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STUDY OF ANTICANCEROUSACTIVITY OF*HEMIDESMUS INDICUS* ON HELACERVICAL CANCER CELL LINE

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ABSTRACT
*Medicinal Plants are important source of traditional and modernsystemof medicine.Hemidesmus indicus (Syn. Indian sarsaparilla) is commonly used in Indian traditional system of medicine to cure various diseases.Traditionally it also been used to treat abdomen and Skin cancer.Ayurveda, the Indian system of medicine mainly uses plant based drugs and formulations to treat various ailments including cancer. The present study Aims to evaluate Methonolic root extract of Hemidesmus indicus for invitro anticancer activity against HeLa cancer cell line causing cervical cancer. The cytotoxicity of samples on HeLa cell lines were subjected to MTTassay in comparison with standard chemotherapeutic agent cyclophosphamide. The assay is based on the principle that the amount of formazan produced is directly proportional to the number of live cells. The result obtained by the MTT assay for HeLa cell line. 1000µg/ml concentration of Root sample extract has shown 14.2 % Cell viability. The IC50 value was found to be at the dilution of **1:4**(extract concentration 125µg/ml).The result shows that at 125/µg/ml Concentration 53% inhibition of cell viability was observed and it indicatesthat Methanolic root extract of Hemidesmus indicus has potential cytotoxicity on HeLa cervical cancer cell line*.

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INTRODUCTION

*Hemidesmus indicus*R.Br.(Tamil:Nannari, Sanskrit, “anantmula”) belongs to the family Ascalepiadaceae. It is a perennial climbing plant, used as the main ingredient in the preparation of the cool and refreshing drink Nannarisherbat. The plant is an Indian Origin and also found in south tropical Asian countries such as Pakistan and Sri

Lanka[1]. *Hemidesmusindicus* hasgrater potentiality to cure inflammatory diseases and also an active antioxidant[2].The plant used in traditional medicine in biliousness, respiratory disorders, eye diseases, epileptic fits in children, urinary disorders, loss of appetite and burning sensation. The plant root is widely used in Ayurveda, Unani, folk medicine for the treatment of inflammation, cuts, wounds, burns, snake bite, skin and blood diseases, ulcers, immunological disorders[3].

Over the past few years, cancer has reminded a major cause of death and the number of individuals living with cancer has been graduallyincreasing. Cancer causes significant morbidity and mortality and is a major health problem worldwide. Medicines derived from plants have played a pivotal role in health care of ancient and modern cultures. Almost 60% of drugs approved for cancer treatment are of natural origin. Vincristine, etoposide, taxanes and camptothecines are all examples of plant-derived anticancer compounds[4]. Herbal remedies are natural products derived from plants and plant extracts have been used traditionally to treat various diseases or to promote general health[5].The search for herbal remedies and natural substances and understanding their mechanisms of action in the body is on the rise. Due to the enormous property of plants, which synthesize a variety of structurally diverse bioactive compounds, the plant kingdom is a potential source of chemical constituents with antitumor and cytotoxic activities. Indian medicinal plants are quoted to be useful in different types of cancer diseases [6] such a plant is *Hemidesmus indicus.*[7,]. The research finding also says that *Hemidesmus indicus* is an important medicinal plan found throughout India. Though almost all of its parts are used in Ayurvedic and Unnani medicine, the extracts from its leaves and roots are most important in the field of medicine and drug[8]

Various studiesreveals anticancer activity of extract derived from mature roots of *Hemidesmus indicus.*[9]

Cancer is a disease of misguide cells that have high potential of excess proliferation without apparent relation to the physiological demand of the process. It is the second largest cause of death in the world. Of all the available anticancer drugs during 1940-2002, 40% were natural products or derived from natural product, with another 8% being natural product mimics[10]. Chemotherapy is one of the methods for the treatment of cancer.

Cancer mostly treated by chemotherapy, radiation therapy and surgery, but each treatment has its own side effects. Cytotoxicity is one of the chemotherapeutic targets foranti-carcinogenic activity. Most of the clinically used anti carcinogenic agents possess significant cytotoxic activity in cell culture system. Cyclophosphamide is a cytotoxic alkylating drug with a high therapeutic index against a variety of cancers. Its side effects include nausea, vomiting, and bone narrow suppression. Hence a major portion of the current pharmacological research is devoted to anticancer drug design customized to fit new molecular targets [11.]

