# Tinospora Cordifolia induces Cell Cycle arrest in Human Oral Squamous Cell Carcinoma Cells

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Original Article

# Tinospora Cordifolia Induces Cell Cycle Arrest in Human Oral Squamous Cell Carcinoma Cells

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## Abstract

Natural products with medicinal value are gradually gaining importance in clinical research due to their well–known property of no side effects as compared to drugs. Tinospora cordifolia (Guduchi) has been used for centuries in Ayurvedic system of medicine for treating various ailments including cancer. In present study, we found that the Tinospora cordifolia extracts (TCE) induced inhibition of proliferation of KB cells was associated with arrest of G0/G1–phase of cell cycle. The effectiveness of TCE in checking the growth of KB cells without altering the growth of normal peripheral blood mononuclear cells (PBMC) indicates that Tinospora cordifolia has differential effect on normal and malignant cells hence, it may have therapeutic potential in cancer.

**Key words:** Tinospora cordifolia, cell cycle, antiproliferative, Oral squamous cell carcinoma.

## Introduction

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries [(1)]. Cancer of the oral cavity ranks from the sixth to eighth most common cancer around the world, with a great variation in incidence among different countries [(2)]. Oral cancer is a common disease in Asian countries and oral squamous cell carcinoma (OSCC) is the most common in Indian males [(3)]. It is well known that OSCC is resistant to cancer chemotherapy mediated apoptosis. Hence often chemotherapy protocol has a limited role in eradication of the disease [(4)].

Out of 121 prescription drugs being used for cancer treatment, 90 have been derived from plant species and 74% of these drugs were discovered by investigating a folklore claim [(5,6)]. A significant emphasis has been given towards the research on complementary and alternative system of medicine that deals with cancer management. Several studies have been carried out on herbs under a multitude of ethno botanical grounds. Hartwell [(7–9)] and Pandey [(10)] has collected data on approximately 3000 plants, those of which possess anticancer activity and subsequently been used as potent anticancer drugs.

Tinospora cordifolia commonly known "Guduchi" is an important drug of Indian Systems of Medicine (ISM) and used in medicines since times immemorial. Many previous studies have provided evidence for the presence of immunomodulator and antioxidant activities in this plant [(10–15)]. Few reports have also demonstrated a potent anticancer activity exerted by the plant extract on different types of cancer [(16–18)]. Some reports demonstrate involvement of immune system [(19–22)] whereas others have shown role of the extracts on radiation induced sensitivity [(23–26)]. However none of these studies demonstrated the mechanism of action and active component of Tinospora cordifolia. Therefore present study was carried out to evaluate the antiproliferative and apoptotic activity of Tinospora cordifolia extracts on malignant oral squamous carcinoma cells.

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## Materials and methods

The present study was conducted at Department of Biotechnology at All India Institute of Medical Sciences (AIIMS), New Delhi. An oral squamous cell carcinoma cell line (KB) was procured from National Center for Cell Sciences (NCSS), Pune, India. The KB cancer cell lines were cultured as monolayers in Dulbecco's modified eagle medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (FCS), 100µg/ml penicillin, and 100µg/ml streptomycin, and maintained in an incubator with a humidified atmosphere of 95% air and 5% CO2 at 37°C. The drug Tinospora cordifolia extract (TCE) was obtained from a standard ayurvedic pharmacy. A stock solution of the drug was prepared in plain medium (DMEM) and desired concentrations (TCE1, TCE2, TCE3 with 25, 50, 75µg/ml respectively) were drawn from time to time. Methotrexate with a concentration of (40µmol/L) was used as positive control.

### Cell cycle analysis by flow cytometry

Subconfluent cells were treated with TCE (25, 50, and 75µg/ml) in culture medium as described above for 24, 48, 96 and 120 hours. The cells were then harvested, washed with cold phosphate buffered saline (PBS), and processed for cell cycle analysis. Briefly, 1x105 cells were resuspended in 200µL of cold PBS, to which cold ethanol (4 ml) was added and the cells were then incubated for 1 hour at 4°C. After centrifugation, the pellet was washed with cold PBS, suspended in 200µL staining buffer containing PBS, RNase (20µg/ml final concentration) and propidium iodide (50µg/ml final concentration) and incubated for 1 hour in the dark. The cell cycle distribution of the cells of each sample was then determined by fluorescent activated cell sorting (FACS) using a BD LSR II Flow Cytometer (BD Biosciences). Modfit LT cell cycle analysis software was used to determine the accurate percentage of cells in the different phases of the cell cycle.

