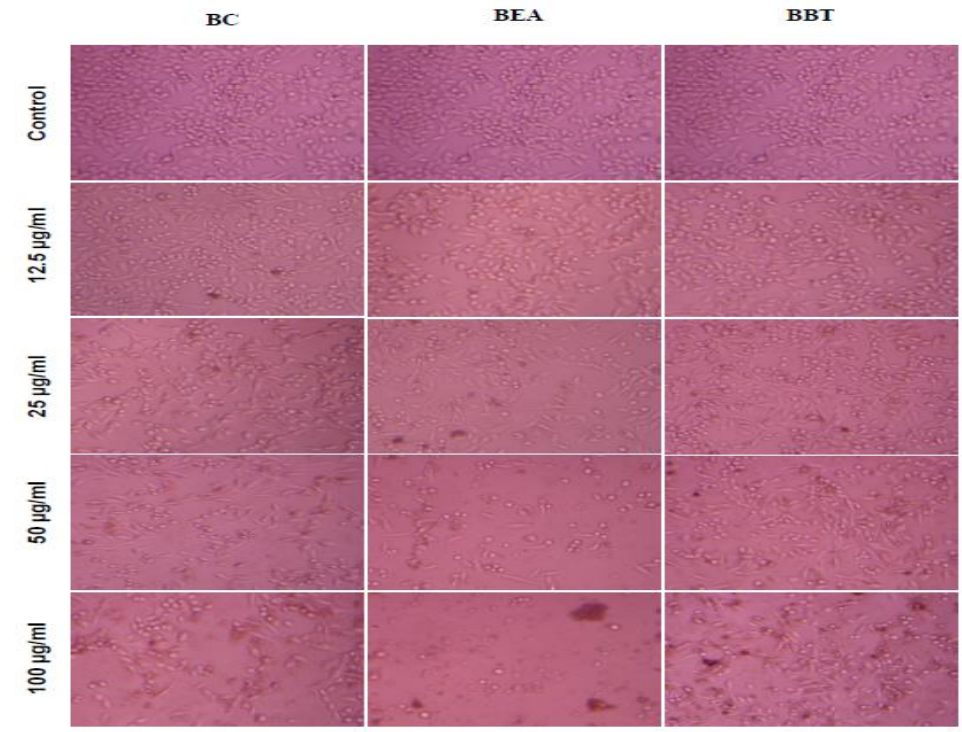

Fig: 1. Effect of HeLa cell line after 48h treatment with various concentration of *Albizia lebbeck* Bark ethanolic extract.

