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Invitro Cytotoxic Activity On Ethanolic Extracts Of Leaves Of *Ipomoea Pes-Tigridis*(Convolulaceae) Against Liver Hepg2 Cellline

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Abstract

The study was aimed to evaluation of the anticancer (liver cancer) activity of the leaves of *Ipomoea pes-tigridis* against liver HEPG2 Cell line. The leaves of *Ipomoea pes-tigridis* hydro alcoholic extract were tested for its inhibitory effect on HEPG2 Cell Line. The percentage viability of the cell line and the cytotoxicity activity of *Ipomoea pes-tigridis* against HEPG2 Cell line was carried out by using MTT assay method. *Ipomoea pes-tigridis* ethanolic extract has significant cytotoxicity effect on HEPG2 Cell Line in the concentration range at 500µg/mL produce 99.87% of cell inhibition. From the above performed assay, ethanolic extract of these drug shows greater activity on HEPG2 cell line that means *Ipomoea pestigridis* can be used as anticancer activity particularly for liver cancer.

Keywords: cytotoxic activity, MTT assay, *Ipomoea pes-tigridis*, HEPG2 Cell line.

INTRODUCTION

Nature has provided a complete store house of remedies to cure all ailments of mankind. The human being appears to be afflicted with more diseases than any other animal species. As per who calculate that about 80% of worlds inhabitants problem should treated by medicinal herbal drug for their primary health care [1-3]. From the last decade, the plants were used in the treatment of cancer.

Some of the plants used for the treatment of advanced stages of various malignancies were catharanthus roseus, angelicagigas, podophyllum pellatum, taxus brevifolia, campotheca acuminata [4]. A uncontrolled multiplication and spread within the body of abnormal form of body's own cells is called as "cancer".Combating cancer is of paramount importance today. An alternative solution to Western medicine which is embodied with severe side effects is the use of herbal preparations to arrest the insidious nature of the disease. Prior to 1983 in United States, approved worldwide between 1983 and 1994, approximately 62% can be related to natural anti oxidants [5]. Free radical damage may lead to cancer. Antioxidants interact with radicals and may prevent some of the damage by free radicals. Laboratory evidence from chemical, cell culture and animal studies indicate that antioxidants may show or possibly prevent the development of cancer [6].

Hepatocellular carcinoma is cancer of the liver. This type of cancer occurs more often in men than in women. It is usually seen in people aged 50 or above. However, the age varies in different parts of the world. The disease is more common in parts of Africa and Asia than in North or South America and Europe. Hepatocellular carcinoma is not the same as metastatic liver cancer,which starts in another organ (such as the breast or colon) and spreads to the liver. In most cases, the cause of liver cancer is usually scarring of the liver (cirrhosis)[7-8] *Ipomoea pes-tigridis* is one of them which not proven anti-cancer activity but in *Ipomoea* species *Ipomoea stans* proved cytotoxic activity. *Ipomoea pes-tigridis* is a spreading or twinning dicotyledons herb belongs to the family convolvulaceae. It was most throughout India ascending up to 4000 ft, plains from the coast, to 750-900m, often in arable lands.The climber flowers were white funnel shaped and it was called in the synonym as tiger foot and morning glory [9] .The herb is used in the treatment of boils, carbuncles and as antidote to dog bites. In Philippines and Indonesia, the leaves are applied as poultices to boils, pimples and sores. The root is used as a purgative [10]. The nutrivive values of *Ipomoea pes-tigridis* was reported[11] . The anti oxidant[12] , anti microbial[13] , anti inflammatory[14] and anti tumour[15] and anti convulsant[16] and antinoceceptive effect[17] was reported in the other species of *Ipomoea.*

MATERIALS AND METHODS

Cell lines and chemicals

HepG2 cells are a suitable *in vitro* model system for the study of polarized human hepatocytes. The human liver cancer cell line (HepG2) was obtained from National Centre for Cell Science (NCCS), Pune, and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Collection of plant material

The plant specimens were collected from Madurai Medical College campus. The plant was identified and authenticated by Dr. L. Stephen, Lecturer, American College and also by Dr. John Britto, Rapinet Herabarium, St. Joseph's College, Tiruchirapalli. The authenticated herbarium sheet had been placed at the Dept. of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai.