## Results

### TCE induces G1–phase cell cycle arrest in KB cells

Based on the preliminary assays in which we found a significant growth inhibitory effect of TCE on KB cells, we determined the possible inhibitory effect of TCE on cell cycle progression. Treatment of KB cells with TCE for 24 hours resulted in a significantly higher number of cells in the G1 phase at the concentrations used, 25 µg/ml (23.7%) (P=0.03), 50µg/ml (43.1%) (p<0.001), and 75µg/ml (35.9%) (p<0.001) compared with non TCE treated control (11.5%) and 8.6% in methotrexate (40µmol/ml) treated KB cells (Fig.1). With each concentration, there was a concomitant reduction in the number of cells in the S phase and significant reduction in TCE1 (48.5%) (p <0.001), TCE2 (27.3%) (p<0.001) and TCE3 (43.6%) (p<0.001) of G2–M phases as compared to control group (70%), these results suggested that TCE induces G1–phase cell cycle arrest in KB cells. Treatment of KB cells with TCE for 48 hours also resulted in an increase in number of cells in the G1 phase at the concentrations used 50µg/ml (10.1%), and 75µg/ml (13.8%) compared

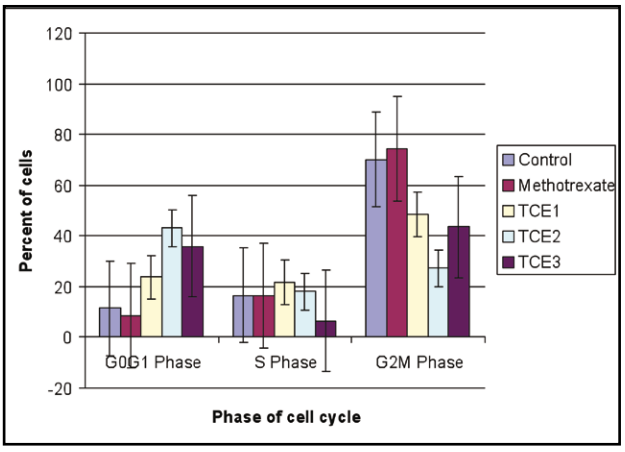


**Fig. 1. Effect of different concentrations of drug on cell cycle parameters at 24 hrs.**

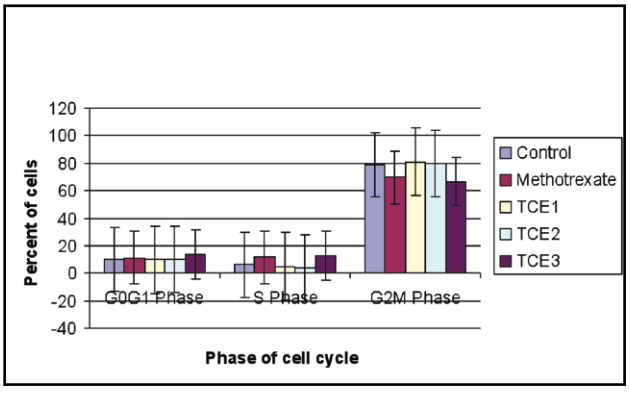


**Fig. 2. Effect of different concentrations of drug on cell cycle parameters at 48 hrs.**

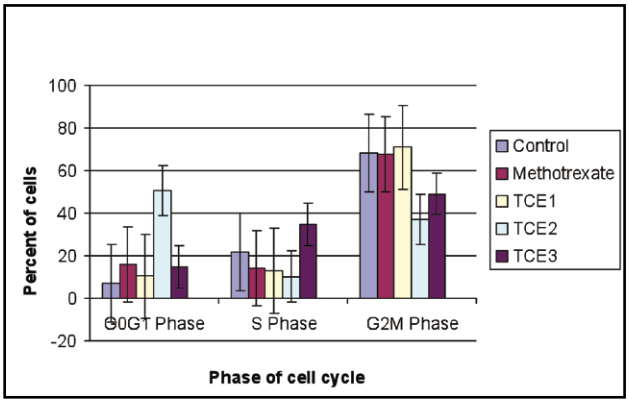


**Fig. 3. Effect of different concentrations of drug on cell cycle parameters at 72 hrs.**

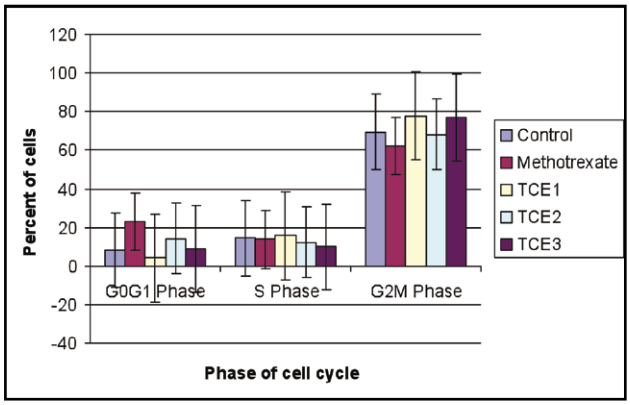


**Fig. 4. Effect of different concentrations of drug on cell cycle parameters at 96 hrs.**

with non TCE treated control (10.0%) and 11.3% in methotrexate (40µmol/ml) treated KB cells, however at 25µg/ml concentration number of cells was 9.7%, which was almost comparable to control (10.0%) (Fig. 2). Similarly a significantly higher number of cells were obtained in the G1 phase at all the concentrations used when the effect of TCE on KB cells was determined at 96 and 120 hours (Fig. 3 & 4).

## Discussion

The evaluation of traditional herbal medicines may indicate novel strategies for the treatment of cancer, which remains the leading cause of cancer related deaths all over the world. In present study, we show that naturally occurring Tinospora cordifolia extracts significantly inhibits the proliferation and reduces the viability of KB cells, which suggests that it may be an effective chemotherapeutic agent against oral cancer cells. Importantly, we found that TCE did not exhibit toxicity to PBM cells under the conditions used, except for a moderate reduction in cell viability at higher concentrations (250µg/ml) when cells were treated in vitro for an extended period of time (120 hrs). There was a decline in the number of