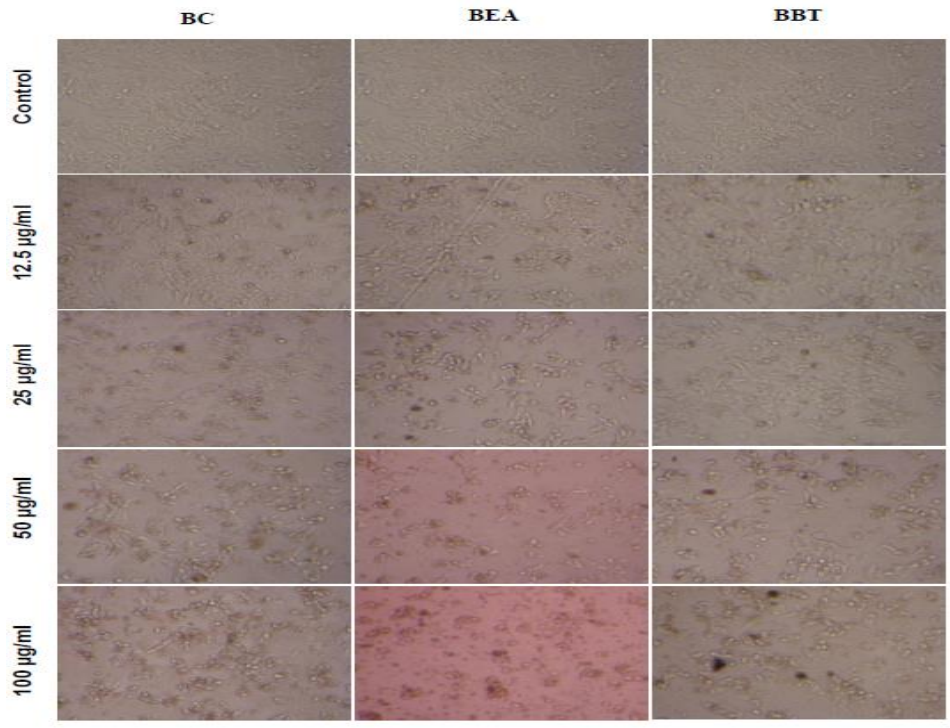

Fig: 2. Effect of Miapaca-2 cell line after 48 h treatment with various concentration of *Albizia lebbeck* Bark ethanolic extract.

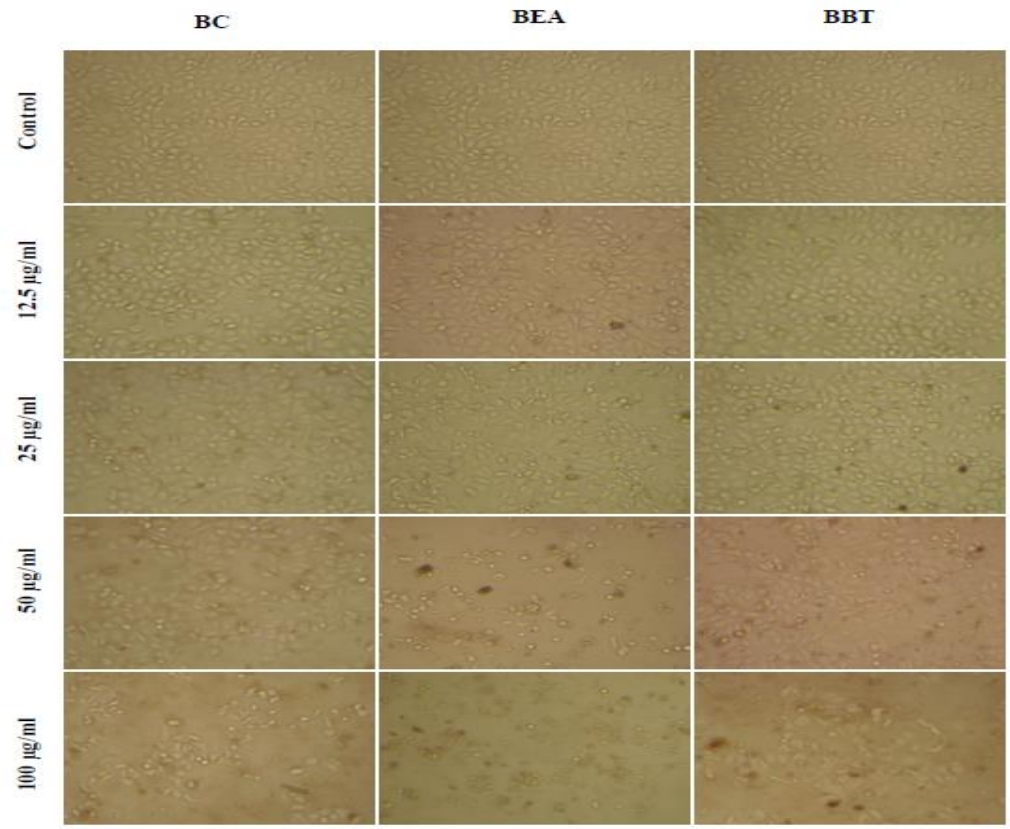

Fig: 3. Effect of HepG2 cell line after 48 h treatmentwith various concentration of *Albizia lebbeck* Bark ethanolic extract.

