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# PLUMBAGIN: A POTENTIAL DRUG CANDIDATE FOR TREATMENT OF ORAL CANCER

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**ABSTRACT**
Oral cancer is the fifth most common type of cancer worldwide. The risk factors for oral cancer include tobacco chewing and cigarette/bidi smoking. Much advancement is made for the treatment of oral cancer, *sans* much promise. Several plant products, like vincristine, vinblastine, paclitaxel, etoposide, plumbagin etc, are promising drug candidates for cancer therapy with their own limitations. Plant products, like plumbagin, are safe and promising drug candidates. Plumbagin is a hydroxy-naphthoquinone that shows potent anti-oxidant, ant-inflammatory, and anti-cancer activities. In this study, we have analyzed the effects of plumbagin on oral cancer cell survival using three oral cancer cell lines. Purified analytical grade plumbagin was procured from and tested on three oral cancer cell lines were used *viz.*, AW13516 (Dwivedi, DWD AW 8507 (Gurav) and KB. Our results demonstrate that increasing concentration of plumbagin ($10^{-7}$ M to $10^{-4}$ M) inhibits growth of oral cancer cells. The LC 50 values show that plumbagin also induces cell death in these cells. We demonstrated here that plumbagin may be utilized as a potential drug candidate for oral cancer, however elaborate mechanistic and pre-clinical studies are warranted. This is the first report showing that plumbagin inhibits the growth/survival of oral cancer cells.

**KEYWORDS**: cell survival, oral Cancer, plumbagin, *Plumbago*.

## INTRODUCTION

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries. It is predicted that by 2020, there will be 15 million new cancer cases and 10 million cancer deaths every year. Oral cancer is considered as the fifth most commonly occurring cancer worldwide (Subapriya *et al*., 2007) which include cancer of the lip and oral cavity (buccal mucosa, gingiva, hard palate, tongue and floor of mouth).

Generally, the highest occurrence of oral cavity cancer is found in the Melanesia, South-Central Asia, and Central and Eastern Europe and is lowest in Africa, Central America, and Eastern Asia. In developing countries such as India, the incidence of oral cancer and other pre-malignant and malignant lesions of the oral cavity are very high. Annual rate of oral cancer worldwide is 3, 00,000 annually and in India it accounts for about 20 per 1, 00,000 population (Sambandham *et al*., 2013).

Smoking, alcohol use, smokeless tobacco products, and HPV infections are the major risk factors for oral cavity cancer (Blot *et al*., 1988). The contribution of each of these risk factors to disease burden varies across regions. Worldwide, smoking accounts for 42% of deaths from cancers of the oral cavity (including the pharynx) and heavy alcohol consumption for 16% of the deaths; the corresponding percentages in high-income countries are about 70% and 30%, respectively (Danaei *et al*., 2005). Smokeless tobacco products and betel quid with or without tobacco are the major risk factors for oral cavity cancer in Taiwan, India, and other neighboring countries (Wen *et al*., 2010). Betel quid is carcinogenic to humans and is an important risk factor among people with this habit in the Asian ethnic minorities residing the UK (Warnakulasuriya, 2002).

Oral squamous cell carcinoma (OSCC) is a wide-spread malignancy that accounts for more than 90% of all oral cancers. Pain is the most frequent symptom and the tongue and floor of the mouth are the most susceptible sites for the development of cancerous lesions. OSCC in its initial stages shows an erytholeukoplastic area without symptoms but in advanced stages, there are ulcers and lumps with irregular margins, which are rigid to touch (Bagan *et al*., 2010). The general symptoms are painless white or red patch in the mouth, hoarseness or change in voice, sore throat, painless lump in the mouth or neck, difficulty in chewing, swallowing, or breathing, frequent nose bleed, hearing loss or ear pain, blood in saliva and weight loss or fatigue.

