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Original article

An invitro study on the anticancer activity of Tinospora Cordifolia Satva on Oral squamous cell carcinoma cell line

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Abstract:

Background: Tinospora Cordifolia satva is an ayurvedic preparation from the stem of Tinospora Cordifolia and commonly used in ayurvedic medicine to treat various diseases. The anticancer activity of the Tinospora Cordifolia satva was not well reported in the literature. Thus, the present stud
y was aimed to find the anticancer activity of Tinospora Cordifolia satva on oral squamous cell carcinoma cell line.

Materials and methods: The Human oral squamous cell carcinoma cell line OECM-1 was procured from Sigma Aldrich and Tinospora Cordifolia satva was procured from standard ayurvedic pharmacy. MTT assay was performed treating the cells with 12.5, 25, 50, 75, 100 and 200 µg/ml concentrations of Tinospora Cordifolia satva. The absorbance was read on a spectrophotometer at 570nm wavelength and the values were recorded, cell viability was calculated and the IC_{50} value was determined.

Results: Tinospora Cordifolia satva had effectively inhibited cell proliferation in a dose dependent manner. The least dose 12.5 µg/ml also showed antiproliferative activity with the IC_{50} value of 148 µg/ml.

Conclusion: Tinospora Cordifolia satva had inhibited cell proliferation effectively in dose dependent pattern. Thus, it can be included in the treatment of oral squamous cell carcinoma. Further, clinical trials can be carried out to fix the optimum dose as it is inhibiting cell proliferation in OSCC cell line with the lowest dose.

Keywords: Tinospora Cordifolia satva, anticancer, oral squamous cell carcinoma, oral cancer.

Introduction:

Cancer is one of the leading causes of death and a significant barrier to improving life expectancy globally.[1] According to estimates from the World Health Organization (WHO) for 2019, cancer is the main cause of death for people under the age of 70 and ranks 1st or 2nd in 112 out of 183 countries, and it ranks 3rd or 4th in 23 additional countries.[2] The rising prevalence of cancer as a primary cause of death is partly due to the fact that mortality rates from stroke and coronary heart disease have declined drastically in many nations when compared to cancer.[1]

On a global basis, oral cancer is ranked in the sixth most common type of cancer and India reports one third of the total cases of the oral cancers.[3] Due to its large impact, this ailment has attracted a great deal of study attention. The 2020 Global Cancer Observatory (GCO) report revealed that there were 377,713 new cases and 177,757 fatalities worldwide that were related to oral cancer.[4,5] Among all the oral cancers 84 to 97% are belonging to oral squamous cell carcinoma (OSCC).[3] India reports about one fourth of all cases worldwide, with 52,000 deaths and 77,000 new cases annually.[6] As the condition has high prevalence, treating it is a difficult and challenging endeavour. Chemotherapy, radiation therapy, and surgical excision are included in the systemic cancer therapy.[7] The chemotherapeutic agents such as doxorubicin and cisplatin are DNA interactive agents, on the other hand methotrexate is an anti-metabolite and most of the chemotherapy drugs have side effects.[8] Thus, there is a need of inventing new herbal anticancer medicine with less side effects and safe.

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Tinospora Cordifolia is an ayurvedic herb commonly known as Guduchi and Amrita is a climbing shrub belongs to the family Menispermaceae.[9] The term Guduchi is a Sanskrit name which means the one protects whole body and Amrita is attributed for its imparting of longevity, vitality, and youthfulness. Tinospora Cordifolia leaves, stem and roots were commonly used to treat various conditions in ayurvedic medicine. Previous studies reported that the phytocomponents present in that the stem of the Tinospora Cordifolia had a good immunomodulator activity, anti-oxidant activity, anti-inflammatory activity, anti-cancer activity and anti-microbial activity.[10] Limited literature was available on the anticancer activity of Tinospora Cordifolia on OSCC cell line and no study was reported till date on the anticancer activity of the Tinospora Cordifolia satva which is a ayurvedic starch preparation from the stem of the Tinospora Cordifolia. Thus, the present study was undertaken to find the anticancer potential of Tinospora Cordifolia satva on oral squamous cell carcinoma cell line.

