“CELL GROWTH INHIBITION AND APOPTOSIS BY EXTRACT OF BASELLA ALBA PLANT ON U937 CELLS”

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ABSTRACT

Leukaemia is one of the leading causes of death in the world particularly developing countries & it is complex group of disease with many possible cause. The synthetic drug and chemotherapies have huge side effects and these are so costly treatment for middle and lower class families. It would be extremely beneficial if an anticancer drug can be discovered from natural edible sources. Therefore our plant of interest an edible plant Basella alba reported as a natural medicinal source on the basis of its potent antibacterial and antioxidant, antiulcer, antidiabetic, hepatoprotective activity. Present study is the evaluation of anti-proliferative effect of the extract & active fraction of Basella alba plants in U937 cell line. Cell viability was studied by Trypan blue exclusion and cell Cytotoxicity study by MTT assay, Morphological study by phase contrast microscopy. DNA laddering assay by gel electrophoresis shows its apoptotic activity. MEBA were found to be responsible for antiproliferative, cytotoxic activity and the morphological images, DNA laddering assay were found to be responsible for the apoptosis of cells. The extract of Basella alba plant was found to be having potent anti-leukemic activity on U937 cells. It can be expected in future to develop an anti-cancer compound for the treatment of human from natural source with less systemic toxicity. Thus, further more investigation is needed for mechanism and to identify the active compound responsible for this activity.

KEYWORDS: Basella alba, Leukaemia, Myeloid, apoptosis

1. INTRODUCTION

Leukaemia refers to cancers that begin in the blood-forming cells of the body. These abnormal cells grow and multiply in an uncontrolled way. As the disease progresses,
leukemic cells move through the bloodstream and the organ such as the spleen, lymph nodes, liver, and central nervous system which are affected by it, this is known as haematological neoplasm. In the hematopoietic malignancies, chemotherapeutic approaches are widely applied in practice. Nowadays, several therapeutic approaches have been taken to overcome the complexities of different cancers. Drug discovery against cancer is ventured throughout the world especially from the natural products.\textsuperscript{[1]} The design of novel drugs from traditional medicine offers new prospects in modern healthcare.\textsuperscript{[2]} The greatest disadvantage in the presently available potent synthetic anti-cancer drugs lies in their toxicity, many side effects and reappearance of symptoms after discontinuation. Treatment for cancer involves surgery, radiotherapy and chemotherapy and often a combination of two or all three is employed.\textsuperscript{[3]} To avoid the disease, it's essential to take advantage of this natural protection. Plants have been playing significant roles in the healing processes, magic rituals, and religious practices of peoples from the five continents.\textsuperscript{[4,5]} It would be extremely beneficial in the field of cancer chemotherapeutics, if a natural anti-cancer agent can be discovered. The edible plant Basella alba (Lam.) of the family Basellaceae was selected for our research project on the basis of ethno-botanical information, which reveals its use in tribal areas. Basella alba possesses various pharmacological activities like antifungal, antioxidant, anticonvulsant, analgesic and anti-inflammatory activities.\textsuperscript{[6,7]} The present planned to study the anti-leukemic property of methanolic extract of Basella alba against human leukemic monocytes lymphoma cell line U937.

2. MATERIALS AND METHODS

2.1. Chemicals

 invitro evaluation

This work was performed at CSIR-IICB, Kolkata & CP University campus. The design of this work mainly covers in-vitro study.

2.2. Cell Culture

The human leukemic cell lines U937 and murine macrophage cell line RAW 264.7 cells were purchased from the National Facility for Animal Tissue and Cell Culture, Pune, India. The cells were maintained in RPMI 1640 medium (Gibco, USA), supplemented with 10% heat inactivated FCS, 100U/ml Penicillin (Biowest, Germany), and 100mg/ml Streptomycin (Biowest, Germany) maintained at 37°C in a humidified atmosphere containing 5% CO2 in air. In all the experiments untreated leukemic cells and RAW 264.7 cells were termed as control group.
2.3. Plant Material
The Basella alba whole plant was collected freshly from South 24 Pargana district, West Bengal in the month of June, 2013 depending upon its easy availability. It was authenticated from Botanical Survey of India by their Taxonomist Mr. V. Prasad against a voucher specimen.

2.4 Extraction, Fractionation & Preparation of Test sample
The Basella alba whole plant powder (1 kg) was defatted by using pet ether for 3 days and then soaked in about 5000 ml methanol for 7 days at room temperature with occasional shaking. The mixture was then filtered by filter paper and the solvent was evaporated by rotary vacuum evaporator & then lyophilized for 4 hrs to produce methanol free extract. The sticky methanolic extract was obtained finally. It was kept in a container, sealed with parafilm & stored at 4° C in airtight container and was designated as methanolic (MEBA) extract of Basella alba for the experiments. 10gms of the Basella alba plant extract extracted with methanol termed as (MEBA) was suspended in 200 ml water & equivalent amount of Pet ether, and then partitioned into water & ethyl acetate to get water fraction & EtAc fraction on the basis of polarity. Stock solution was prepared as 1mg/ml in PBS, from here the desired amount was taken as required for different experimentation. The fractions are termed as Ethyl acetate fraction of Basella alba (EBA) & water fraction of Basella alba (WBA).

