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Anticancer Activity From The Leaf Acetone Extract of Piper Betle Using Human Lung Cancer Cell Line (A_{549})

KEYWORDS: P. betle, MTT assay, lung cancer, antiproliferative.

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ABSTRACT *Piper betle Linn. commonly known as paan in India belongs to family Piperaceae. The leaves are used for treating cough, foul smell in mouth, ozoena, bronchitis, clear throat and stypne. Lot of research work has been carried out on antibacterial, antiprotozoan, antifungal, antioxidant, immunomodulatory, antimutagenic, antileishmanial, neurostimulatory properties of piper betle leaves. However not much data is available on the antitumor properties and their derivatives.*

SUMMARY

The present study was undertaken to check the antiproliferative activity from the leaves of acetone extract of Piper betle using human lung cancer cell line (A549). The leaves of P. betle was identified in and around Guindy. Fresh leaves were further processed for extract preparation. Lung cancer cell line (A549) was obtained from NCCS, Pune and was maintained in minimum essential media supplemented with 10% FBS, Pencillin (100U/mL) and streptomycin (100µg/mL) in humidified atmosphere of 5% CO2 at 37°C. IC50 values represented the maximum activity. An increasedrate of cell death was significantly observed with increase in concentration of leaf extract irrespective of the cell type.

INTRODUCTION

Piper betle Linn. belongs to family *Piperaceae*, is a tropical, perennial, dioecious, semi woody plant (creeper) commonly found in Malaysia, Indonesia, India, Srilanka and Philippines and cultivated in many other Asian and East African countries (wealth Asia,1997). Betle leaf (*Piper betle*) has many medicinal uses and has been recommended in the ancient scriptures of Ayurveda and is known for its acrid, antiseptic, aphrodisiac, aromatic, astringent, bitter, carminative, hot and stimulant properties (Parmer *et al.*,1997). Betle leaf is in use from ancient times as a digestive edible. Applying juice of leaves on wound is a common rural practice. In treatment of gout, arthritis and orchitis betle leaf plays a good role. This herb is also an effective external application for boils. Juice of betle leaves with honey or *gulkand* (rose pedal marmalade) is a good tonic. Studies have shown that betle leaves contain tannins, sugar, carotenes, ascorbic acid and essential oils.

Cancer is the uncontrolled growth and spread of cells that can affect almost any part of the body. Lung cancer is one of the five most common cancers prevalent in world for both men and women. More than 11million people are diagnosed with cancer every year. It is estimated that there will be 16 million new cases every year by 2020 (WHO, 2013). The best possible way to find out the activity of carcinogenic and non-carcinogenic compounds is to do trialingon cell lines. Cell lines play an important role in the cancer biology and are an easy approach to understand the mechanism of carcinogenicity in *in vitro* condition.

Even though lot of research has been done on various biological properties and medicinal uses of *P. betle* our knowledge of *P.betle* and its derivatives in cancer treatment is very limited. Our present investigation aimed at antiproliferative activity of acetone extract of *P. betle* leaves against human lung cancer cell line (A_{549}).

MATERIALS AND METHODS

Plant Material

The plant material of *Piper betle* leaves were collected from in and around Guindy.It was identified using standard books. The leaves and stem were shade dried and crushed into fine powder with electric blender. The powdered sample was sealed in polythene bags and was stored in desiccators until further uses.

Preparation of acetone extract

Dried and powdered betel leaves (500 g) were extracted using soxhlet with 100% acetone (1:5 W/V) for about 72 hours. The extracts was removed and it was concentrated to dryness in rotary vacuum evaporator below 50° C and stored until needed for the bioassays at -4 °C.

IN VITRO ANTICANCER ACTIVITY

Cell line and culture

Lung Cancer Cellline (A_{549})was obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), andstreptomycin (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 laboratorychemicals Mumbai). All of other chemicals and reagents wereobtained from Sigma Aldrich Mumbai.

In vitro assay for Cytotoxicity activity (MTT assay).

The Cytotoxicity of samples on $A_{549}$was determinedby the MTT assay(*Mosmann et al.*,1983). Cells (1 × 10^5/well) wereplated in 1ml of medium/well in 24-well plates (Costar Corning, Rochester,NY). After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence samples for 24 - 48hours at 37°C. After removal of the sample

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solution and washing with phosphate-buffered saline (pH 7.4), 100µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromidecells(MTT) phosphate- buffered saline solution was added. After4hours incubation, Viable cells weredetermined by the absorbance at 570nm. Measurements were performed and theconcentration required for a 50% inhibition of viability(IC_{50}) was determined graphically. The absorbance at 570 nm was measured with a UV- Spectrophotometer usingwells without sample containing cells as blanks. The effect of the samples on the proliferation of A_{549} was expressed as the % cell viability, using the followingformula:

% cell viability = A570 of treated cells / A570 of control-cells × 100%.