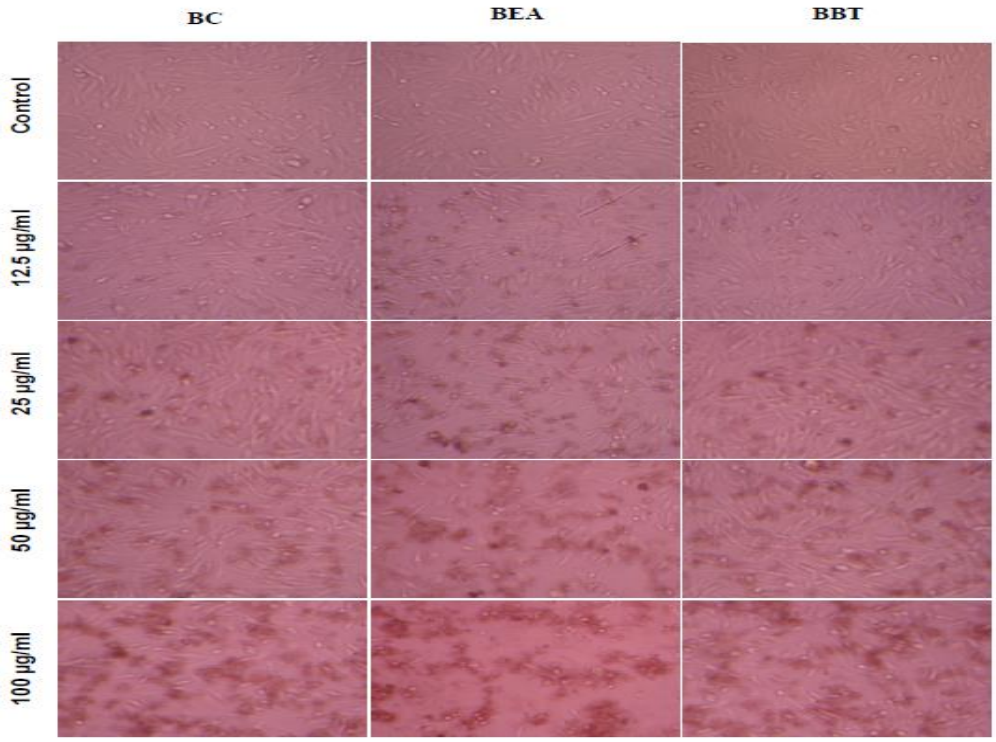

Fig: 4. Effect of AGS cell line after 48 h treatmentwith various concentration of *Albizia lebbeck* Bark ethanolic extract.