Materials and methods:

The Human oral squamous cell carcinoma cell line OECM-1 was procured from Sigma Aldrich (St Louis, MO, USA). The Tinospora Cordifolia satva an ayurvedic preparation from the stem of the Tinospora Cordifolia plant was procured from the ayurvedic pharmacy. The cells were maintained in Alpha-MEM media supplemented with 10 % FBS along with the 1% antibiotic, antimycotic solution in the atmosphere of 5% CO_2, 18-20% O_2 at the temperature of 37^0C in the CO_2 incubator. They were sub-cultured for every two days. In a 96-well plate, 200µl cell suspension was seeded at the required cell density without the test agent and allowed the cells to grow for about 24 hours. The attached cells were treated with appropriate concentrations of the Tinospora Cordifolia Satva 12.5µg/ml, 25 µg/ml, 50µg/ml, 100 µg/ml and 200 µg/ml. A well without adding any drug was considered as negative control and incubated the plate for 24 hours at 37°C in a 5% CO_2 atmosphere. After the incubation period, the plates were taken out from incubator, and removed the spent media and MTT reagent was added to a final concentration of 0.5mg/mL of total volume. The plate was wrapped with aluminum foil to avoid exposure to light and the plates were returned to the incubator and incubated for 3 hours. MTT reagent was removed and then 100µl of DMSO was added. Gentle stirring in a gyratory shaker was done to enhance dissolution. The absorbance was read on a spectrophotometer at 570nm wavelength and the values were recorded. The percentage of cell viability is calculated. The IC_{50} value was determined by using linear regression equation.

Statistical analysis:

The MTT assay was performed in triplicate and the results were shown as mean and standard deviation of the values. The data was analysed by using linear regression GraphPad Prism 8 software and $p< 0.05$ was considered as level of significance.

Results:

There was a decrease in the cell proliferation with increase in the concentration of Tinospora Cordifolia satva. The inhibition of cell proliferation was observed in a dose dependent manner as the cell viability was gradually decreasing from 100 % in untreated samples to 32.90% in 200 µg/ml of Tinospora Cordifolia satva concertation. The mean absorbance of the controls and the different concentrations of the Tinospora Cordifolia satva were tabulated in (Table 1; Figure 1 & 2).

The data was analysed using linear regression and observed that the Tinospora Cordifolia satva had shown considerable cytotoxic potential ($P<0.0001$) at a 95% confidence interval, with an R2 value of 0.9982. Tinospora Cordifolia satva at its lowest concentration of 12.5µg/ml also exhibited an apoptotic effect. With an IC_{50} value of 148.18 ug/ml, Tinospora cordifolia demonstrated notable cytotoxic potential properties against OECM-1 cell lines, according to the observations in the statistical data of the MTT cytotoxicity research. The least dose of Tinospora Cordifolia satva was 12.5µg/ml also showed apoptotic effect on OSCC cell line. The observations in statistical data of MTT cytotoxicity study suggested that, Tinospora Cordifolia satva showed significant cytotoxic potential properties against OECM-1 cell lines with the IC_{50} concentration at 148.18µg/ml.

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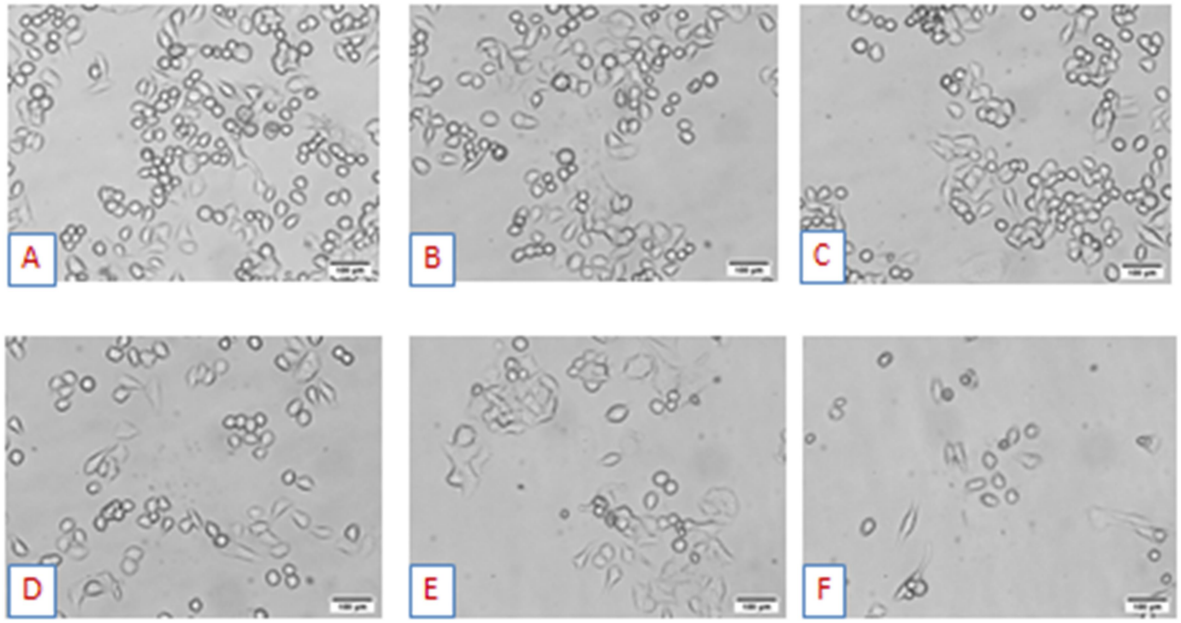
Table 1: Showing the mean absorbance, SD, SE and percentage of cell viability of different concentrations of Tinospora Cordifolia satva and controls.