2.5 Anti-Carcinoma Study
2.5.1 Cell Cytotoxicity Study
Log phase cells (U937) at a concentration of 1x10^5 cells (100μl cell suspension) were seeded in 96 well tissue culture plates. They were treated with freshly prepared 1mg/ml stock solution of MEBA in various concentrations between 10μg - 200μg for 24, 48, 72 hrs at 37°C in a humidified atmosphere containing 5% CO2 in air. Untreated cells served as control. The Cytotoxicity studies were performed by MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-2,5-dipheniltetrazoliumbromide] assay & the absorbance of the colour solution was measured at a wavelength of 492 nm by microplate manager (Reader type: Model 680 XR Bio-Rad laboratories Inc) [8] IC50 values were obtained for 24 hrs.

2.5.2 Toxicity study on RAW 264.7 cells by MTT assay
Toxicity studies of MEBA were studied on normal RAW 264.7 by MTT assay. 100μl RAW 264.7 cells (1x10^5) suspension per well in a log phase were seeded in 96 well tissue culture
plates. Treatment regime of 10μg, 50μg, 100μg, 150 μg, 200μg doses of MEBA were used for a period of 24 hrs & incubated at 37°C in a humidified atmosphere containing 5% CO2 in air, untreated cells served as control. The absorbance was measured at 492nm by micro-plate manager (Reader type: Model 680 XR Bio-Rad laboratories Inc).

2.6 Cell viability study by Trypan blue exclusion method
For cell viability studies U937 cells 1x10^5, 100μl cell suspension per well in a log phase were seeded in 96 well tissue culture plates. They were treated with MEBA freshly prepared 1mg/ml stock solution extract in a concentration of 10μg -200μg for 24, 48, 72 hrs at 37°C in a humidified atmosphere containing 5% CO2 in air. Untreated cells served as control. After treatment 1mg/ml trypan blue dye was prepared and 10μl of dye was mixed with all the wells containing cells accordingly. The dye exclusion test is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as trypan blue, whereas dead cells do not. In the protocol presented here, a viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm. The unstained (viable) and stained (nonviable) cells, counted separately in the haemocytometer.

2.7 DNA fragmentation by Agarose Gel Electrophoresis Study
U937 cells (1x 10^6) were treated with IC50 dose of MEBA & Ara-C as standard for U937 were taken. The cells were resuspended in 500μl of lyses buffer (50Mm Tris-HCl, Ph- 8.0, 10Mm EDTA, 0.5%S DS) 10μg/ml of Proteinase - K was added and kept for incubation at 50°C for 1h and then 37°C for overnight. After incubation was done by following the general phenol-chloroform extraction procedure\[^9\] and kept at -20°C overnight. After centrifugation, DNA precipitates were washed with 70% ethanol, dried and evaporated at room temperature and dissolved in TE buffer at 4°C overnight. To detect the DNA fragments the isolated DNA samples were electrophoreses overnight at 20V in 1% agarose gel and stained with ethidium bromide. DNA fragmentation was observed in UV transilluminator.\[^10\]

2.8 Morphological study by light microscopy
U937 cells (1x10^6) were treated with IC 50 dose of MEBA and kept for 18 hrs of incubation another set remain as untreated. After 18hrs of incubation the treated and controls cells observed under light microscope.
2.9 Statistical Analysis
Statistical analysis was done by Student’s t-test. P < 0.05 was considered as significant.
The percentage cell inhibition was calculated by the following formula:

\[%\text{Cell inhibition} = 100 \times \frac{(\text{O.D of control} - \text{O.D of treated})}{\text{O.D of control}}\]

O.D= Optical Density.
The percentage cell viability was calculated by the formula: -

\[\text{Viable cells (\%)} = \left(\frac{\text{Total number of viable cells per ml}}{\text{Total number of cells per ml}}\right) \times 100.\]

3. RESULTS
3.1 Cytotoxicity study by MTT assay
In the MTT assay MEBA at concentrations of 10μg to 200μg significantly inhibited the growth of U937 cells compared with that of the control cells after 24, 48 & 72 hrs of treatment in a concentration-dependent manner (Fig.1). These observations show the cytotoxic nature of MEBA. The IC50 calculated after MTT assay and MEBA in U937 is found to be 24.54 μg/ ml.