Herbal remedies are natural products derived from plants and plant extracts have been used traditionally to treat various diseases to promote general health [12].The search for herbal remedies and natural substances and understanding their mechanisms of action in the body is on the rise. Due to the enormous property of plants, which synthesize a variety of structurally diverse bioactive compounds, the plant kingdom is considered as a potential source of chemical constituents with antitumor and cytotoxic activities[19].

Methanolic extract of the roots of *Hemidesmus indicus*, was investigated against human colon cancer cell line (HT29) to explore its anticancer potential[13].Anticarcinogenic and cytotoxic potential of Hemidesmus indicus methanolic root extract (HiRe) was reported against Ehrlich Ascites Tumor. The extract showed a significant in vitro cytotoxic activity against Ehrlich Ascites Tumor (EAT) cell line[14]. The study showed IC50 value for EAT cell line was 274.83µg.The anticarcinogenic activity of the extract was determined by using EAT cell line induced ascites tumor model in mice and its

comparison with standard anticancer drug cyclophosphamide. The treatment with methanolic root extract of Hemidesmus indicus (50 mg/kg and 100 mg/kg body weight) significantly increased the body weight of ascites tumor model. The life span of treated animal was increased up to 67.78%.The results were more significant in mice treated with 100 mg/kg body weight. This study revealed that Hemidesmus indicus may have a great potential to be exploited for the search of anticancer drugs[20].

With all these wide spectrum of medicinal properties, the present study aims to evaluate the anti-carcinogenic and cytotoxic activities of methanolic root extract of *Hemidesmus indicus* against HeLa Cervical Cancer cell line in comparison with standard chemotherapeutic agent cyclophosphamide.

MATERIALS AND METHODS

Collection of plant material and Extraction:

The Dried roots of Hemidesmus indicus was collected from a authenticated crude drug supplier from Kanya kumari District Tamil Nadu. The plants were identified and confirmed by a taxonomist. The roots were shade dried and powdered in the mill. The powdered shade dried plant roots were extracted with methanol using soxhlet extraction apparatus.3-4 cycles of solvent running was allowed to ensure complete extraction. After 24 hrs, extract was collected from the apparatus. The extraction was allowed to evaporate to dryness and the residue thus obtained was used for anticarcinogenic analysis.

Cell line and culture

HeLa cell lines were obtained from King Institute, Guindy, and Chennai. The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

Reagents:

MEM was purchased from Hi Media Laboratories Fetal bovine serum (FBS) was purchased from Cistron laboratories Trypsin, methylthiazolyldiphenyl- tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

Preparation of Materials:-

The MTT assay was prepared by mixing Methylthiazoly di phenyl tetrazolium bromide in PBS (5mg/ml). 0.04M HCL Prepared by mixing in absolute isopropanol. 0.1%Dimethylsulphoxide (DMSO), Phosphate Buffered saline solution were prepared separately and 24 well plates used in the method. Freshly prepared materials are stored at 4^0 Centigrade Protected from light.

In vitro assay (MTT) for Cytotoxicity activity:-

The Cytotoxicity of samples on HeLa was determined by the MTT assay[15]. This is a colorimetric assay that measures the reduction of yellow 3-(4,5 dimethyl -2-thiazolyl) 2, 5 diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to a insoluable, coloured (dark purple) formazon product. The cells are then solubilized with an organic solvent and the released, solubilized formozon reagent is measured spectrophotometric 570nm. Since reduction of MTT can only occur in metabolically active cells the level of activity is measure of the viability of the cells.

Plate Cells (1×10^5/well) were plated in 1ml of medium/well in 24-well plates. After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide cells (MTT) phosphate- buffered saline solution was added. After 4h incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance at 570 nm was measured with a UV-

Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of HeLa was expressed as the % cell viability, using the following formula:

% cell viability = O.D. of treated cells / O.D of control cells × 100%.