RESULT

Anticancer study was performed with acetone extract followed by DMSO control(used to dissolve extract). In order to confirm the anticancer activity of *P. betle* leaf extracts on A_{549} cell line, cell viability percentage was observed by MTT assay. The percentage cell survival of the control cell have been shown in (Table 1, figure 1) which is about 100%.

The viability of A_{549} cells treated with acetone extract decreases in a concentration dependent manner, lower extract concentration exhibited stronger anticancer activity(Table1;Figure1).Anticancer activity was found to be concentration dependent. Concentration of cell death was attained with least value of 11.4% respectively. The IC_{50} value of leaf acetone extract of *P. betle* was found to be 120µg/mL respectively.

Maximum cell cytotoxicity of 88.7% was observed at higher concentration of 1000µg/mL respectively. (Figure 2) represents the confluency of cells showing cell division and extent of cell proliferation. In control (normal cell) (Figure a) the cells are clearly visible whereas when the cells are treated with various concentration significant percentage inhibition was found.

At higher concentration of 1000µg/mL (Table 1, Figure 1) the cell toxicity was found to be 88.7% and in lower concentration least cell death was attained at 11.4% respectively.

DISCUSSION

Cancer present a serious clinical problem and pose significant social and economic impacts on the health care system. Despite improved imaging and molecular diagnostic techniques, the disease still impacts millions of patients worldwide (Eisenberg, 1998). Interfering with tubulin's normal biological function is a clinically proven approach for treating various types of tumors. Compounds that bind to b-tubulin, such as taxanes and the Vinca alkaloids form interfere with tubulin polymerization and microtubule depolymerization and thereby disrupting the normal cell division and commit the cell to apoptosis (Wang , *et al.,* 1994; Jordan *et al.*, 1998; Kuo *et al.*, 2004) .

Living organisms, including plants, microbes, and marines organisms, provide rich sources of chemically diverse bioactive compounds. More than 40% of the chemicals, thus far identified as natural products, have not been chemically synthesized. Natural products and their derivatives include vinblastine, paclitaxel, and etoposide already play critical roles in cancer chemotherapy (Schwartsmann *et al.,* 2002; Zou *et al.,* 2004; Kragelj *et al.,* 2005; Kugler *et al.,*1997). Chinese herbal medicine books (such as Shen Nung Pen Tsao Ching [220 AD] and the Pharmacopoeia of China) provide a wealth of information about plants and anticancer herbal formulations that are a useful starting point for the identification of new anticancer compounds (Schwartsmann *et al.,* 2002; Jemal *et al.,* 2002;Cheng *et al.,* 2004; Kao *et al.,* 2001 and Vickers, 2002).

Various phytochemical tests proved that the leaves extract was also rich in different bioactive molecules like flavonoids and phenols. This has been reported in one of our earlier study (WHO, 2013).

Significant inhibition in the growth of the lung cancer line (A_{549}) was obtained in acetone extract of *P. betle*. Infact the percentage inhibition in the acetone extract was found to be more. This may be due to the presence of other bioactive molecules having antiproliferative activity in the crude acetone extract.

From the present experiment it was concluded that the leaf extracts of *P.betle* shows significant anticancer activity on lung cancer cell line (A549). (Table 1, Figure 1&2). According to Santhakumari *et al.,* 2003; Shun *et al.,* 2007 the maximum antiproliferative activity was obtained in *P. betle* leaves of petroleum ether extract.

The antitumor property of *Piper betle* may be due to the phytochemicals present in it, including polyphenols and alkaloids, most of which are potent free radical scavengers. Phenolic compounds such as epigallocatechin gallate, catechin, genistein and quercetin suppressed growth of breast cancer cells implying the importance of antioxidants towards the anti-proliferative effects of cells. Anti-cancer agents with antioxidant activities may exert their beneficial effects by balancing levels of ROS so as not to cause further proliferation of cancer cells while still allowing apoptosis to occur (Abrahim*et al.,*2012).

