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An in-vitro evaluation of cytotoxic activity of *Wrightia tinctoria*

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ABSTRACT

The objective of the study was to analyze the anticancer property of the leaves of *Wrightia tinctoria* on HeLa Cells. Cancer has been estimated as the second leading cause of death in humans. So there has been an intense search on various biological sources to develop a novel anti-cancer drug to combat this disease. The anti-cytotoxic effect of methanolic extract was evaluated in-vitro by employing MTT assay. The potency of each plant extract concentration was calculated in terms of percent decrease in viable HeLa cells as compared to the control value. The extract showed dose dependent anticancer activity. The MTT assay showed an antiproliferative activity (IC_{50}) at 76.1 µg/ml of crude extract.

Keywords: Wrightia tinctoria, HeLa cell line, MTT assay, Cytotoxic, Methanolic extract

INTRODUCTION

Cancer is one of the leading causes of mortality worldwide. On a yearly basis in India, cervical cancer is a leading cancer among women with annual incidence of about 13000 cases and 70-75000[1]. As per WHO calculate that about 80% of the world's inhabitant's problem should treated by medicinal herbal drugs for their primary healthcare[2,3]**.** Most of the anticancer drugs currently used in chemotherapy are cytotoxic to normal cells and cause immuno-toxicity which affects not only tumor development, but also aggravates patient's recovery. The discovery and identification of new anticancer drug with low side effects on immune system has become an essential goal. With this aim, many attentions have been paid to natural compounds in plants, marine and microorganisms.

Plants are playing an important role as a source of anticancer drugs and the mechanism of interaction between many phytochemicals and cancer cells has been extensively. *Wrightia tinctoria* is a small deciduous, perennial tree. It is distributed in south India specifically in Annamalai and Kanyakumari regions of Tamilnadu. It is wildly available in subtropical and tropical areas of the world. Plant grows well near costal areas. Plant belongs to the family, Apocynaceae. Commonly known as dudhi in hindi and asita kutuj in Sanskrit. Leaves of the plant are green in color with acute apex and entire margin and flowers are white in color [4-5]. Ethanobotanical Survey of the *Wrightia tinctoria* plant reveals that it is used traditionally as hepatoprotective, antitumour, antifungal, antiepileptic antihypertensive, analgesic, antinflammatory, antidairrhoeal, colon protective and antidiabetic[6-10].

Wrightia tinctoria is a potential herbal alternative as anticancer agent and one of the active principles reported to be responsible for this action is β-amyrin[11-12]. A HeLa cell is an immortal cell line used

in medical research. The cell line was derived from cervical cancer cell taken from Henrietta Lacks, who died from her cancer in 1951. Initially, the cell line was said to be named after a "Helen Lane" in order to pressure lack's anonymity[13].

This study was aimed to evaluate anti-cytotoxic activity of different concentrations of plant extract on HeLa cell lines (immortal cell lines) in MTT assay protocols as per the standard guidelines.

MATERIAL AND METHODS

Reagents

Trypan blue, MTT roche applied science, cat no. 11, 465007
DPBS (Dulbecoo's phosphate buffer saline) SRB dye, MTT salt
DMEM (Dulbecoo's Modified Eagels medium)
FBS (Fetal Bovine Serum)

Plant material collection and identification: The leaves of *Wrightia tinctoria* were collected from forest of Ghatigaon, Gwalior, Madhya Pradesh. Leaves of *Wrightia tinctoria* were identified at Maharaja College, Chattarpur, Madhya Pradesh.

Preparation of methanol extract: For preparation of methanol extract, 1000 g of fresh dried leaves were crushed thoroughly using mortar and pestle. The crushed leaves were completely exhausted by adding small quantities of methanol several times followed by filtration, to yield final volume of ten liter. The extract was filtered and concentrated to dryness under reduced pressure and controlled temperature (40^0C to 50^0C) in a rotary evaporator. 100mg of the extract was dissolved in 1ml of dimethyl sulphoxide (DMSO) to prepare stock solution (100mg/ml).

Pharmacological Studies

Antitumor activity screening: Cell line used: Cervical cancer cell line (HeLa)

Microculture tetrazolium (MTT) assay: This colorimetric assay is based on the capacity of mitochondria succinate dehydrogenase enzyme 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[14,15].

