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RESEARCH ARTICLE

IN VITRO CYTOTOXITY EFFECT OF METHANOL EXTRACT OF *WATTAKAKA VOLUBILIS* (leaf) AGAINST BREAST CANCER CELL LINE.

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Abstract

Cancer is the second leading cause of death worldwide. World Health Organization estimates that 80% of the world';s population still rely mainly on traditional medicines for their basic health care. During the last decades of the 20th century, medical researchers have developed new methods for cancer treatment by combining surgery with chemotherapy, radiations and various phytochemicals obtained from different plant species. It is important to note that chemotherapy not only kills the cancer cells but it also has some side effects on normal cells too. Medicines obtained from plants have less or no side-effects. Present investigation is mainly concerned with the documentation of anti-cancer plant species around the globe. The present study has been formulated to understand the *in vitro* anticancer property elicited by Wattakaka volubilis Wight. The cytotoxic activity of extract of *Wattakaka volubilis* leaves was determined by MTT assay against cell lines of breast cancer (MCF-7) and HeLa . Cell proliferation was measured based on the ability of metabolic active cells to cleave the yellow tetrazolium salt MTT [3- (4, 5- dimethyl–thiazole- 2- yl) - 2, 5- diphenyl tetrazolium bromide] to form insoluble purple formazan crystals. The findings of the present study suggest that the methanolic extract of leaves of *Wattakaka volubilis* possess excellent anticancer potential that might be used for therapeutic purposes for cancer treatment with proper evaluation procedures.

Introduction :-

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries (Jemal et al., 2011, WHO 2006) estimates that 84 million people will die of cancer between 2005 and 2015. Chemotherapy is one of the most frequently used therapeutic modalities for the treatment of cancer, but it does not achieve a satisfactory therapeutic result if it is used alone. Breast cancer is a major cause of death in the female population. According to worldwide data in 2008 of American Cancer Society, there are 1,383,500 new cancer cases (23 %) and 458,400 cancer deaths (14 %) (Ferlay et al., 2010). The most common treatment for breast cancer is radiotherapy, hormonal therapy and chemotherapy. The patients often experience many extreme side effects from anti-cancer drugs. Intensive treatment with radiotherapy or chemotherapy is usually associated with adverse side effects ranging from nausea to bone marrow failure. Moreover, tumor cells are usually resistant to chemotherapy (Raguz and Yague 2008). Medicinal plants have been used as remedies for human diseases for centuries. The reason for using them as medicine lies in the fact that they contain chemical components of therapeutic value. Plant materials have served as medicines across cultures and throughout time. Knowledge about plants that were found to be most effective against particular ailments was passed down to the succeeding generations. Therefore, phytochemicals from medicinal plants may be an option for treatment.

Wattakaka volubilis (Linn. f.) Benth ex. Hook f. Syn: Dregea volubilis (Linn. f.) Stapf; Marsedenia volubilis (Cooke) belongs to the family Asclepiadaceae and is commonly known as "Jukti" in Bengal. It is a tall woody

climber with height of 11 m and a girth of 95 cm with densely lenticulate branches. It occurs throughout the hotter parts of India and Car Nicober Islands ascending to an altitude of 1500m1. The parts of the plant are used traditionally as medicines. The juice of the plant is used as a sternutatory and the leaves are employed in the treatment of boils and abscesses. The roots and tender stalks are used as emetic and expectorant. It has been reported that the ethyl alcohol (50%) extract of the plant has activity on the central nervous system as well as anti-cancer activity against Sarcoma 180 in mice (panda et al., 2006), the reported maximum tolerated dose being 500 mg/kg body weight of albino mice. Two pregnane glycosides, dregeosides Ap1 and A01, isolated from this plant, collected from Thailand, showed antitumor activities against melanoma B-16 in mice1. Reichstein and co- workers2 studied the components of the seeds of the plant and the structure of drevogenins A, B, D and P. Subsequently, the isolation and characterization of twelve polyhydroxy C/D cis-pregnane glycosides from the same plant collected from Thailand was reported (Sahu et al., 2002). Isolation of - sitosterol, kaempherol-3- galactoside, a 2-deoxy sugar, drevogenin A, drevogenin P, D–cymarose and L-olendrose from the plant has also been reported (Panda et al., 2003; Mahato et al., 1992). It has not been reported in breast cancer cell lines. Present investigation was aimed to assess the cytotoxic potential of methanol extract of *Wattakaka volubilis* leaf against MCF-7 and HeLa human breast cancer cell line.

