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ABERRANT EXPRESSION OF WNT/BETA-CATENIN SIGNALING PATHWAY AND *IN - VITRO* CYTOTOXIC ACTIVITY OF *TRADESCANTIA SPATHACEA* MEDICINAL PLANT USED TO TREAT HUMAN BREAST ADENOCARCINOMA (MCF-7 CELL LINES)

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ABSTRACT: Cancer plays crucial role in human life and critical disease to cure. Signaling pathways involved in stem cells development process. Especially, Wnt/β-catenin signaling pathway plays important role in stem cells self-renewal, differentiation and proliferation. Despite, over expression and mutation of Wnt/β-catenin signaling pathway induced variety of cancer development. Anticancer activities of several natural and synthetic agents were used to treat many cancer types. Many synthetic agents were used to cure various cancer but they have their toxicity. So, plant derived chemotherapeutic agents are going to investigate against cancer without side effect. In this paper we evaluate anticancer properties of *Tradescantia spathacea* medicinal plant and aberrant expression of β-catenin protein in human breast adenocarcinoma cell line (MCF-7). The plant leaves were collected, shade dried and extracted with hydroalcohol using soxhlet. Cytotoxic activities were assayed with MTT colorimetric procedure against MCF-7 cell lines. From the result, plant leaves extracts showed nearly 50 % MCF-7 cell line inhibition at 229.7µg/ml tested dose.

INTRODUCTION: Natural products from plants have been valuable sources for anticancer drug discovery [1]. A screening program was initiated by Leven et al., (1979) that identified many antibacterial antifungal, antiviral, antiparasitic, and other pharmacologically active substance activities in higher plants [2]. Herbal or 'botanical', medicines, recorded in developing countries with ancient civilizations, such as Egypt and China, provide an abundant Pharmacopoeia of products that have been prescribed for many diseases over many centuries. The natural products underlying traditional medicines have received increased scientific attention recently [3, 4].

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Since there are national and indigenous rights over plant derived resources, basic scientific investigations based on medicinal plants and indigenous medical systems have increased in developing countries [3, 5]. Ancient herbal medicines may have some advantages over single purified chemicals [4]. Often the different components in an herb have synergistic activities or buffer toxic effects. Mixtures of herbs are even more complex and so might have more therapeutic or preventive activity than single products alone.

In fact, several studies have demonstrated that extracts from several herbal medicines or mixtures had an anticancer potential *in-vitro* or *in-vivo* [5-8]. Phenolic and flavonoid contents provide antioxidant activities that may underlie the anticancer potential [9]. The cytotoxic screening models are the preliminary methods for selection of active plant extracts against cancer. A huge

reservoir of bioactive compounds exists in many species of plants of Earth, only a small percentage of which have been examined and continued to be an important source of anticancer agents. Worldwide effects are ongoing to identify new anticancer compounds from plants. With the current decline in the number of new molecular entities from the pharmaceutical industry, novel anti cancer agents are being sought from the traditional medicines. Ethno medicinally important plants in cancer from Indian medicinal plants which used to treated various types of cancer. It will be helpful to explore the medicinal value of the plants and for the new drug discovery from it for researchers and scientists around the globe.

Cancer is an abnormal types of tissue growth in which the cells exhibit an uncontrolled division, relatively in an autonomous fashion, leading to a progressive increase in the number of dividing cell [10]. The limited success of clinical therapies including radiation, chemotherapy, immune-modulation and surgery in treating cancer, as evident by the high morbidity and mortality rates, indicates that there is an imperative need of new cancer management. Chemoprevention involves the use of pharmacological, dietary bio-factors, phytochemicals and even whole plant extracts to prevent, arrest, or reverse the cellular and molecular processes of carcinogenesis due to its multiple intervention strategies [11].

Tradescantia spathacea, or Moses-in-the-Cradle, is a herb native to Mexico with fleshy rhizomes. It has rosettes of waxy lance-shaped leaves. Leaves are dark to metallic green above, with glossy purple underneath. These will reach up to 1 foot (30 cm) long by 3 inches (7.5 cm) wide. They are very attractive foliage plants that will reach 1 foot (30 cm) tall. They are hardy in USDA zones 9-12. It is invasive exotic to South Florida. The current research was based on the *In-vitro* cytotoxicity activity in *Tradescantia spathacea.* This was first report for the plant and no systematic work has been undergone in this plant.