Hydroxychavicol, a component of (Chang *et al.,*2002). Antioxidants may inhibit carcinogenesis through other non-antioxidant action such as by modulating signalling pathways involved in cellular functions such as proliferation, cell growth and differentiation, by influencing activities of cancer-related enzymes such as cyclooxygenase-2 and phase I or II metabolizing enzymes or by inducing cell cycle arrest . *P. betle* leaf showed anti-proliferative effect towards oral carcinoma cell line (Wang *et al.,*2011)

The intension of the present study was to check the efficacy of *P. betle* leaf extracts against anticancer acticvity of A_{549} cell line. Betel leaves are also reported to possess antioxidant activity besides antimutagenic and anticarcinogenic properties, particularly against the tobacco carcinogens, due to the presence of ingredients like hydroxychavicol and chlorogenic acid in it. The latter compound is also reported to kill the cancerous cells without affecting the normal cells unlike the common cancer drugs and relevant therapeutic means. Therefore, possibility of manufacturing a new blood cancer drug from it cannot be ruled out (Guha *et al.,*2006).

Table 1: Anticancer effect of leaf acetone extract of *P. betle* using A_{549}cell line

S.No	Concentration(µg/ml)	Cellviability(%)	Cytotoxicity(%)
1	1000	11.3±0.79	88.7±6.20
2	500	22.7±1.58	77.3±5.41
3	250	29.5±2.06	70.5±4.93
4	125	43.1±3.01	56.9±3.98
5	62.5	52.2±3.65	47.8±3.34
6	31.2	65.9±4.61	34.1±2.38
7	15.6	81.8±5.72	18.2±1.27

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8	7.8	88.6±6.20	11.4±0.79
9	Cell control	100	0
IC 50 Value (µg/ml)			120.19

IC $_{50}$Value- concentration of drug requires to scavenge 50% of the radicals.

Figure 1: Determination of *in vitro* assay for cytotoxicity (MTT assay)

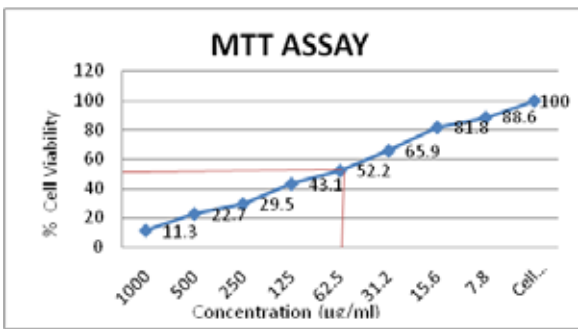
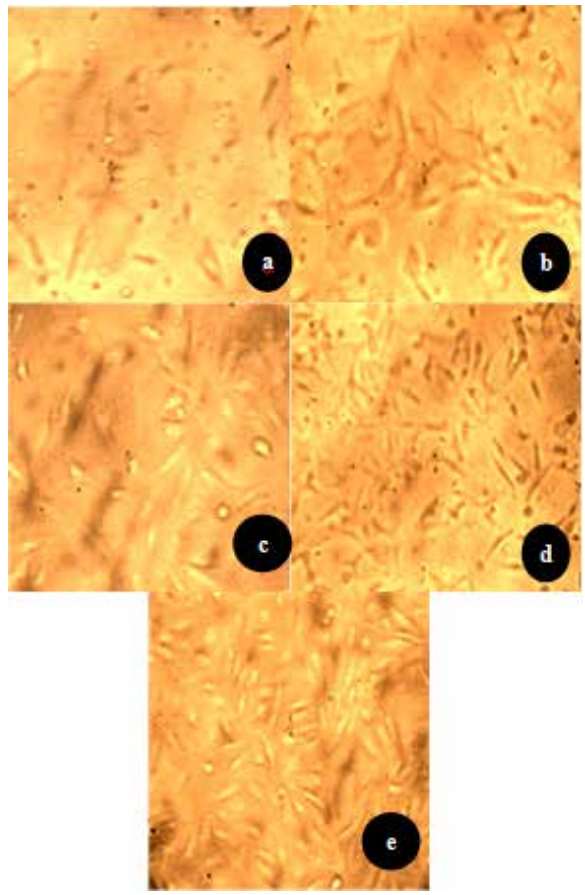


Figure 2: Cytotoxicity of *P.betle* leaf acetone extracts on lung cancer cell line



Control (normal cell); b. 15.6µg/mL ; c. 62.5µg/mL ; d. 250µg/mL
1000µg/mL

CONCLUSION

P. betle leaves have potent anticancer properties due to the presence of phytochemicals, free radical activity as well as inducing selective toxicity against cancerous cells. It can also be used for the treatment of various ailments including human lung cancer.

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