Materials and methods:-

Plant material

The leaves of *Wattakaka volubilis* were collected from Trichirappalli. The plant material was taxonomically identified by Dr. John Britto Rabinet Herbanium. St. Joseph's College, Trichy.

Preparation of extract

The leaves of *Wattakaka volubilis* were dried in shady condition and powdered. The 200 g of powdered material was dissolved with 250 ml of 95% methanol and extract was prepared using soxhlet apparatus for 48hr. The extract was filtered and concentrated in rotatory evaporator at 35-40ºC under reduced pressure (yield: 28.5% w/w) and was stored in refrigerated condition for further use.

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

MCF-7 and HeLa cell lines were 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 (100Mg/ml) an D amphotericin B(5µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent . The cells were dissociated with TPVG solution 90.2% 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:

Principle:

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium . The assay depends both on the number of cells present and on the mitochondrial activity per cell. 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 colored 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)

Procedure:
The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 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 performed , the supernatant was flicked off , washed the monolayer once with medium and 100µl of different test concentrations of test drug were added on to the partial monolayer in microtitre plates.The plates were than incubated at 37°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°C in 5% CO_2 atmosphere. The supernatant was removed and 100µl 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.

$$\% \text{ Growth inhibition} = 100 - \left\{\frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100\right.$$

Results:-

The effect of Methanolic extract of *Wattakaka volubilis (MEWV)* on MCF-7 (Breast cancer) by MTT assay method.The extracts were tested against a panel of normal and MCF-7 (breast cancer) cell lines at a range of 62.5 to 1000 µg/ml. The CTC50 values were shown for normal and cancer cell lines as in table 1 and the CTC50 for study are depicted in fig.1. The methanol extract of Wattakaka volubilis exhibited moderate cytotoxicity against MCF-7 cancer cell lines, showing higher affinity towards cytotoxicity as (CTC_{50}) was found to be below 1000 µg/ml.
To determine the cytotoxicity of methanol extract of Wattakaka volubilis to human breast adenocarcinoma HeLa cells, it was treated with increasing concentrations of plant extract and viable cells detected with MTT assay. The results depicted in table 2 summarize the cytotoxic effects of the extract on HeLa breast cancer cell lines. The methanol extract of Wattakaka volubilis showed cytotoxic activity on the breast adeno carcinoma (HeLa) cell line in a concentration-dependent manner. The extract OF Wattakaka volubilis on HeLa cell line produced a 50% of net killing (CTC 50) at a range of 62.5 to 1000 µg/ml at 210 µg/ml respectively.

Table 1: Cytotoxic activity of Wattakaka volubilis against MCF-7 cell line.

S.NO	Name of Test sample	Test Concentration(µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1.	MEWV	1000 500 250 125 62.5	50.44±0.4 21.29±0.4 17.90±0.3 14.34±0.5 12.05±0.5	1000±0.00

