Anticancer Activity From The Leaf Acetone Extract of Piper Betle Using Human Lung Cancer Cell Line (A549)

INTRODUCTION

Piper betle Linn. belongs to family Piperaceae, is a tropical, perennial, dioecious, semi woody plant (creeper) commonly found in Malaysia, Indonesia, India, Sri Lanka 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 11 million 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 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 (A549).

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. The extracts were stored until needed for the bioassays at -4 °C.

IN VITRO ANTICANCER ACTIVITY

Cell line and culture

Lung Cancer Cell line (A549) 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) and streptomycin (100 μg/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.

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 increased rate of cell death was significantly observed with increase in concentration of leaf extract irrespective of the cell type.

KEYWORDS

P. betle, MTT assay, lung cancer, antiproliferative.
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 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 A₅₄₉ was expressed as the % cell viability, using the following formula:

\[ \text{% cell viability} = \frac{A_{570 \text{ of treated cells}}}{A_{570 \text{ of control cells}}} \times 100\% \]

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 A₅₄₉ 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 A₅₄₉ 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 IC₅₀ 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 β-tubulin, such as taxanes and the Vinca alkaloids 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 marine 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 (Schwartzmann 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 (Schwartzmann 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 (A₅₄₉) was obtained in acetone extract of P. betle. In fact 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 epiigallocatechin 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 activity of A₅₄₉ 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 (Guhu et al.,2006).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration(µg/ml)</th>
<th>Cell viability(%)</th>
<th>Cytotoxicity(%)</th>
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<tbody>
<tr>
<td>1</td>
<td>1000</td>
<td>11.3±0.79</td>
<td>88.7±6.20</td>
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<td>2</td>
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<td>22.7±1.58</td>
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<td>5</td>
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<td>52.3±3.65</td>
<td>47.8±3.34</td>
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<td>6</td>
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<td>65.9±4.61</td>
<td>34.1±2.38</td>
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<td>7</td>
<td>15.6</td>
<td>81.8±5.72</td>
<td>18.2±1.27</td>
</tr>
</tbody>
</table>

Table 1: Anticancer effect of leaf acetone extract of P. betle using A₅₄₉ cell line.
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

**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.

**REFERENCE**