Procedure: The monolayer cell culture was trypsinized and the cell count was adjusted from 2.5 to 4 lake cells/ml using medium containing 10% new born calf serum. To each well of 96 well microtitre plates, 0.1 ml of diluted cell suspension was added.

After 24 hours, when the monolayer formed the supernatant was flicked off and different concentrations of test compound were added to the cell in microtitre plates. The stock solution of 100µg/ml was made by using solvent DMEM (Dulbecoos modified Eagles medium) and DMSO (Dimethyl Sulphoxide). Then the different concentrations of stock solution were made by serial dilution with DMEM (Dulbecoos modified Eagles medium). The final concentrations were 1.23µg/ml, 3.7 µg/ml,11.11 µg/ml,33.33 µg/ml and 100 µg/ml. these concentrations were added to the cells in microtitre plates and kept for incubation at 37^0C in 5% CO_2 incubator for 72 hours and cells were periodically checked for granularity, shrinkage and swelling.

After 72 hours, the sample solution in wells was flicked off and 50µl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37^0C in 5% CO_2 incubator. The supernatant was removed, after that 50µl of Propanol was added and plates were gently shaken to solubilize the formed formazan.
The absorbance was measured using a microplate reader at a wavelength of 550nm[16].

The percentage growth inhibition was calculated using the formula below
% Cell inhibition= 100{(At-Ab)/(Ac-Ab)}×100
Where
At= Absorbance value of test compound
Ab= Absorbance value of blank
Ac= Absorbance value of control
% Cell inhibition= 100-Cell survival

Data interpretation: Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely, a higher absorbance rate indicates an increase in cell proliferation. Rarely, an increase in proliferation may be offset by cell deaths. Evidence of cell death may be inferred from morphological changes

RESULTS AND DISCUSSION

The effect of methanolic extract of Wrightia tinctoria plant on the growth of the HeLa cell line was examined by MTT assay. The extract was screened for its cytotoxicity activity at different concentrations to determine the IC50 (50% growth inhibition) value.
A chart was plotted using the % cell inhibition in Y axis and concentration of the plant extract in X axis. Cell control was included in each assay to compare the full cell inhibition in cytotoxicity and antitumor activity assessments. The results are tabulated in

table 1 and figure 1 and 2. When HeLa cells were treated with the methanolic extract of leaves of Wrightia tinctoria, there was a concentration dependent cytotoxicity effect. As the concentration increased from 1.23-100µg/ml, percentage of inhibition increases from 6.93%-47.53%. The IC_{50} value was found to be 71.6µg/ml from the non-linear regression equation.

Table1: The inhibition pattern against HeLa cell line at different concentrations.

S.No.	Concentration (µg/ml)	Viability (%)	Inhibition (%)
1	Control	100	0
2	1.23	93.07	6.93
3	3.7	83.89	16.11
4	11.11	73.40	26.6
5	33.33	66.66	33.33
6	100	52.43	47.57

+All experiment are triplicates (n=3): mean±SD, *P>0.05 when test group compared with standard.