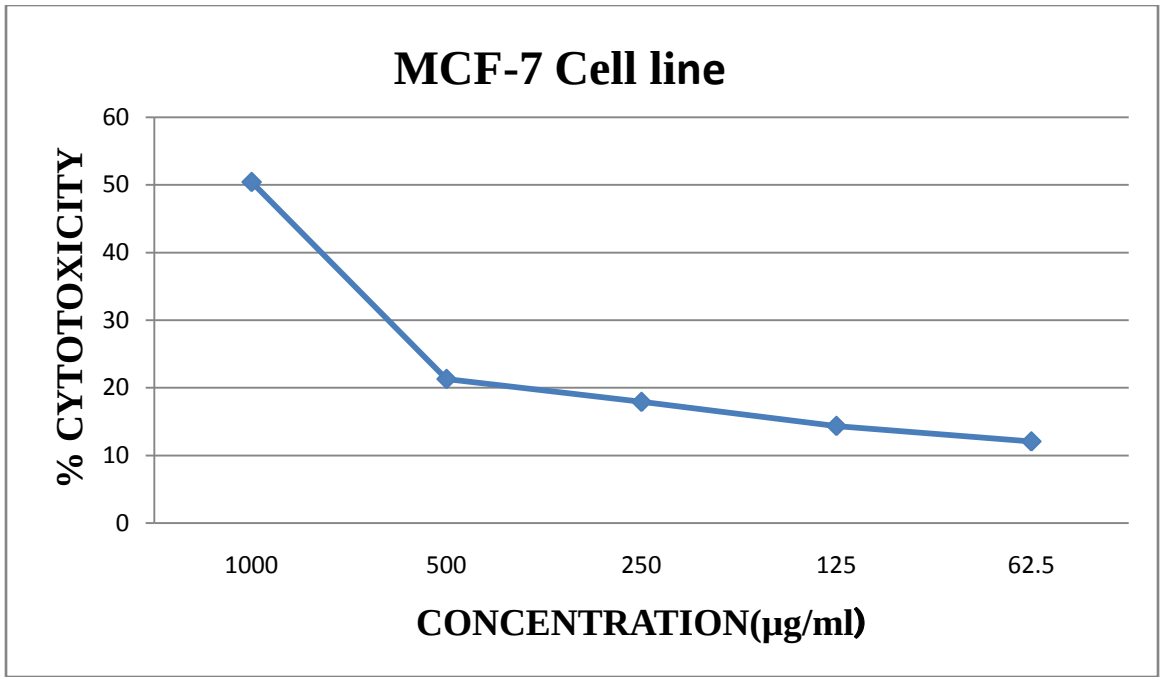

Fig: 1- Cytotoxic activity of Wattakaka volubilis against MCF-7 cell line.

Table2: Cytotoxic activity of Methanol extract Wattakaka volubilis against HeLa cell line.

S.NO	Name of test sample	Test concentration	% cytotoxicity	CTC $_{50}$ (µg/ml)
1.	MEWV	1000 500 250 125 62.5	75.27±0-5 69.51±0-2 66.54±0.4 21.36±0.2 16.09±0.4	210.00±0.00