  

The main treatment package for patients with oral cancer includes surgery and radiotherapy. Chemotherapy is also used to treat oral cancer, but it is usually used in patients with metastasis (Liu *et al*., 2012). There is an emerging trend for the use of chemotherapy in combination with radiation therapy and surgery for patients with advanced, recurrent, and metastatic head and neck cancer, although there is limited evidence showing any substantial survival benefit when used for treating patients with oral cavity carcinoma (Day *et al*., 2003). Although great improvements have been made in treatment of oral squamous carcinoma using a combination of anti-cancer treatments, the 5-years overall survival rate is still kept around 60%, (Liu *et al*., 2012).

Recently, a greater re-emphasis has been given to research in natural plant products that may provide prevention and/or treatment of cancer, without the side-effects, as seen with most chemo-radio therapies. Plumbagin is a plant secondary metabolite obtained from the medicinal plant *Plumbago* of the family Plumbaginaceae is a semi-climbing shrub that grows throughout Asia and Africa. It exhibits highly potent biological activities, Roots of plant have potential therapeutic properties like anti-anthrogenic, carditoxic, hepatoprotective, neuroprotective, anti-atherogenic, cardiotonic, hepatoprotective and neuroprotective properties (Tilak, *et al*., 2004). In traditional Indian medicine, P. zeylanica L. has been assigned medicinal properties and is used in formulations of a number of ayurvedic compounds (Kirtikar, K.R. and Basu, B.D. 1993)). In southwestern Nigeria, P. zeylanica L. is used in folk medicine to treat parasitic diseases, scabies and ulcers (Daiziel, J.M. 1959). Pharmacological studies carried out by severalworkers have indicated that P. zeylanica L. extract has antiplasmodial (Simonsen *et al*., 2001), antimicrobial (Ahmad I.et al., 2000), antihyperglycemic (Olagunju, J.A. *et al*., 1999), and antiallergic (Dai, Y., *et al*, 2004) properties. It is cytotoxic for tumor cell activities (Lin, L.C., *et al*., 2003). Chemically, plumbagin is a simple hydroxy-naphthoquinone, (5-hydroxy-2-methyl-1, 4-naphthoquinone, chemical formula- $C_{11}H_8O_3$). Plumbagin has been reported to suppress NF-κB activation in tumor cells, and hence might affect the biological functions of leukocytes participating in various immune responses (Padhye *et al*., 2012).

Plumbagin exhibits highly potent biological activities that have been attributed to its effects on multiple signaling and apoptotic pathways, and its ability to undergo redox-cycling property generating ROS (Padhye *et al*., 2012). It efficiently induces apoptosis in breast cancer cells. (Ahmad *et al*., 2008). Several other mechanistic studies confirm the potential of plumbagin as a therapeutic compound against various types of cancers. It is reported that plumbagin is capable of suppressing constitutive NF-κB in various cancer cells, which ultimately leads to suppression of downstream NF-κB-regulated pro-survival mechanisms (Sandur *et al*., 2006). Anticancer activity of the plumbagin has been reported against breast cancer (Kuo *et al*., 2006), prostate cancer (Aziz *et al*., 2008), ovarian cancer (Srinivas *et al*., 2004), liver cancer (Shih *et al*., 2009), and cervical cancer (Srinivas *et al*., 2004). It is somewhat surprising that the anti-cancer effects of plumbagin have not been previously investigated thoroughly on oral cancer.

We postulated, based on previous reports in several types of cancers, that plumbagin might have a potent anti-cancer activity against oral cancer cells. In the present work, we have tried to demonstrate the effects of plumbagin on oral cancer cell survival.