Wnt/β-catenin signaling in malignant breast tumors

β-catenin was first identified as cell adhesion molecules and further distinguished as proto-ongogene. In *Drosophila*, it was identified as homologous protein called as armadillo. β-catenin protein involved both of transcriptional activation and cell-cell adhesion. A Wnt gene (Wnt1) was first discovered in mouse mammary tumor [12, 13]. Wnt signaling, β-catenin plays crucial impact in stem cells development and carcinogenesis. Overexpression and mutation of β-catenin associated many cancer types including malignant breast cancer, prostate cancer, lung cancer, ovarian and liver cancer. Aberrant expression and mutation of β-catenin stimulated malignant breast tumors via Wnt/β-catenin signaling pathway [14].

MATERIALS AND METHODS:

Collection of Plant material

The leaves of *Tradescantia spathacea* Collected and the specimen were deposited in the Alpha Omega Hi- Tech Bio research centre. The fresh leaves Tradescantia *spathacea* were authenticated by ABS Botanical conservation, Research & Training Centre, Salem (Dt).

Extraction of the plant material

The fresh plant materials were washed with running tap water and shade dried. The leaves were crushed to coarsely powdered by grinder. These coarse powders (5g) were then subjected to successive extraction in 250ml of each solvent (hexane, ethyl acetate and methanol) by using Soxhlet apparatus. The collected extracts were stored and then taken up for further investigations. The DMSO (Dimethyl sufloxide) is act as dissolved solvents for these extracts.

Anticancer activity

MTT ASSAY

MTT-Assay-Chemicals

3-(4, 5 – dimethyl thiazol – 2 – yl) – 5 – diphenyltetrazolium 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.

Determination of Cell Viability by MTT Assays

The MTT assay, based on the conversion of the yellow tetrazolium salt-MTT, to purple-formazan crystals by metabolically active cells, provided

quantitative determination of viable cells. Cells were placed on to 96 well plates at a cell density of 2×105 mL-1 per well in 100 µL of RPMI 1640 and allowed to grown in CO2 incubator for 24 h (37 ˚C, 5 % CO2). The medium was removed and then replaced by fresh medium containing different concentrations of sample for 48 h. The cells were incubated for 24-48 h (37 ˚C, 5 % CO2). Then, 20 µL MTT ([3- (4, 5-dimethylthiazol-yl)-2, 5-diphenyltetrazolium bromide]) stock solution (5 mg/mL in PBS) was added to each well, then the β-Catenin was added and incubated for 5 h. The medium was removed and 200 µL DMSO was added to each well to dissolved the MTT metabolic product. Then the plate was shaken at 150 rpm for 5 min and the optical density was measured at 560nm. Untreated cells (basal) were used as a control of viability (100 %) and the results were expressed as % viability (log) relative to the control.

% Growth inhibition =

$$100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

RESULTS:

In - vitro Cytotoxicity activity MCF- 7 **(Table 1** and **Figure 1)** cell line for the test drug with β-Catenin **(Figure 2)** for test concentration 31.25 to 1000 µg/ml was tested and the highest % Cytotoxicity of 76.13 is seen in Test concentration of 1000µg/ml with CTC50 value of 29.7µg/ml **(Figure 3)**.

TABLE 1: *IN-VITRO* CYTOTOXICITY ACTIVITY MCF- 7 CELL LINE

Test Drug + β- Catenin	Test Conc in µg/ml	% Cytotoxicity	CTC_{50} in µg/ml
MCF-7	1000	76.13	229.7
	500	61.49	
	250	52.76	
	125	40.84	
	60.5	24.95	
	31.25	00.51	