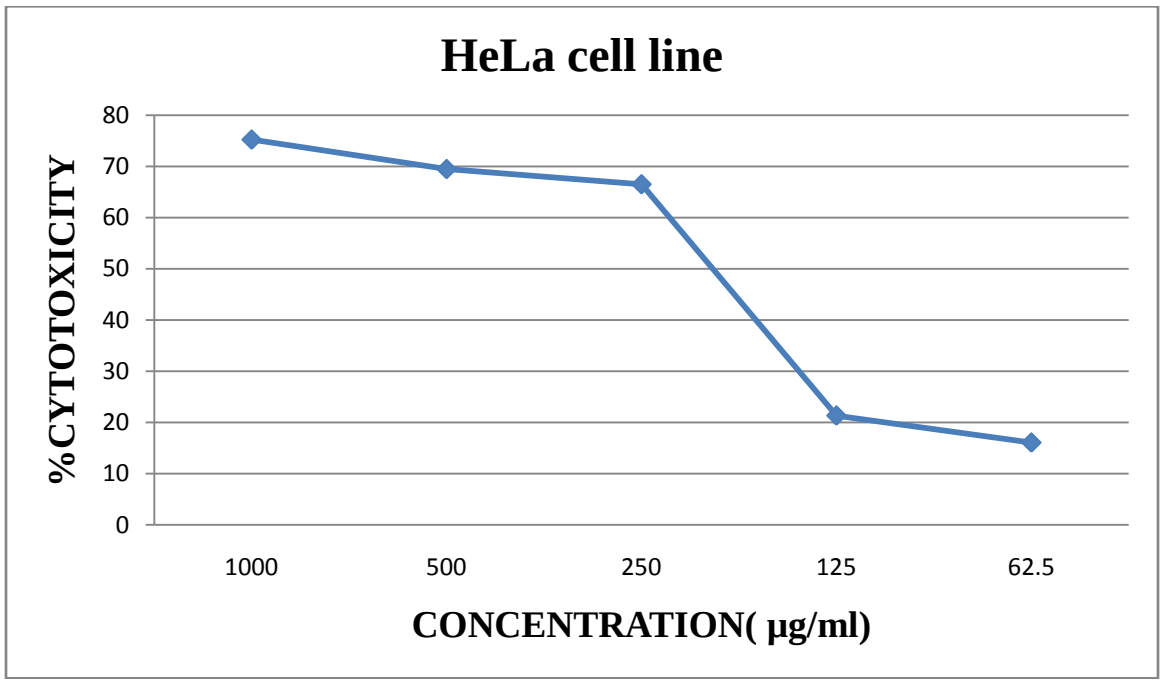

Fig: 2- Cytotoxic activity of Methanol extract Wattakaka volubilis against HeLa cell line.

Discussion:-

Medicinal plants constitute a common alternative for cancer prevention and treatment in many countries around the world (Desai et al., 2008; Guilford Pezzuto, 2000) (Soobrattee , 2006). Approximately, 60% of the anticancer drugs currently used have been isolated from natural products from the plants. More than 3000 plants worldwide have been reported to possess anticancer properties. Extracts of these medicinal plants are believed to contain a wide array of polyphenolic compounds which might possess cancer preventive and/or therapeutic properties (Dai and Mumper 2010). According to the US NCI plant screening program, a crude extract is generally considered to have *in vitro* cytotoxic activity if the IC_{50} value (concentration that causes reduction in cell viability to 50%) is less than 30µg/ml (Boik, 2001). Time-and concentration-dependent manner of the extract activities reflects the logical pharmacokinetics and pharmacodynamics on the cancer cells (Lees et al., 2004) (Hsieh and Korfmacher 2006) This is normally indicated in the cellular uptake across membrane and the metabolic disturbance within the cells (Le Coutre et al., 2004).These cellular pathways of activities are concerned with necessary signaling transduction through cytosol and nucleoplasm. The study of drug response and development of drug response model using these cell lines is the key to determine safety and hazardous levels and dosages of the extracts to which the cells are exposed (Schriner et al., 2005) .The search for anticancer agents from natural sources has been successful worldwide. Active constituents that have been isolated nowadays are used to treat human diseases. The Ethnopharmacological knowledge is helpful to lead the search for plants with potential cytotoxic activity (Marina Galvez et al., 2003) previous results examined the anti-proliferative effect of sapogenin mixture(WVSM) and the polyhydroxy pregnane glycoside (PPG) on human colon cancer HT-29 cells at various concentrations (1-50µM). WVSM and PPG decreased the proliferation of HT-29 cells by 62 and 55% respectively (Jadhav et al., 2008). Present study *Wattakaka volubilis* potent cytotoxicity against cancerous MCF-7 and HeLa cell lines with the average CTC_{50} values of 1000 µg/ml and 240 µg/ml respectively. The plant species of *wattakaka volubilis* have been reported to be utilised as remedies against skin diseases , diabetes, cough, jaundice, position bites and purifying blood . No such studies on crude leaf extract of *wattakaka volubilis* have been reported earlier so the present study explore that the *Wattakaka volubilis* leaf extract has growth inhibitory and cytotoxic effects on human breast adenocarcinoma (MCF-7) and HeLa cell line . This preliminary results could be helpful to find out the major components present in methanol extract of *Wattakaka volubilis* against the cancer activities .

Conclusion:-

Medicinal plants are an important resource to traditional society's health care systems. Most anticancer drugs have been discovered through random screening of plant materials. In today's world the percentage of people using chemicals and drugs are increasing with their side effects. "The boon given to our earth is the herbs", which needs to be utilized in sustainable manner. Many of today's drugs are derived from plant sources. The cytotoxicity assay indicated the potential of the methanol extract of leaves of *Wattakaka volubilis* could be a source of anticancer therapeutic agent against both MCF-7 and HeLa cell lines. Hence, the need to exploit the potentials of these plants especially in areas of traditional medicine and pharmaceautical industries arises.

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