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RESEARCH ARTICLE

# ANTITUMOR ACTIVITY OF METHANOLIC EXTRACT OF *VERNONIACINEREA*LESS BY HUMAN CELL LINES

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**ABSTRACT**

*Vernoniacinerea* (L.) Less a member of family compositae (Asteraceae), is an important medicinal plant has already been in used as antibacterial, Analgesic, antipyretic, anti-inflammatory preparation. In this study an attempt of the plant has been made to evaluate the anticancer activity of the methanolic extract of the plant, as stated in many hypothesis. Themethanolic extract of leaves of *Vernoniacinerea*L. Were screened for their anticancer activity by cell *line*studies was carried out by two methods Tetrazolium salt assay and Tryphan blue dye exclusion method. In MTT Assay the total growth inhibition (TGI) of methanol extract was found to be >10 mg/ml on both cell lines (HEp 2 and HT29). The relative cell survival progressively decreased in dose dependant manner. In addition to that, Short term Cytotoxicity studies by Tryphan Blue exclusion method also confirmed the anticancer activity of vernoniacinerea L. (1mg/ml showed 77% of Cytotoxicity inhibition). Our studies point the possibility of developing *Vernoniacinerea* L. as a novel potential agent in the area of cancer chemotherapy. Further investigation has to be carried out in isolation and characterization of active constituents and the mechanism involving in antitumor and cytotoxic effect.



## INTRODUCTION

Over 60% of currently used anticancer agents are derived in one way or another from natural sources. Scientific literature is the collection of research information and as such, serves as the reservoir of knowledge about a subject [1][2]. As the scientific literature of information should occur, helping to advance the science of these plants. [3][5][6][7] Hartwell, in his review of plants used against cancer, lists more than 3000 plant species that have reportedly been used in the treatment of cancer.

The search for anti-cancer agents from plant sources started in earnest in the 1950s with the discovery and development of the vinca alkaloids, vinblastine and vincristine, and the isolation of the cytotoxic podophyllotoxins [6] [7] [13] [14]. *Vernoniacinerea* (L.) Less a member of family compositae (Asteraceae), is an important medicinal plant found throughout the Indo-Malaysia region, tropical Africa, Australia and New Zealand. It is the one of the most common weeds in India, found throughout the country to an altitude of 2500m in the Himalayas. Iwalewa E O, *et al.,*[15] has reported an analgesic, anti-inflammatory, antipyretic and behavioral activity from the chloroform, methanolic and ether extracts of *Vernoniacinerea* Less leaf. Mazumder U K, *et al.,*[16] has reported anti-inflammatory activity from the methanolic extract of the whole plant of *Vernoniacinerea* Less. Malaya Gupta, *et al.,*[17] has reported antibacterial activity from The benzene extract of *Vernoniacinerea* Less. Mary Latha R, *et al.,*[4] has reported the anti-inflammatory effect of an alcoholic extract from the flower of *Vernoniacinerea* Less was tested in adjuvant arthritic rats. MamtaTandon, *et al.,*[18]Insect Antifeed ant Principles from *Vernoniacinerea* Less. Adeboye J O, *et al.,*[19] proposed a study on diuretic and anti-diuretic potency of the leaf extracts of *Vernoniacinerea* in albino rats. Amritpal Sing, *et al.,*[21] has reported anti-inflammatory activity from the alcoholic extract of the flower of Vernoniacinerea Less. Sotheara Hout, *et al.,*[22] The plant extracts were tested for in vitro Antiplasmodialactivity against a chloroquine resistant *Plasmodium falciparum* strain.Yuan-Chuen W, *et al.,*[23] has reported that plant posses anti-Helicobacter pylori activity in *Vernoniacinerea* Less. Cheeptham N, *et al.,*[25] has reported Antimicrobial activity from the leaf of *Vernoniacinerea* Less shows Light-mediated activity tested against *Bacillus subtilis*, *Staphylococcus aureus* K147 methicillin-sensitive (Ms), *Escherichia coli* DC10, *E. coli* (wild), *Pseudomonas aeruginosa*187 (wild), *Candidaalbicans* and *Aspergillusfumigates.* Henrik T S, *et al*., [26] has reported the ethanol extract of the whole plant of *Vernoniacinerea* Less. Shown antiplasmodial activity by *invitro* method. Valsaraj R, *et al.,*[27] has reported Antimicrobial screening of *Vernoniacinerea* Less of Different concentrations of 80% ethanol extracts were tested, using the agar dilution method, against four bacteria.

