World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 3.347

EVALUATION OF *IN VITRO* CYTOTOXIC ACTIVITY OF ETHANOLIC EXTRACT OF ROOT OF *IPOMOEA BATATAS* (L) (EERIB) AGAINST THE HUMAN COLORECTAL ADENOCARCINOMA HT-29 CELL LINE

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Article Received on 21/10/2016 Article Revised on 10/11/2016 Article Accepted on 30/11/2016

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ABSTRACT

HT-29 is a human colorectal adenocarcinoma cell line with epithelial morphology. These cells are sensitive to the chemotherapeutic drugs 5-fluorouracil and oxaliplatin, which are standard treatment options for colorectal cancer. In addition to being a xenograft tumor model for colorectal cancer, the HT-29 cell line is also used as an in-vitro model to study absorption, transport, and secretion by intestinal cells. Under standard culture conditions, these cells grow as a nonpolarized, undifferentiated multilayer. Altering culture conditions or treating the cells with various inducers, however, results in a differentiated and polarized morphology, characterized by the redistribution of membrane antigens and development of an apical brush-border membrane. The main aim and objective of the present research work was the evaluation of in vitro Cytotoxic activity of ethanolic extract of root of Ipomoea batatas (L) (EERIB) against the human colorectal adenocarcinoma HT-29 cell line**.** The *in vitro* cytotoxic activity was carried out by SRB assay. The results obtained from the *in-vitro* studies performed by SRB assay by using human colorectal adenocarcinoma HT-29 cell line displayed that the EERIB possessed

a very good cytotoxic activity. From the present studied it had been concluded that EERIB was exhibiting the potential cytotoxic action on HT-29 cell line which was proved by using standard drug 5-FU and it was found that **EERIB** showed the highest **90.95%** growth inhibition at 10 µg (**IC_{50} = 2.7 µg/ml**. The IC_{50} value of standard drug 5-FU was found to be **1.3 µg/ml** with **95.97%** growth inhibition at concentration 75 µg/ml.

KEYWORDS: Epithelial morphology, chemotherapeutic drugs, nonpolarized, adenocarcinoma, cytotoxic activity, IC_{50} etc.

INTRODUCTION

The sweet potato (Ipomoea batatas) is a dicotyledonous plant that belongs to the family convolvulaceae. Its large, starchy, sweet-tasting, tuberous roots are a root vegetable. Literature survey on the plant showed that leaves have a high content of polyphenolics - anthocyanins and phenolic acids, with at least 15 biologically active anthocyanins with medicinal value. The aim of the present study is to prepare the aqueous extract of Ipomoea batatas and to evaluate invitro anti-inflammatory activity by membrane stabilizing method. Phytochemical analyses of IBAE showed the presence of phenols, flavonoids, tannins, anthraquinones, and reducing sugars. It has been shown that the anti-inflammatory activity is may be because of phenols and flavonoids.[1]

Ipomoea batatas (L.) Lam. from the family Convolvulaceae is the world's sixth largest food crop. The tubers of Ipomoea batatas commonly known as sweet potato are consumed as a vegetable globally. The tubers contain high levels of polyphenols such as anthocyanins and phenolic acids and vitamins A, B and C, which impart a potent antioxidant activity that can translate well to show wound healing effects. To check their effects on wound healing, the peels and peel bandage were tested on various injury models in rats in the present study. The methanolic extracts of the peels and peel bandage of Ipomoea batatas tubers (sweet potato) were screened for wound healing by excision and incision wound models on Wistar rats. Three types of gel formulations were prepared, viz., gel containing 3.0% (w/w) peel extract, gel containing 6.0% (w/w) peel extract and gel containing 10% (w/w) peel extract. Betadine (5% w/w povidone iodine cream) was used as a reference standard. In the incision wound model, Tensile strength of the skin was measured. Epithelization time, wound contraction, hydroxyproline content of the scab, and ascorbic acid and malondialdehyde content of the plasma were determined in the excision wound model.[2]

MATERIALS AND METHODS

Plants material collection and authentification: Fresh tubers of sweet potato were collected from kodad market. The plant roots of Ipomoea batatas were collected in was collected from the areas around Nalogonda, India in the month of Jan-2016. The plant were identified and authenticated by Dr. S. Baburaj, Botanist, Department of Botany, Thyagarajar College of arts and science, Madurai. The root was washed with water to remove soil and other extraneous matter. This was then dried under shade for few days. Then the shade dried root was homogenized to get coarse powder and was stored in air tight containers.