RESULTS AND DISCUSSION

Cervical Cancer is among the most widespread forms of cancer that affect women in common. The degree of spread of this deadly disease decides on the type of treatment. The most appropriate type of treatment mainly depends on the size of the tumour and how far the cancercell has proliferated. If the tumour has already spread to tissue in the surrounding areas, doctors usually recommend having surgery to remove the entire womb. And also often recommended for Radiotherapy and Chemotherapy. A major complication of chemotherapy is its toxicity to normal cells, which is due to the inability of drug differentiate between normal cells and malignant cells. These often impact the efficacy of the treatment and even make it impossible to cure the pations . One of the requisites of cancer chemo preventive agent is elimination of damaged or malignant cell through cell cycle inhibition or induction of apoptosis with less or no toxicity to normal cells.[16] Cancer chemoprevention, utilizing chemical compounds or natural products reverts orinhibits malignant cell transformation, and prevents invasion, The use of herbal medicine or dietary agents is being increasingly utilized as an effective way for the preventive and management of many cancer treatments.[17]The greatest recent impact of plant derived drugs is observed in the area of antitumor research, where compounds such as taxol, vinblastine, vincristine, and camptothecin have dramatically improved the effectiveness of chemotherapy against dreaded canceres.[18]Hence there is a great potential for the development of anticancer drugs essentially from the untapped reservoir of the plant Kingdom. The present study reveals that thetheMethonolicroot extract of Hemidesmus indicus has cytotoxicity activity (Graph1,) against HeLa cervical cell Lines. MTT is a yellow tetrazolium salt that is converted into a blue formazan by dehydrogenases of a live cell .The assay is based on the principle that the amount of formazan produced is directly proportional to the number of live cells. The result obtained by the MTT assay for *HeLa* cell line (Table 1.).1000μg/ml concentration of Root sample extract has shown 14.2 % Cell viability. The IC50 value was found to be at the dilution of **1:4**(extract concentration 125µg/ml). (Plate1.Fig.3). Thus, it hypothetically indicates that Hemidesmus indicus root extract has significant cytotoxic activity against Cervical Cancer cell lines.

Table:1 Effect different concentrations of Hemidesmus indicus Root Extracts on HeLa Cervical Cancer cell line.

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.07	14.2
2	500	1:1	0.12	24.4
3	250	1:2	0.21	42.8
4	125	1:4	0.26	53.0
5	62.5	1:8	0.29	59.1
6	31.2	1:16	0.34	69.3
7	15.6	1:32	0.38	77.5
8	7.8	1:64	0.42	85.1
9	Cell control	-	0.49	100

Graph .1. Effect of Methonolic root extracts of Hemidesmus indicus on HeLaCell

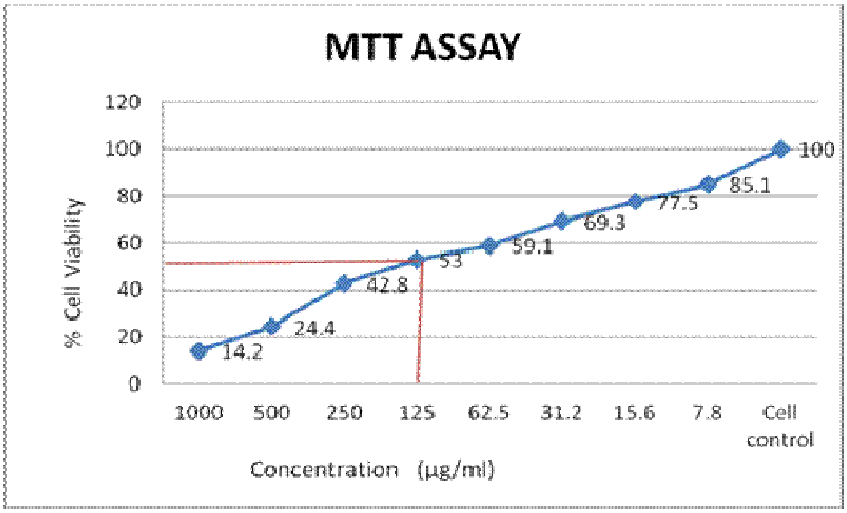

Plate 1. Effect of different concentration of Hemidesmus indicus root extracts on HeLa cervical cancer cell line