Extract preparation

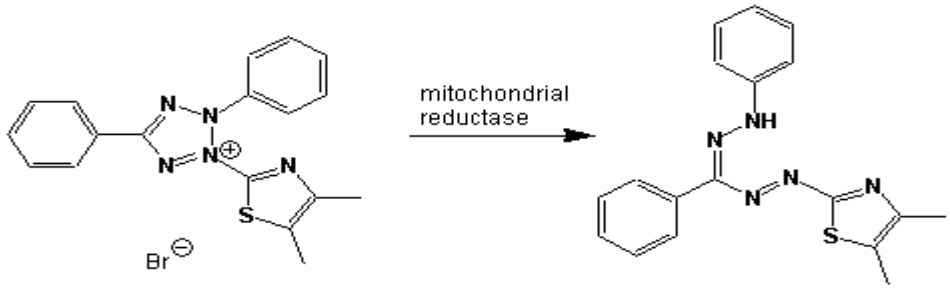
The leaves were collected during the month of September and washed thoroughly and dried in shade. About 500g of the dried powdered leaf of *Ipomoea pes-tigridis* was defatted with 1.5L petroleum ether ($60\text{-}80^0$ C) by maceration. The solvent was removed by filtration and the marc was dried. To the dried marc, 1.5L of 70% ethanol was added and the extraction was performed by triple maceration (72h process). It was then filtered and the combined filtrate was evaporated to a cohesive mass using rota vapour.

Extracts were prepared by dissolving in DMSO to a concentration of 10mg/mL and further diluted to a concentration of 500, 250, 125, 62.5 and 31.25µg/mL for the experiments.The control cultures were treated with equivalent concentrations of DMSO alone.

Microculture tetrazolium (MTT) assay

Principle

The method is based on the capacity of Mitochondria succinate-dehydrogenase enzymes in living cells which convert the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble purple coloured formazan product which is measured spectrophotometrically[17] . Therefore, the amount of formazan produced is directly proportional to the number of viable cells.



MTT **FORMAZAN CRYSTALS**

Methodology

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium with 5% FBS to give final density of $1x10^5$ cells/ml. 100µL/well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24h the cells were treated with serial concentrations of the extracts and fractions. They were initially dissolved in dimethylsulfoxide (DMSO) and further diluted in serum free medium to produce five concentrations. 100µL/well of each concentration was added to plates to obtain final concentrations of 500, 250, 125, 62.5 and 31.25µg/ml. The final volume in each well was 200µL and the plates were incubated at 37^0C, 5% CO_2, 95% air and 100% relative humidity for 48h. The medium without samples served as control. A triplicate was maintained for all concentrations.

. After 48h of incubation, 15µL of MTT (5mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37° C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µL of DMSO and then the absorbance was measured at 570nm using micro plate reader.The % cell inhibition was determined using the following formula

% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % cell inhibition and Log_{10} concentration and IC_{50} was determined using Graph Pad Prism software. The results obtained are presented in Table 1and Figs. 1& 2.

RESULTS AND DISCUSSION

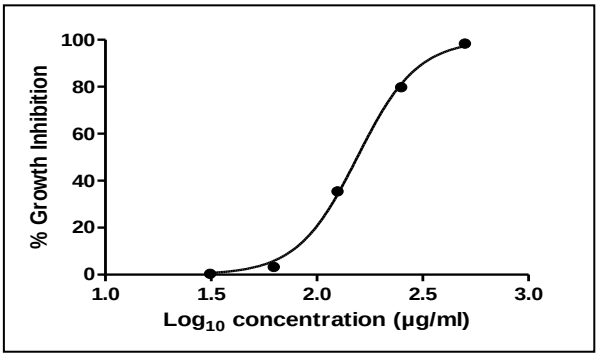
The cytotoxic activity of 70% ethanolic extract of *Ipomoea pes-tigridis* on liver cancer cell lines by MTT assay which was presented in Table1 and Figs.1& 2. A decrease in the cell count was observed with the increase in the concentration of the extract. There was a dose depended increase in the cytoxic activity. The extract at low concentration (31.25µg/mL) showed 0.36 % cell inhibition and at high concentration (500µg/mL) 98.83% cell inhibition and the same is seen in Table 1.

Table 1: Cytotoxic activity using liver HEP G2 cell lines by MTT assay method

S. No	Conc. µg/mL	% cell inhibition	IC_{50} µg/mL
1	31.25	0.3646	**155.2**
2	62.5	3.2818	
3	125	35.4604	
4	250	79.7630	
5	500	98.3592	

The 70% ethanolic extract of *Ipomoea pes-tigridis* was studied for their *in vitro* effects on liver HEPG2 cell lines. The selection of the crude plant extracts for screening has the potential of being more successful in initial steps than the screening of pure compounds which are isolated from natural products [120, 121]. In this study the 70% ethanolic extract exhibited the most effective cytotoxicity at 500µg/mL (98.83%) which is depicted in **Table 1.**