Drugs and chemicals: The standard drug 5-FU purchased from Local Retail Pharmacy Shop and solvents and other chemicals used for the extraction and phytochemical screening were provided by Institutional Store and were of LR and AR grade.

Cell culture: The human colorectal adenocarcinoma HT-29 cell line was provided by Centre for Cell and Molecular Biology (CCMB), Hyderabad and was grown in Eagles Minimum Essential Medium (EMEM) which contained 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 100% relative humidity, 5% CO2, 95% air and the culture medium was changed twice a week.

Methodology: First the dried powder of root of ***Ipomoea batatas*** is placed into the thimble made of stout filter paper and the apparatus is fitted up. The flask containing suitable solvent like ethanol is heated on water bath or on a heating mental Soxlet apparatus is set up**.** As the solvent boil, its vapors rise through the side tube up into the water condenser. The condensed liquid drops on the solid in the thimble, dissolves the organic substances present in the powdered material and filters out into the space between the thimble and the glass cylinder. As the level of liquid here rises, the solution flows through the siphon back into the boiling flask. The solvent is once again vaporized, leaving behind the extracted substance in the flask. In this way a continuous stream of pure solvent drops on the solid material, extract the soluble substance and returns to the flask. At the end of the operation the solvent in the boiling flask is distilled off, leaving the organic substance behind.[3] Afterwards the ethanolic extracts are transferred in a clean and dried beaker separately and are concentrated by placing on a water bath and cool and then **ethanolic extract of root of *Ipomoea batatas* (EERIB)** is obtained and keep all these extracts in a freeze. From this concentrated extract the preliminary phytochemical screening has to be carried out.

Phytochemical screeninig[4, 5, 6, and 7]: The preliminary phytochemical analysis of EERIB showed the presence of various phytochemical constituents like Carbohydrates, Phenol, flavonoids, Tannins etc.

Screening of *in vitro* cytotoxic activity by SRB assay[8, 9]

Principle: Sulforhodamine B (SRB) is a bright pink amino xanthine dye with two sulfonic acid group. Under mild acidic conditions, SRB dye binds to basic amino acid residues in trichloroacetic acid (TCA) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of visible at least two order of magnitude**.**

Procedure: The monolayer cell culture was trypsinized and the cell count was adjusted to 0.5-1.0x10^5 cells/ml using a medium containing 10% new born sheep serum. To each well of the 96 well micro titre plates, 0.1 ml of the diluted cell suspension (approximately) 10,000 cells was added. After 24 hrs, when a partial monolayer was formed, the supernatant was flicked off, washed once and **10 µg/ml, 20µg/ml, 30µg/ml, 40 µg/ml** of different concentration of EERIB was added to the cell in a microtitre plate. The plates were incubated at 37^0c for 72 hrs in 5% CO_2 incubator, microscopic examination was carried out and observations were recorded every 24 hrs. After 72 hrs, 25µl of 50% TCA was added to wells gently such that it forms a thin layer over the test extracts to form overall concentrations 10%. The plates were incubated at 4^0c for 1 hr. The plates were flicked and washed five times with tap water to remove traces of medium sample and serum and were then air dried. The air dried plates were stained with 100µl SRB and kept for 30 mints at room temperature. The unbound dye was removed by rapidly washing four times with 1% acetic acid. The plates were then air dried. 100 µl of 10 Mm Tris base was then added to the wells to solubilize the dye.[10] The plates were shaken vigorously for 5 mints. The absorbance was measured using microplate reader at a 540 nm. The % growth inhibition was calculated by the following formula: **% cell growth inhibition = 100-{(At-Ab/Ac-Ab)}x 100**

At = Absorbance value of test compound

Ab = Absorbance value of blank

Ac = Absorbance value of control

RESULT AND DISCUSSION

Table 1: For percentage (%) of cell growth inhibition of EERIB on HT29 Cell lines by SRB Assay.

