

EFFECT ON CANCER CELL GROWTH BY METHANOLIC EXTRACT OF *GORDONIA OBTUSA* WALL. EXUT AND ARN, AN ENDEMIC WILD TEA PLANT

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ABSTRACT

The methanolic extract of *Gordonia obtusa* (Theaceae) was evaluated for its effects on growth in two malignant cell lines including a Human Lung cancer cell lines (A549) and Colon cancer cell lines (HT-29) using MTT assay. In these cell lines studied, the extract decreased cell viability, inhibited cell proliferation, and induced cell death in a dose dependent manner. The ethanolic extract of *G. obtusa* showed good cytotoxicity and their IC₅₀ values were found to be 35.12 µg/mL and 6.65 µg/mL respectively. It could be a reliable source of potent pharmacophore for treatment of disease like cancer.

KEY WORDS: *Gordonia obtusa*, Colon cancer, Lung cancer.

INTRODUCTION

Cancer is a deadly disease and affects a considerable number of people worldwide. Because of the complexity of human cancer, alternative management may be needed to improve the efficacy of therapeutic treatments and the quality of patients^[1]. Natural products have long been a fertile source to cure the cancer. There are different anticancer herbs from plants that have been used by different cultures throughout time for medicinal purposes^[2]. Consumption of tea has been associated with many health benefits, and tea's role and mechanism in cancer chemoprevention have been extensively reviewed and one of the key advantages of GT as a cancer preventative is its nontoxicity^[3]. *Gordonia obtusa* wall. exut and Arn is a wild tea plant in the tea family, Theaceae. *Gordonia* is a genus of flowering plants in the family

Theaceae, of the roughly 40 Sps. all two are native to South east Asia in Southern China, Taiwan. The remaining species are native to South East North America. They are evergreen trees growing to 10-20 M tall. The bark is thick and deeply fissured. The leaves are alternatively arranged, simple, serrated, thick, leathery, glossy and 6-18 cm. long. The flowers are large and conspicuous. The fruit is dry 5-valved capsule with 1-4 sees in each section. They have inflammatory, antioxidant medicinal properties.

MATERIALS AND METHOD

Cytotoxicity Study: Cell line used, Cell line and culture.

Human Lung cancer (A549) and Human colon adenocarcinoma (HT-29) cell lines were obtained from National centre for Cell Sciences, Pune (NCCS). The cells were maintained in RPMI-1640 supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO₂ at 37 °C.

Cell proliferation kit: MTT assay kit.

Reagents

RPMI-1640 was purchased from GIBCO/BRL Invitrogen (Caithershurg, MD). Fetal bovine serum (FBS) was purchased from Gibco laboratories Trypsin, methylthiazolyl diphenyl-tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from Sisco research laboratory chemicals, Mumbai. All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

Glass wares and plastic wares

96-well micro titer plate, Tissue culture flasks, Falcon tubes, Reagent bottles

Equipments

Fluorescence inverted microscope (Leica DM IL), Biosafety cabinet class II (Esco), cytotoxic safety cabinet (Esco), CO₂ incubator (RS Biotech, mini galaxy A), Deep freezer, ELISA plate reader (Thermo), Micropipettes

Preparation of plant extract

The plant *Gordonia obtusa* was collected from Nilgiris and authenticated by Dr. M. Murugesan , Scientist, SACON, Coimbatore, Tamilnadu. Accurately weighed and dried in lab condition. The dried materials were then ground ed to powder.10 mg. of air dried powder was taken in 100 ml. of organic solvent (methanol) in a flask, plugged with cotton wool and

kept on a rotary and shaken for 2 days. Then filtered and supernatant was collected and evaporated, and made the final volume one-fourth of the original volume and stored in air tight bottles^[4].

Microculture tetrazolium (MTT) assay principle

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is an indication of the viability of the cells.

Procedure *In vitro* assay for Cytotoxicity activity (MTT assay).

The Cytotoxicity study of samples on cancer cells was determined by the MTT assay^[5]. Cells (1×10^5 /well) were plated in 100 μ l of medium/well in 96-well plates (Hi media). After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20 μ l/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide cells(MTT) phosphate- buffered saline solution was added. After 4h incubation, 0.04M HCl/ isopropanol was added. Viable cells were determined by the absorbance at 570nm with reference at 655nm. Measurements were performed in 3 times, and the concentration required for a 50% inhibition of viability (IC₅₀) was determined graphically. The absorbance at 570 nm was measured with a microplate reader (Bio-Rad, Richmond,CA), using wells without sample containing cells as blanks. All experiments were performed in triplicate. The effect of the samples on the proliferation of human colon and lung cancer cells were expressed as the % cell viability, using the following formula: % cell viability = A₅₇₀ of treated cells / A₅₇₀ of control cells \times 100%.