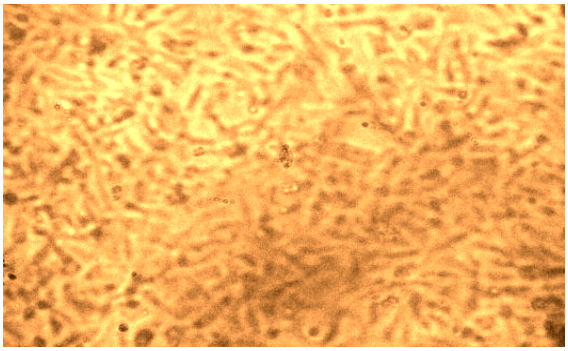

Fig.1.Normal HeLa cell line

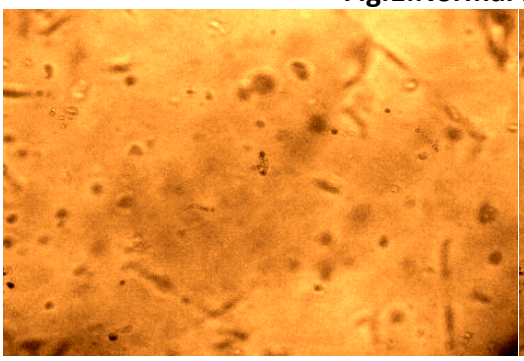

Fig.2 Toxicity- 1000µg/ml

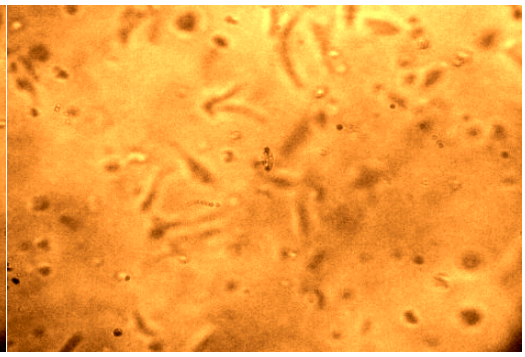

Fig.3 Toxicity- 125µg/ml

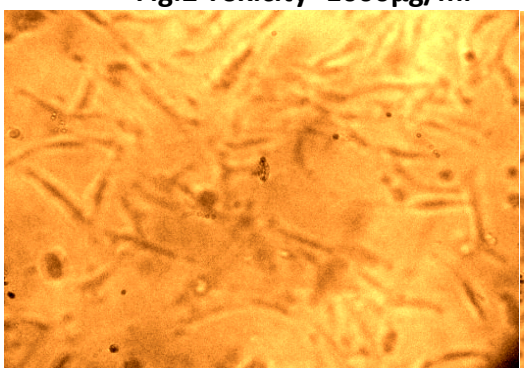

Fig.4Toxicity- 62.5µg/ml

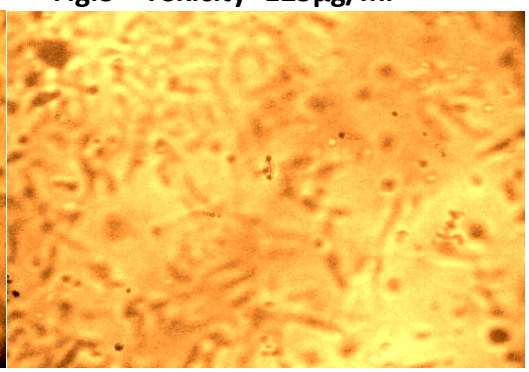

Fig.4 Toxicity- 31.2µg/ml

CONCLUSION

The study results shows that the Hemidesmus indicus root extracts has potential bio active compounds which can helps to treat cervical cancer. Further more the results provides strong evidence to suggest that Hemidesmus indicus root extracts have in vitro cytotoxicity against HeLa cells. The results reveals that the Hemidesmus indicus Plant could be used to develop effective approaches towards the prevention and management of cervical cancer.

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