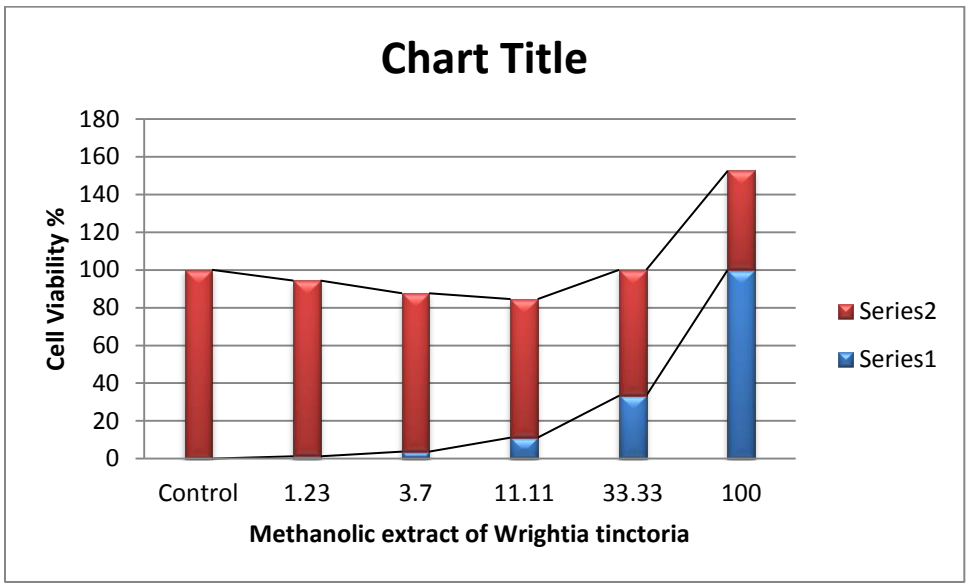

Figure1: Percentage Viability of HeLa cell growth at various concentrations of methanolic extract of *Wrightia tinctoria*

Traditionally many medicinal plants, which possess the ability to prevent and even to stall the progress of cancer, were in use. Plants possess certain chemicals, which have the ability to modify the physiological function of cells and hence act as anticancer drugs to arrest the proliferation of cancer cells. The mode of action of the drugs is unknown but successfully integrating our documented knowledge of plant properties and modern technological tools, effective anti-cancer drugs can be derived from plant sources and their mechanism can be elucidated [17-18]. The present need is to develop drugs that can potentially target cancer cells by means of their inherent differences to normal cells. The development of such drugs with differential action will be very valuable in cancer chemotherapy without the observed side effects. The methodology involves use of cancer cells lines to test the efficacy of the plant extracts in vitro.

Conclusion

The potential use of *Wrightia tinctoria* as therapeutic agent holds great promise as the isolation of one or more cytotoxic chemicals from crude extract and the judicious use of such chemicals can control the progression of cancer and also can prevent the formation of tumor in individuals who are highly susceptible to developing a tumor.

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Conflict of interest: Conflict of interest declared none

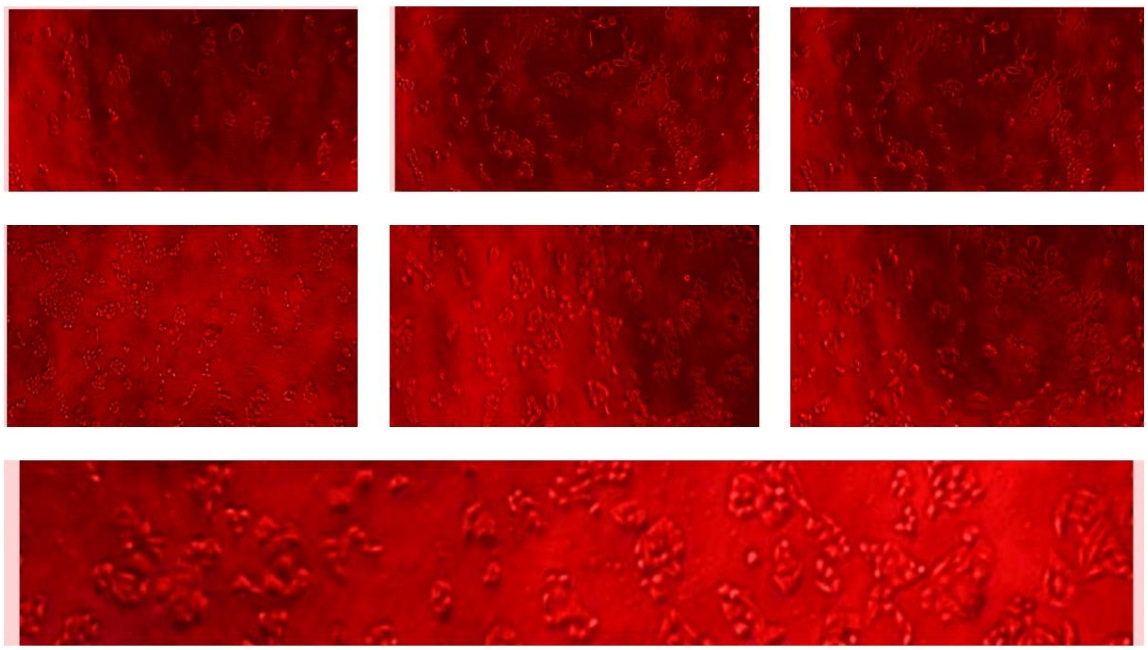

Figure 2: Combined Inhibitory effects of methanolic extract on HeLa cells

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