RESULTS AND DISCUSSION

In vitro confirmation of this cytotoxicity of the *G. Obtusa* extract on Human Lung cancer cell lines (A549) and Colon cancer cell lines (HT-29) were reported. This extract was screened for its cytotoxicity against two cell lines at different concentrations and determined the IC₅₀ values (50% growth inhibition) by MTT assay. The present results are tabulated (Table 1).The dose dependent responses for the Human Lung cancer cell lines (A549) and Colon cancer cell lines (HT-29) are also graphically represented in the Figure 1. Upon

treating A 549 cancer cells and HT-29 cancer cells, the methanolic extract of *G. obtusa* shows good cytotoxicity and with an IC₅₀ value of 35.12 µg/mL and 6.65 µg/mL respectively.

Table 1: *In vitro* cytotoxicity effect of *Gordonia obtusa* extract on Colon and Lung cancer cell lines.

S.No	Concentration (µg/mL)	Colon Cancer (HT-29)	Lung cancer (A549)
1	250	21.56±0.44	5.84±0.35
2	125	35.59±0.68	8.63±0.37
3	62.5	42.59±0.37	14.40±1.09
4	31.25	51.29±0.58	24.43±0.64
5	15.625	59.29±0.30	34.40±0.64
6	7.8125	68.45±0.45	54.73±1.24
7	3.906	77.62±0.61	74.00±0.94
8	Cell control	100	100
IC₅₀		35.12 µg/ML	6.65 µg/mL

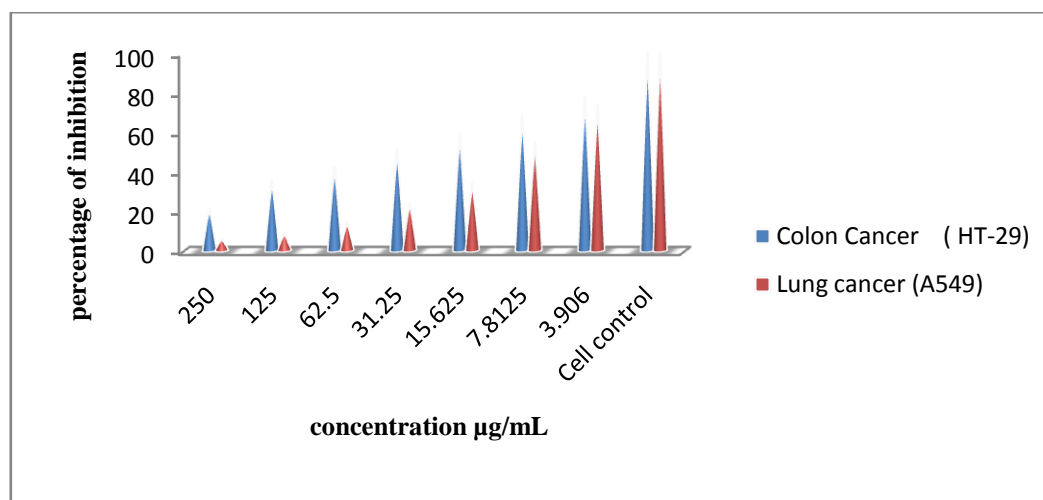


Figure 1: *In vitro* cytotoxicity of different concentrations of methanolic extract of *Gordonia obtusa*.

Green tea is rich in poly phenolic compounds called catechins, which accounts for the one - third of the dry leaves. GT consists of four different types of catechins: epigallocatechin-3-gallate(EGCG), epigallocatechin, epicatechin-3-gallate and epicatechin^[6]. EGCG is the most abundant and powerful antioxidant in green tea for cancer chemoprevention^[7]. The previous study showed that EGCG enhanced the effects of ginseng compounds in the inhibition of colon cancer cell growth, indicating that green tea could be an effective synergist with anticancer drugs for cancer chemoprevention^[8]. Tea catechins appear to inhibit human

melanoma cells; both EGC and EGCG suppressed growth of human melanoma cell line UACC-375^[9]. Studies investigating the association of green tea consumption with lung cancer risk have reported inconsistent findings. In support of green tea consumption on reduced lung cancer risk, a meta-analysis was conducted by a literature search in PubMed from 1966 to 2008. The overall evaluation of 22 relevant studies suggests that high consumption of green tea but not black tea may be related to the reduction of lung cancer risk^[10].

CONCLUSION

The role of plant derived polyphenols in chemoprevention of cancer is an emerged and important area. The study results showed that the methanolic extracts of *Gordonia obtusa* is promisingly cytotoxic against human colon cancer and human lung cancer cell lines. Therefore, purification of *G. obtusa* extract in future studies is suggested.

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