## MATERIALS AND METHODS

### Methods

Three oral cancer cell lines were used *viz.*, AW13516 (Dwivedi, DWD), AW 8507 (Gurav) and KB. Out of these three, AW13516 and AW 8507 are derived from Indian patients. The cell lines were selected from the cancer cell line collection of 'Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Navi Mumbai, INDIA. Purified analytical grade plumbagin was procured from SIGMA-ALDRICH (P7262 SIGMA). The experiments to analyze the effect of plumbagin on the survival of oral cancer cells were carried out by 'Anticancer Drug Screening Facility, (ACTREC), Kharghar, Navi Mumbai-410210', using Sulphorhodamine B colorimetric assay for cytotoxicity screening. (Vichai & Kirtikara, 2006). Plumbagin was dissolved in DMSO and was diluted to give different concentrations viz., $10^{-7}$, $10^{-6}$, $10^{-5}$, and $10^{-4.}$ Adriamycin, was used as a positive control compound. From the selected oral cancer cell lines medium was removed and cells were washed with PBS. Then using 0.25% (wt/vol) trypsin in versene –EDTA homogenous cell suspension was obtained. Cell count was measured by using trypan blue solution and adjusted to seeding density (1.9 * $10^4$ cells per well). Plates were incubated at 37 0 C in a humidified incubator with 5 % CO2 for 72 hrs. Then 100 ul of 10% (wt/vol) TCA was added to each well and incubated at 4 0C for 1 hr. Then plates were washed and allowed to dry. Then 100 ul of 0.057% (wt/vol) SRB solution was added to each well and

  

incubated at room temperature and quickly rinsed with 1% acetic acid. After 30 mins SRB is solubilised in 10Mm Tris base solution and OD was taken at 510 nm in microplate reader. The percentage cell growth inhibition was calculated using the formula.

$$\% \text{ of control cell growth} = \frac{\text{mean } OD_{sample} - \text{mean } OD_{day0} * 100}{\text{Mean } OD_{control} - \text{mean } OD_{day0}}$$

% growth inhibition = 100 - % control cell growth.

LD value were determined from the dose response relationship between the compound concentration and the percentage of cells killed using the formula

$$\% \text{ cells killed} = 100 - \text{mean } \frac{OD_{sample} * 100}{\text{Mean } OD_{day0}}$$

The study was approved by the Institutional Ethics Committee (IEC) of Dr. D. Y. Patil Biotechnology & Bioinformatics Institute, Pune, India. Student's T-test was used to calculate the 'p values'.

## RESULTS

Resistance of cancer cells to drug-induced apoptosis is a major obstacle for anti-cancer therapeutics; hence, novel therapies are much needed. This study examined the effect of plumbagin on oral cancer cells and reports the potential of plumbagin against this debilitating condition. Our results demonstrate that increasing concentration of plumbagin ($10^{-7}$ M to $10^{-4}$ M) inhibits growth of oral cancer cells (Fig 1, Table 1). All the experiments were done in triplicate and the 'mean' is plotted in the graph. Plumbagin demonstrated significant inhibition ($p<0.001$) of cell growth in all the three cell lines. Moreover, plumbagin also induces cell death in these cells (Table 2; LC50 values), indicating towards its mechanism of action. This is the first direct report showing the efficacy of plumbagin as a potential drug candidate in oral cancer.

**Table 1. Effect of Plumbagin on % Control Growth in Cell Lines.**

| | % Control Growth in molar concentration | | | |
|---|---|---|---|---|
| **Molar Drug Concentrations** | **$10^{-7}$M** | **$10^{-6}$M** | **$10^{-5}$M** | **$10^{-4}$M** |
| **Human Oral Cancer Cell Line AW13516** | 100.0 | 100.0 | -64.6 | -70.7 |
| **Human Oral Cancer Cell Line AW8507 (Gurav)** | 90.5 | 85.8 | 0.5 | -36.7 |
| **Human Neso-Pharyngeal Cancer Cell Line KB** | 100.0 | 100.0 | 86.3 | -79.2 |

(Each Cell line Control growth data is average of 3 experiments)

Table 2. Anticancer Activity of Plumbagin on three Oral Cancer Cell Line.

| **Cell lines** | **µMolar** drug concentrations calculated from graph (fig. 1) | | |
|---|---|---|---|
| **Molar Drug Concentrations** | **LC50** | **TGI** | **GI50*** |
| **Human Oral Cancer Cell Line AW13516** | 78.1 | 40.8 | 3.5 |
| **Human Oral Cancer Cell Line AW8507 (Gurav)** | >100 | 61.7 | 14.7 |
| **Human Neso-Pharyngeal Cancer Cell Line KB** | 85.0 | 57.3 | 29.7 |