Serial no.	Concentration of the Extracts	Absorbance of extracts	Inhibition of cell growth (%)
1	10 µg/ml	0.036	90.95
2	20 µg/ml	0.055	85.58
3	30 µg/ml	0.069	80.88
4	40 µg/ml	0.091	77.17
5	75 µg/ml (5-FU)	0.012	95.97
6	Control	0.298	0

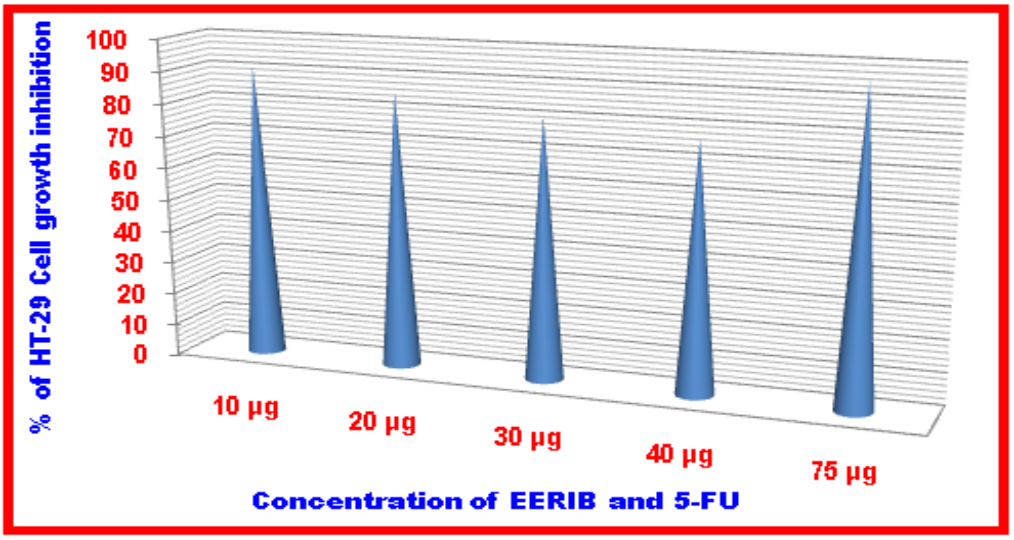

Fig-1: Percentage of Cell growth inhibition

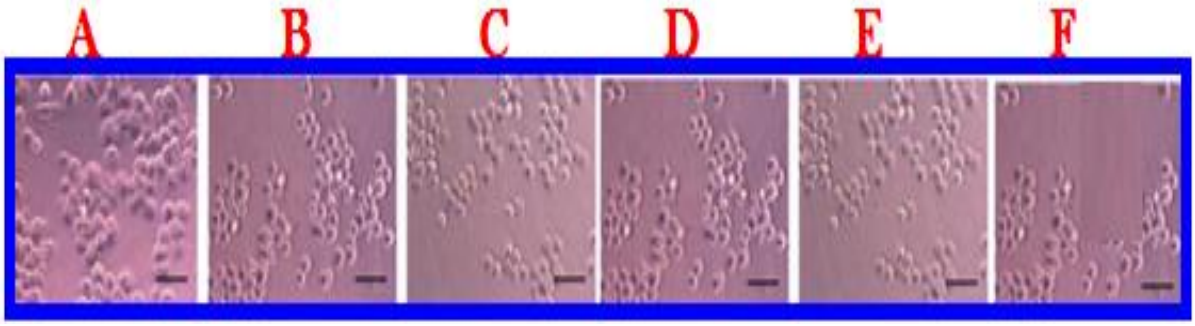

Fig-2: Inhibition of growth in human colon cancer cell line HT-29 by EERIB in various concentrations and by std, drug 5-FU (A= CONTROL, B= 10 µg, C= 20 µg, D= 30 µg, E= 40 µg of EERIB, and FU= 75 µg).

The cell growth inhibition by the extracts such as **EERIB** against HT 29 cell lines for various concentrations is shown in table 1. As the concentration increase, there is an increase in the cell growth inhibition and it was found that **EERIB** with the highest **90.95%** growth

inhibition at 10 µg (**IC_{50} = 2.7 µg/ml**. The IC_{50} value of standard drug 5-FU was found to be **1.3 µg/ml** with **95.97%** growth inhibition at concentration 75 µg/ml.

CONCLUSION

The results obtained from the *in-vitro* studies displayed that the **EERIB** possessed a very good cytotoxic activity against human colorectal adenocarcinoma HT-29 cell line. From the present experimental data it had been concluded that EERIB was exhibiting the potential capability to inhibit the growth of cancer cell when compared with standard drug 5-FU and the cell growth inhibition of EERIB was found to be the highest 90.95% at 10 µg (IC_{50} = 2.7 µg/ml).

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