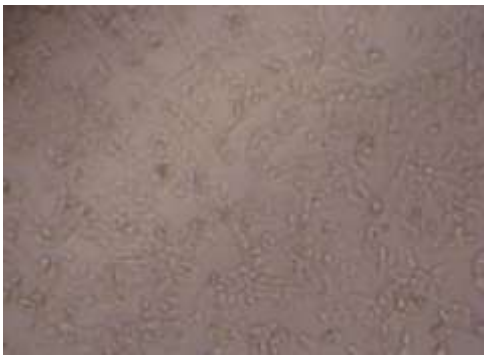
Fig. 1: Cytotoxic activity (% growth inhibition) using Liver HEP G2 cell lines by MTT assay method



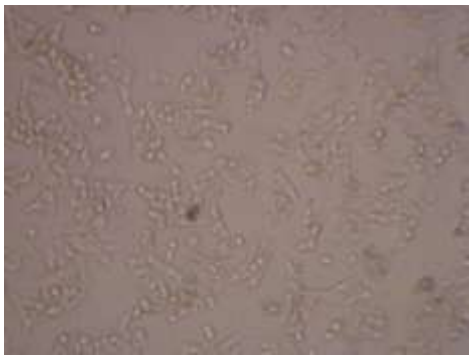
The inhibitory concentration (IC_{50}) value was calculated using the regression analysis and was found to be155.2µg/mL which is shown in **Fig. 1.**

Fig. 2: Cell inhibition (formazan crystals produced) at various concentrations of 70%ethanolic extract after MTT treatment by using HEPG2 cell lines

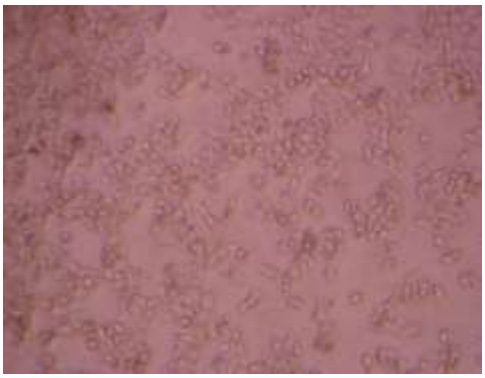
31.25µg/mL



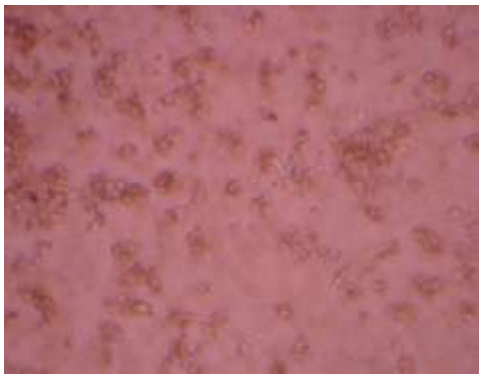
62.5µg/mL



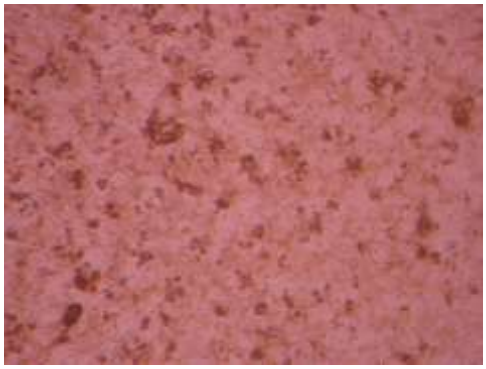
125 µg/mL



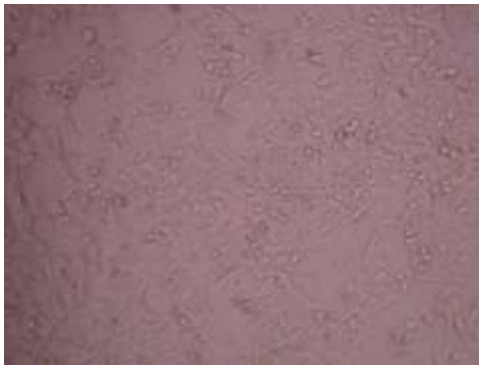
250µg/mL



500 µg/mL



CONTROL



The amount of formazan crystals produced by MTT is directly proportional to the number of viable cells which and the same is depicted in **Fig. 2.**

From the above study the crude extract has pharmacological effect [122] which may be due to the synergistic effects of the various components present in the crude extract. The active constituents namely flavanoids and terpenoids may be responsible for reducing the cancer risk factors [123-125]. Thus the 70% ethanolic extract of *Ipomoea pes-tigridis* exhibited significant anti cancer effect particularly for liver cancer. The **anti cancer effect** particularly against **liver cancer on HEP G2** cell lines showed that the plant possesses anti proliferative effect comparable to that of *Ipomoea stans*.

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