Fig 1; The histogram shows the effect of MEBA on U937 cell line. The cell growth is compared to the untreated control cells and MEBA treated U937 cells respectively. Reduction in the number of cells is observed in a time and concentration dependent manner. Data are mean ± S.E.M. denotes significant decrease in cell count from control values p<0.05.
3.1.1 Toxicity study on RAW264.7 cells by MTT assay

In the Cytotoxicity study or MTT assay on RAW 264.7 cells, there was no significant reduction in the O.D. values compared to that of the untreated control cells after treating the cells with MEBA at the doses of 10 μg, 50 μg, 100 μg, 200 μg for 24 hrs. The O.D of the MEBA is almost similar to that of the control cells, thereby it indicates that MEBA has selectivity for leukemic cells and exerts no effect on the RAW264.7 cells. (Fig.2)

![24hrs cytotoxicity study on RAW 264.7 CELLS](image)

Fig-2 Histogram shows the insignificant effect of MEBA on RAW 264.7 cells

3.2 Cell viability study by Trypan blue exclusion method

Cell growth inhibition study performed with the freshly prepared MEBA (1mg/ml stock), significantly inhibited the cell growth in a concentration and time dependent manner at the concentration of 10 μg, 25 μg, 50 μg, 100 μg, 150 μg for 24, 48, 72 hrs on U937 human leukemic cell line. The increase in the percentage inhibition of cell growth according to concentration and time after 24, 48, 72 hrs of treatment are plotted in the graphs for U937 cell line.
Fig: 3 Histogram shows effect of MEBA on cell growth inhibition, observed on U937 human leukemic cell line after 24, 48 and 72hrs of treatment. The cell count is compared to the untreated control cells and treated with MEBA cells. Reduction in no. of cells is observed in a time and concentration dependant manner. Data are mean ± S.E.M. denotes significant decrease in cell count from control values p<0.05.

3.3 Agarose gel electrophoresis study
After Agarose gel electrophoresis, the DNA samples isolated from the untreated control U937 shows intact DNA bands whereas the DNA samples from U937 cells treated with MEBA and standard showed fragmented DNA bands in the form of ladders. (Fig.6) So, the observations confirmed that the treatment with MEBA, caused apoptosis in U937 human leukemic cell lines.
Fig 4: Lane 1 represents U937 control cell lane 2 Ara- C (100μg), lane 3 represents MEBA IC 50 dose for U937 cell.

3.4 Morphological Studies for Detection of Apoptosis

The effect of MEBA to produce cell death in the U937 cell lines is observed by inducing apoptosis. After the Phase contrast microscopic study, the image showed that untreated control cells, having intact, no such changes take place whereas MEBA treated cells with condensed chromatin, fragmentation of cell were obtained. Morphologically this indicates that the treatment with MEBA causes apoptotic change in the LEUKEMIC cells compared to the untreated control.
4. DISCUSSION

Chemoprevention is a novel approach emphasizing on the prevention or delay of carcinogenesis by means of pharmacological, biological & nutritional intervention and recently, cancer chemoprevention with strategies using foods and medicinal herbs has been regarded as one of the most visible fields for cancer control.\[11\] A significant part of drug discovery in the last few years has been focused on agents to prevent or treat cancer (leukemia). Treatment for leukemia involves surgery, radiotherapy & chemotherapy. Natural product derived drugs are taking a great role in preventing the leukemia.\[12\]

The present study reveals that the MEBA (Methanolic extract of Basella alba) has a potent anti-leukemic activity, Cytotoxicity. The MEBA significantly inhibits the cell proliferation in human leukemic cell line U937. Then from the cell viability study TRYPAN BLUE exclusion method MEBA showed a significant reduction in cell count U937 cell line.[fig1&3]The Cell Cytotoxicity study done by the MTT assay in case of RAW 264.7 and leukemic cells showed that MEBA selectively kills the leukemic cells and MEBA has no damaging effect on normal RAW cells. [Fig1, 2, 3]

Further evidence the MEBA showed an apoptogenic activity from gel patterns of Agarose gel electrophoresis. MEBA treated cells showed a degraded DNA bands in the form of ladder,
atypical indication of apoptosis, whereas the untreated control cells showed intact DNA bands when observed in UV transilluminator. [Fig 4] The light microscopy study has also showed that control cells were with intact nuclei whereas MEBA treated cells indicated apoptotic changes like nuclear disintegration and formation of apoptotic bodies, which clearly indicates the apoptotic nature of MABA towards leukemic cell. [Fig 5]

From the above performed experiments it can be confirmed that MEBA Methanolic fraction has significant Antileukemic activity on human leukemic cell line U937.

5. CONCLUSION
So from the above total experiments confirmed that the methanolic extract of plant Basella alba has a apoptogenic activity against human leukemic cell line. Some synthetic drug discovered but they are very costly not available to normal people. So here a natural edible compound Methanolic extract of Basella alba can prevent the dramatic disease leukemia then that may be as an Antileukemic drug & available to normal people also.

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7. REFERENCES
2. Mule SN patil SB, Naikwade NS, Magdum CS. Antinociceptive and anti-inflammatory activity of stems of Gynandropsis pentphylls Linn. Int J Green Pharm, 2008; 2; 87-90