total cells, similar to the results of Jagetia et al. [27] and Thippeswamy et al. [28] who have shown a dose dependent decline in the clonogenicity of Ehrlich ascites tumor (EAT) cells after treatment with different concentrations of Tinospora cordifolia. Several recent studies have reported that various extracts of Tinospora cordifolia plant possess bioactive components which inhibit cellular proliferation in various in vitro models and also show antineoplastic [18], antitumor [11,28,29], anti–angiogenesis [28,30] and anti–metastatic activity in various in vivo models [18,29,30].

Control of cell cycle progression in cancer cells is considered to be a potentially effective strategy for the control of tumor growth [31,32] as the molecular analyses of human cancers have revealed that cell cycle regulators are frequently mutated in most common malignancies [33,34]. Our in vitro data indicated that treatment of KB cells with TCE resulted in significant G1–phase arrest of cell cycle progression, which indicates that one of the mechanisms by which TCE may act to inhibit the proliferation of cancer cells is inhibition of cell cycle progression. The results of this study are similar to the results obtained by Thippeswamy et al. [28] in which TcHf (Hexane extracts of Tinospora cordifolia) decreased cell number and inhibited the proliferation of Ehrlich ascites tumor (EAT) cells by blocking cell cycle progression in the G1 phase. G1–phase arrest of cell cycle progression provides an opportunity for cells to either undergo repair mechanisms or follow the apoptotic pathway. Similarly berberine, a naturally occurring isoquinoline alkaloid, produced by Tinospora cordifolia, has been shown to exert potent anti–inflammatory and antitumor effects in in vitro as well as in vivo systems. Berberine induced antiproliferative effects against prostate carcinoma cells was associated with G1–phase arrest that correlated with inhibition of expression of cyclins D1, D2 and E; cyclin dependent kinases (Cdk 2, Cdk4 and Cdk6) and increased expression of Cdk inhibitory proteins p21Cip1 and p27Kip1 [16]. A number of mechanisms including alteration of antioxidant activity, expression of some apoptotic proteins and immune status linked parameters have been attributed to antiproliferative effects of TCE. But from the results of present study, it seems that Tinospora cordifolia, exerts its antiproliferative activity by arresting the G1–phase of cell cycle.

In conclusion results of present study indicate that a naturally occurring Tinospora cordifolia extracts significantly inhibit the proliferation and reduces the viability of KB cells, which suggests that TCE may be an effective chemotherapeutic candidate for human carcinomas.

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