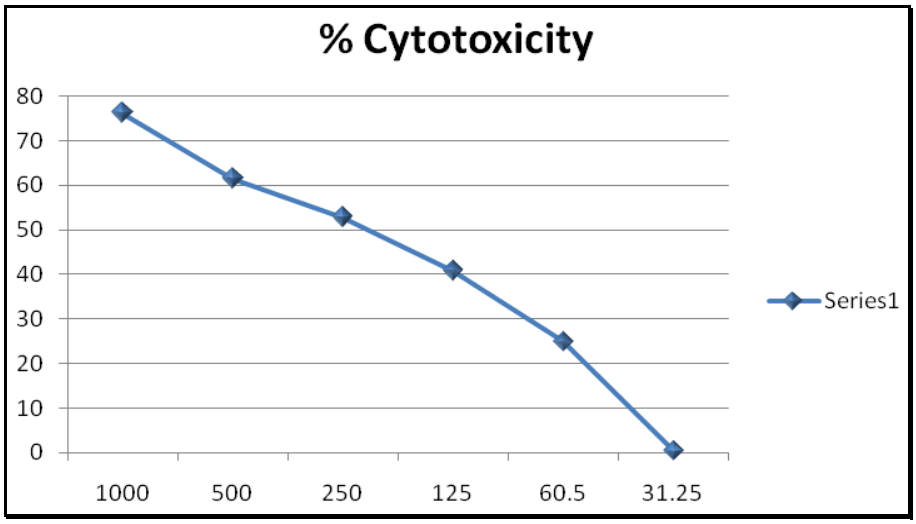

FIGURE 1: *IN-VITRO* CYTOTOXICITY ACTIVITYMCF-7 CELL LINE

DISCUSSION: Anticancer or cytotoxic properties of *Tradescantia spathacea* medicinal plant showed in human breast adenocarcinoma cell line. Anticancer activity of *Tradescantia spathacea* in MCF-7 cell line was spotted by used different concentrations. From the result, 50 % MCF-7 cell line inhibition at 229.7µg/ml or final CTC_{50} is 229.7. Further, signaling pathway also was observed in MCF-7 cell line such as Wnt/β-catenin signaling pathway. Over expression and mutation in β-catenin protein induced variety of cancers.

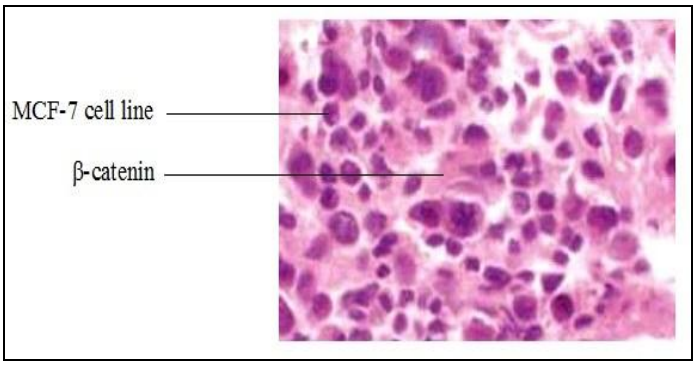

FIGURE 2: OBSERVATION OF B-CATENIN EXPRESSION AND MCF-7 CELL LINE

In breast cancer, overexpression of β-catenin was observed [14]. In each concentration, cytotoxic activity of *Tradescantia spathacea* medicinal plant was also shown β-catenin expression in human breast adenocarcinoma cell line. Deregulation of β-catenin signaling majorly regulated breast cancer in female. Medicinal plant *Tradescantia spathacea* leaves extracts were inhibited β-catenin protein expression. This plant drug was also suppressed or prevents overexpression of β-catenin that showed by each concentration. Finally, CTC_{50} is 229.7 and β-catenin expression suppressed by leaves extracts of *Tradescantia spathacea* medicinal plant [15].