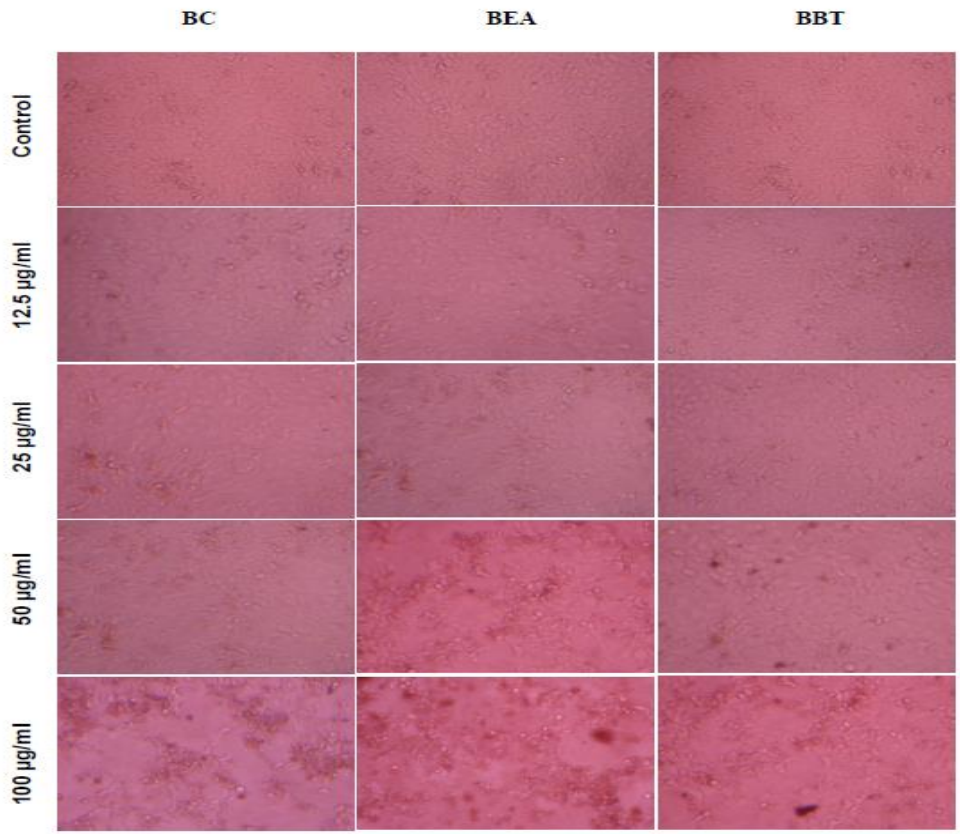

Fig: 5. Effect of U373MG cell line after 48 h treatment with various concentration of *Albizia lebbeck* Bark ethanolic extract.

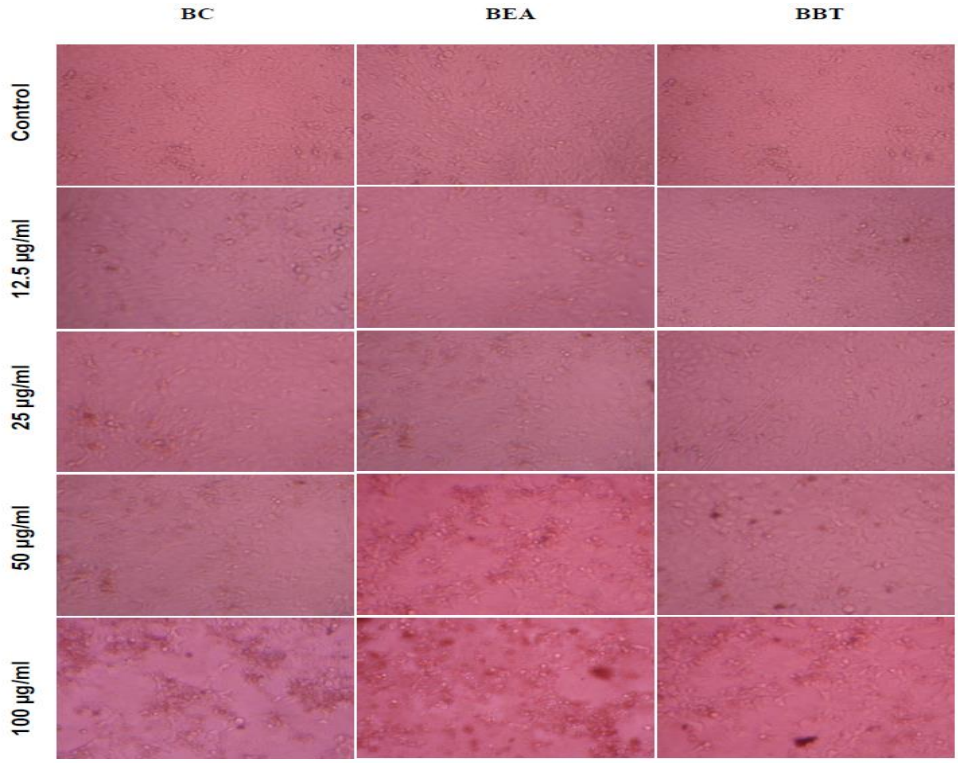

Fig: 6. Effect of A431 cell line after 48 h treatmentwith various concentration of *Albizia lebbeck* Bark ethanolic extract.

CONCLUSION

This study reveals that the in vitro cytotoxic and apoptotic activity of methanol extracts of *Albizia lebbeck* in Human breast carcinoma cells, thus possibly suggesting a new potential chemotherapeutic agent for the treatment of breast cancer.

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