



Elucidation of Inhibitory Potentials of *Eclipta prostrata* on Oral Cancer Cell Lines

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ABSTRACT

The aim of the study is to analyse the cytotoxicity activity of *Eclipta prostrata* on oral cancer cell lines. KB cells were used for the present study. The extract of *Eclipta prostrata* was prepared. The cytotoxicity of *Eclipta prostrata* on the oral cancer cell line was evaluated by MTT assay. The *Eclipta prostrata* showed significant cytotoxicity activity on the oral cancer cell lines. The significant cytotoxic activity of *Eclipta prostrata* on oral cancer cell lines was concluded in this study. Further research on this area would be a thrust area for future research.

Keywords: Cytotoxicity, *Eclipta prostrata*, KB cell lines, MTT assay.

INTRODUCTION

Cancer which can affect any part of the body is a group of diseases caused by loss of cell cycle control. It is associated with uncontrolled and abnormal cell growth. Oral cancer is the sixth most common cancer affecting mankind, which also presents with low rate of survival. More than 90% of oral cancers are histopathologically squamous cell carcinomas (SCC).¹ Oral SCC's typically affects males with 40 years of age with a history of regular exposure to etiological risk factors like tobacco products, alcohol, betel quid or micronutrient deficiency.

However today even younger patients with lower cumulative tobacco or alcohol consumption are presented with OCSS's. These early onset of oral squamous cell carcinoma are often located at the base of the tongue, tonsils and oropharynx and are associated with human papilloma virus.¹

The important pharmacological activities are hepatoprotection, antibacterial, analgesic, antidiabetic hair growth, etc.² There are 10.9 million new cases, 6.7 million deaths, and 24.6 million persons alive with cancer.³

Treatments like chemotherapy, surgery and radiation are available but it has failed in many aspects reducing the life span of the patients. These treatments kill the cancer cells along with the normal cells. Natural therapies, such as the use of the plants or plant derived natural products are being beneficial to combat cancer. The Indian system of holistic medicine known as "Ayurveda" uses mainly plant based drugs or formulations to treat various ailments, including cancer.

Recent phytochemical examination of plants which have a suitable history of use in folklore for the treatment of cancer. Among many recent advances in cancer chemotherapy, plant natural products play an important role in having contributed considerably to the approximately 60 available cancer chemotherapeutic drugs.³

Eclipta prostrata is an annual herbaceous plant, commonly known as false daisy. It is an erect or prostrate, much branched, roughly hairy, annual, rooting at the nodes; the leaves are opposite, sessile and lanceolate. Belonging to family Asteraceae. It is also known as Bhringaraj and Karisilakanni, which is found a common weed throughout India ascending up to 6000 ft.

The specific *Eclipta prostrata* means white which refers to the color of the flowers. Main active principles consist of coumestans like wedelolactone, desmethyl wedelolactone, furanocoumarins, oleanane & taraxastane glycosides. *Eclipta prostrata* has been used in various parts of tropical and sub-tropical regions like South America, Asia, Africa.^{4,7}

In this present study the inhibitory potential of *Eclipta prostrata* on commercially bought oral cancer cell line was studied.

MATERIALS AND METHODS

Preparation of the plant extract

Eclipta prostrata leaves were collected. The leaves were thoroughly washed and the leaves were shade dried and coarsely powdered in a grinder.⁵ Shade dried powder was extracted with methanol. Methanol extract was prepared by cold percolation and it is concentrated under reduced pressure using rotatory evaporator at 4^o C.



Finally crude extract was obtained. The crude extract was stored at 4°C until further use.^{6, 8, 9} Three different concentrations of the extract (100, 200, 300 µg) were prepared.

Cell line and cell culture

Oral Squamous cell carcinoma cell lines (kb cell line) were procured from the National Institute of Cell Sciences, Pune, India. The cells were maintained in 96 well microtitreplate in DMEM medium supplemented with 10% Fetal Bovine Serum at 37°C in humidified atmosphere with 5% CO₂.

Treating oral cancer cell lines with various concentrations of *Eclipta prostrata*

The MTT assay (Mossman, 1983) is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product. The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2×10^4 cells /well and allowed to attach overnight at 37°C. The medium was then discarded and cells were incubated with different concentrations of the samples (100, 200 & 300 µg) for 24 hours.

Cell viability assay

After the incubation, medium was discarded and 100µl fresh medium was added with 10µl of MTT (5mg/ml). After 4 hours, the medium was discarded and 100µl of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570nm in a microtitre plate reader. Cyclophosphamide was used as a positive control.

Cell survival was calculated by the following formula:

$$\text{Viability \%} = (\text{Test OD} / \text{Control OD}) \times 100$$

$$\text{Cytotoxicity \%} = 100 - \text{Viability\%}$$

RESULTS AND DISCUSSION

This study elucidated the inhibitory potential of *Eclipta prostrata* on oral cancer cell lines. Cytotoxicity analysis using various concentrations of *Eclipta prostrata* extract (100,200,300 micrograms) was performed. The viability of the KB cell lines shows a gradual decrease as the concentration of the extract is increased.

This exhibits the cytotoxicity of *Eclipta prostrata* with increasing concentration. The graph 1 given exhibits the cytotoxicity analysis.

The main chemical constituents like Wedelolactone [1.6%], desmethywedelolactone, desmethylwedelolactone-7-glucosidde, stigmasterol^{4, 11} contributed to the cytotoxic effect of *Eclipta prostrata* on oral cancer cell lines.

The use of herbs and other plant products as medicine is practiced worldwide from time immortal. In India, the major way of treating diseases in ancient time was Ayurveda which mainly concentrates on using natural plant products as therapeutic agents.¹ *Eclipta prostrata*

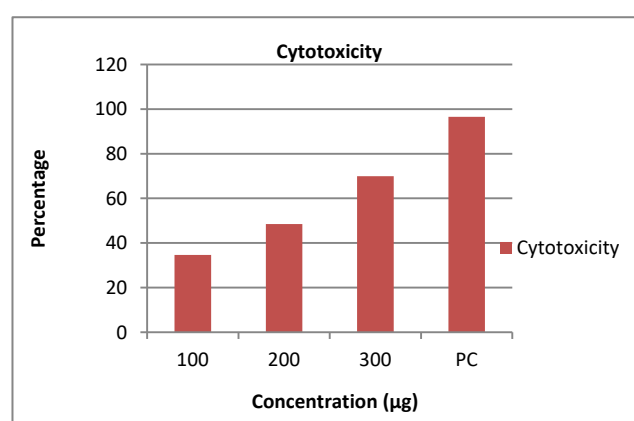
commonly called as Karisalankanni is found in tropical regions.¹⁰

The leaf aqueous extract of *Eclipta prostrata* mediated synthesis of titanium dioxide nanoparticles were also studied earlier.¹⁰ The herbal synthesis of silver nanoparticles using the plant *Eclipta prostrata* was studied by Swaminathan et al.¹²

The inhibitory effect of the crude ethyl acetate extract of *Eclipta prostrata* has been tested in vitro against the cancer cells using MTT assay.

This was a preliminary in vitro study wherein the inhibitory potential of *Eclipta prostrata* on oral cancer cell lines was observed. There is a significant effect of Karisalankanni on oral cancer cells.

Graph 1



CONCLUSION

This study exposed the cytotoxic potential of *Eclipta prostrata*, a medicinal herb. The potential to exploit *Eclipta prostrata*, as an anticancer drug is a thrust area for future research.

Day by day a lot of research works have shown the potential of phytochemicals as health benefactors because of their ability to neutralize free radicals activity, oxygen species or oxidants responsible for the cell damage.

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