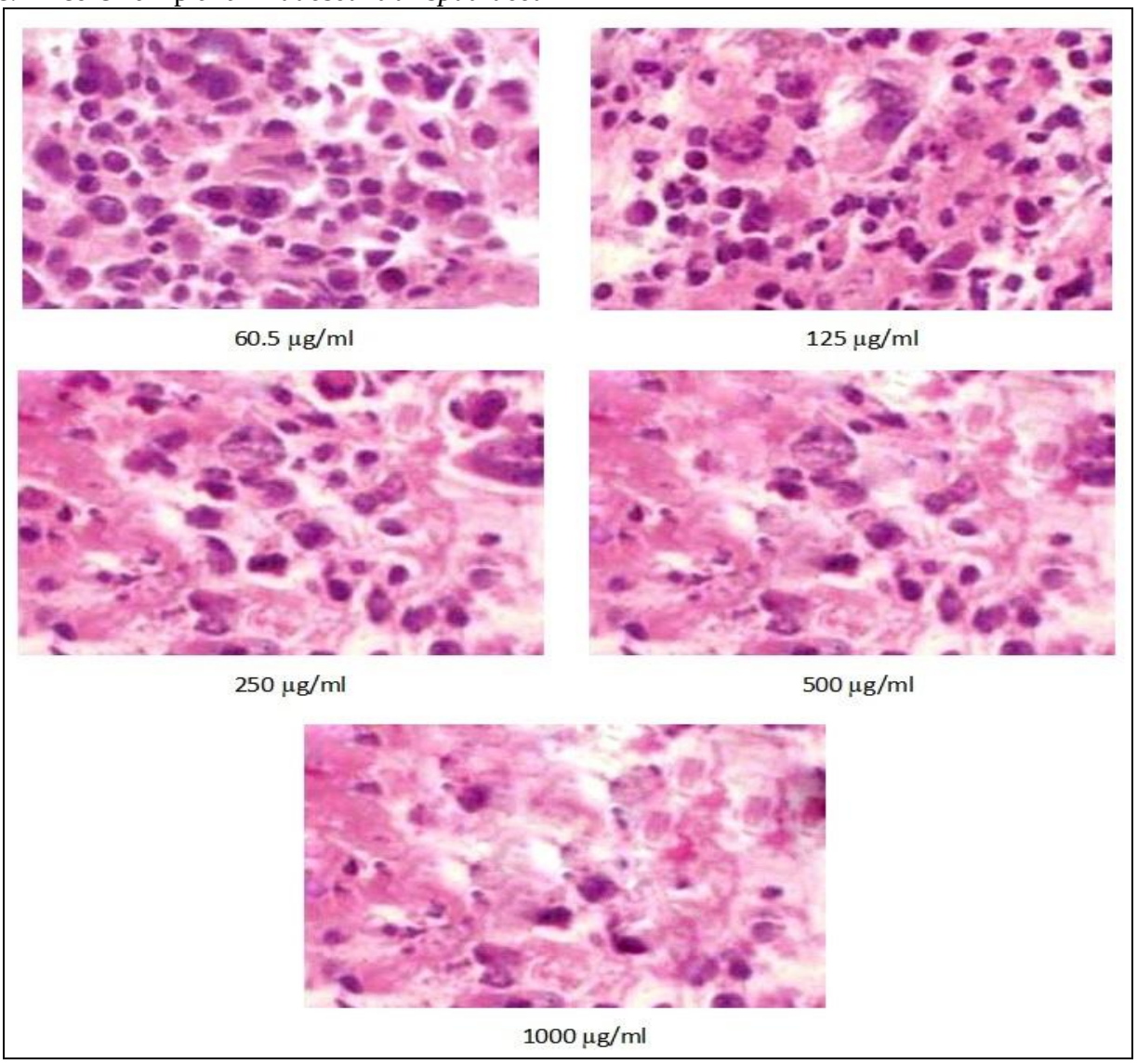

FIGURE 3: *IN -VITRO* CYTOTOXICITY ACTIVITY IN MCF-7 CELL LINES AND OVEREXPRESSION OF B-CATENIN

CONCLUSIONS: We well known about signals are way to communicate, transfer all message one cell to another cell and also within cell. Especially, Wnt/β-catenin signaling pathway was transferred message of stem cells self-renewal, differentiation and proliferation. Over expression of Wnt/β-catenin signal induced cancer progression mainly in mammary gland. Most of breast cancer occurred via mutation or overexpression of β-catenin protein in breast. Anticancer agents like nature and synthetic biological or chemical agents cure cancer but they also express their toxicity. Hence, plant derived chemotherapeutic agents were investigated against breast cancer. Here, we reported cytotoxic activities of *Tradescantia spathacea* medicinal plant, final CTC_{50} is 229.7 and inhibition of overexpression of β-catenin protein in human breast adenocarcinoma cell line (MCF-7).

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