**Definitions:**

1) LC50 = Concentration of drug causing 50% cell kill
2) GI50 = Concentration of drug causing 50% inhibition of cell growth
3) TGI = Concentration of drug causing total inhibition of cell growth

The data demonstrates that the growth/survival of all the three oral cancer cell lines is inhibited by increasing concentrations of plumbagin. One group of cells was kept untreated, i.e. without plumbagin treatment. This was used as 'negative control' and no inhibition of cell growth was observed in this group, i.e. 100% growth (unpublished observation).

  

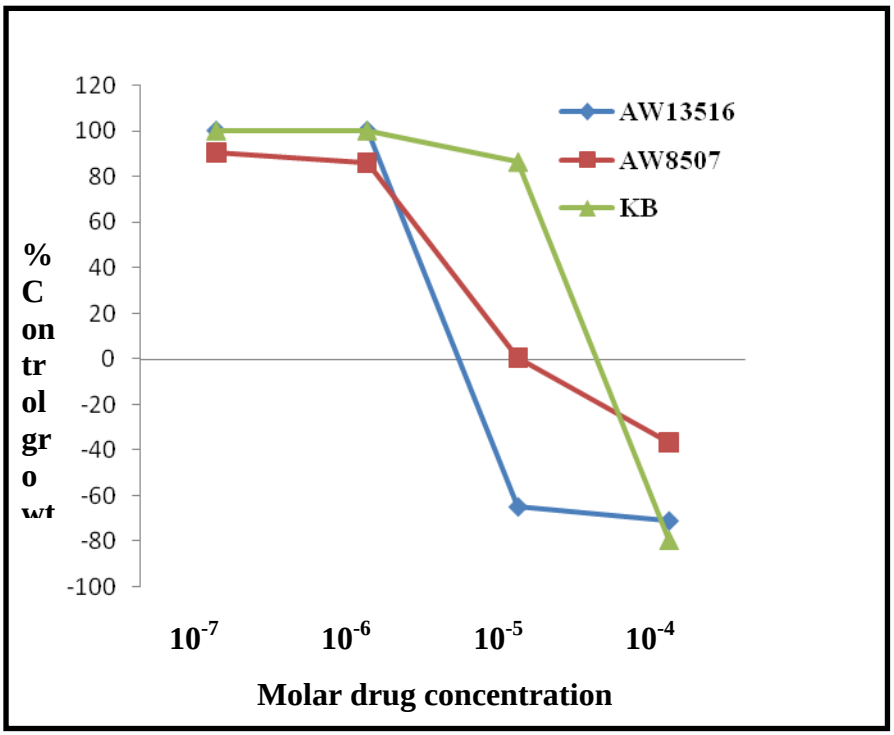


**Figure 1. Growth curve showing effect of plumbagin on three oral cancer cell lines.**

## DISCUSSION

One of the few mechanistic studies on plumbagin by Kanjoormana A. Manu *et al* in 2011 shows that plumbagin inhibits the expression of CXCR4 in oral adenosquamous carcinoma cells (CAL27). Interaction of CXCR4 with its ligand CXCL-12 is a critical process of metastasis that accounts for more than 90% of cancer related cell deaths. In our study, we found that plumbagin has differential effects on cell survival in the three cell lines chosen (Fig 1, Table 1 & 2). This needs further evaluation to elucidate the molecular targets of plumbagin that may modulate cell survival pathways leading to cancer cell death.

## CONCLUSIONS

Based on our cell growth retardation data, we can state that ours is the first direct report of the use of plumbagin as a potential therapeutic drug candidate for oral cancer, although, extensive *in vitro* and *in vivo* studies are warranted to delineate the exact mechanisms of plumbagin-induced cell growth retardation in oral cancer cells.

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