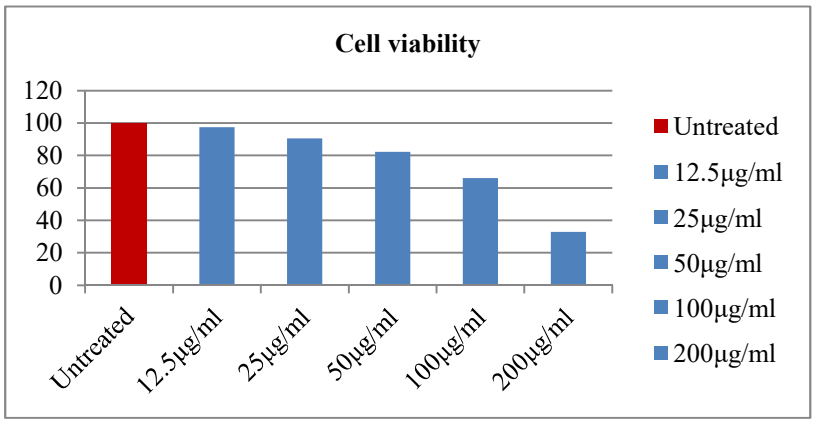
Sample concentration	Untreated	Tinospora Cordifolia satva concentrations (µg/ml)				
		12.5	25	50	100	200
Mean Absorbance	0.9735	0.949	0.8845	0.8085	0.6565	0.3485
Standard deviation	0.01626	0.0028	0.0120	0.009	0.009	0.054
Standard error	0.0115	0.002	0.0085	0.0065	0.0065	0.0385
Cell Viability %	100	97.36	90.44	82.28	65.96	32.90

Figure 1: Showing the change in the OSCC cell viability and OSCC cell inhibition with increase in the concentration of Tinospora Cordifolia Satva.



(A- Untreated; B- 12.5µg/ml; C- 25µg/ml; D- 50µg/ml; E 100µg/ml and F- 200µg/ml)

Figure 2: Bar graph showing the percentage of OSCC cell viability values of Tinospora Cordifolia satva on the OECM-1 cell lines by MTT study.



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Discussion:

Tinospora Cordifolia is commonly used plant in ayurveda and it's satva is an ayurvedic preparation in the form of aqueous extract and called starch of Tinospora Cordifolia which is an active part of the stem. Present days medical research is aimed to discover new anticancer agents as the cancer is one of the most common health issues that causes highest number of deaths globally. The synthetic drugs cause more side effects so, the natural plant-based drug discovery plays a crucial role in the development of anticancer drugs. The presents study was aimed to find the anticancer effect of Tinospora Cordifolia satva on the OSCC cell line

In the present study, the anti-cancer activity of Tinospora Cordifolia satva was evaluated by treating the OSCC cell line with 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml and found that it effectively inhibited proliferation of cells and proved that it has anticancer activity as its least dose 12.5µg also showed antiproliferative activity. The anticancer activity of Tinospora Cordifolia satva was observed to be in dose dependent manner as the dose was increasing from 12.5 µg/ml to 200 µg/ml the cell viability was reducing significantly with the IC_{50} value 148 µg/ml. These results were comparable to those of Shankar Gouda Patil et al., who found that AW13516 oral cancer cells exhibited efficient, dose-dependent apoptosis in response to Tinospora Cordifolia stem extract.[11] In another study by Parveen Bansal et al., Tinospora Cordifolia effectively arrested the cell cycle in the KB cell line of oral cancer, however, they did not specify which portion of the plant extract was utilized.[12] According to Rumana Ahmad et al., a cytotoxic effect of the methanolic extract of the stem of Tinospora Cordifolia was observed on a breast cancer cell line.[13] As of now, no literature was available on the anticancer activity of Tinospora Cordifolia satva which is a traditional ayurvedic preparation of aqueous extract starch.

Conclusion:

Tinospora Cordifolia stem extract satva effectively inhibits cell proliferation in a dose dependent manner in Oral squamous cell carcinoma cell line. The least dose used in the presents study 12.5 µg/ml was also inhibited cell proliferation and showed anticancer activity. Thus, Tinospora Cordifolia extract can be used considered in the treatment of oral squamous cell carcinoma and further clinical research studies can be carried out to fix the dose in the humans.

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