Cancer (medical term: malignant neoplasm) is a class of diseases in which a group of cells display uncontrolled growth (division beyond the normal limits), invasion (intrusion on

and destruction of adjacent tissues), and sometimes metastasis (spread to other locations in the body via lymph or blood).Cancer may affect people at all ages, causes about 13% of all human deaths. According to the American Cancer Society, 7.6 million people died from cancer in the world during 2007. The Main objective of this studies to find out the possibility of developing *Vernoniacinerea* L. as a novel potential agent in the area of cancer chemotherapy.

## MATERIAL AND METHODS

### *Collection and authentication of plant material*

The leaves of *Vernoniacinerea*Lesshas been procured from Kanyakumari district, Tamilnadu. The plant material wasidentified by botanist, Prof. Dr.S. Jayaraman, plant anatomy Research centre (PARC) chennai.

### *Preparation of plant extracts*

Freshly collected leaveswere washed, shade dried under room temperature for a period of three weeks. The dried plant material was made into a coarse powder. A weighed quantity of the powder (750g) was passed into sieve number 40 and subjected to hot solvent extraction in a soxhlet apparatus using methanol at a temperature range of 40-80$^0$c before and after every extraction the marc was completely dried and weighed. The filtrate was evaporated to dryness at 40$^0$c under reduced pressure in a rotary vacuum evaporator.

### *Antitumor study-Human cell lines*

### *Tryphan Blue Dye Exclusion Technique*

Tryphan Blue was a vital dye. The reactivity of Tryphan blue was based on the fact that thechromophorewas negatively charged and does not interact with the cell unless themembrane was damaged. Therefore, all the cells which exclude the dye are viable.

### *Procedure*

Place 0.5 ml of a suitable cell suspension (dilute cells in complete mediumwithout serum to an approximate concentration of 1 x 105 to 2 x 105 cells per ml) in a screw cap test tube. 0.1 ml of 0.4% Tryphan Blue Stain added and Mix thoroughly. This mixture is allowed to stand for 5mintues at15 to 30°C (room temperature). Fill this resulting mixture in a Haemocytometer for cell counting and observed under microscope to confirm staining and non-staining of non-viable and viable cells respectively

Cell count = Number of cells X Dilution / (Area X Thickness of fluid film)

Percentage of cell viability = (live cell count/total cell count) x 100

### *MTT-Assay*

MTT (3-(4, 5-dimethyl thiazolone 2-yl)-2, 5-diphenyl Tetrazolium bromide) measures the metabolic activity of the viable cells. The reaction between MTT and mitochondrial dehydrogenase produce water insoluble formazan salt. The method involves culturing the cells in a 96 well microtitreplate, and then incubates with MTT solution for 2hrs. During the period viable cells convert MTT into a water insoluble formazan dye and it can be colorimetrically detected at 595 nm. The absorbance directly correlates with the cell.

### *Procedure*

Cell lines at exponential growth phase were washed, trypsinized and re suspended in RPMI 1640 medium cells kept at a concentration of $10^5$cells/well in 96 well micro titer plates. The cells were treated with different concentration of tested drug (10, 5 and 1.25mg/ml), control which contain only the medium and incubated for 24hrs. MTTsolution was added to each well to make the final volume concentration of 400µg/ml and further incubated at37$^0$c incubate for 3hours.

The reaction resulted in the reduction of concentration MTT bythe mitochondrial dehydrogenase of viable cells to a purple formazan product. After 3 h, 150 µl of dimethylsulfoxide (DMSO) was added to all the wells to dissolve the formazan crystals and the optical density (OD) was measured at a wave length of 595 nm [2][5][9][12][37][38][39][40][41][42][43][44][45].

CELL VIABILITY (%) = Mean OD/Control OD x 100

OD = Optical Density.

## RESULTS

### *Antitumor studies by Human cell lines*

In the present study the Cytotoxicity of methanol extract of *Vernoniacinerea* L. using Human cancer cell line were evaluated with MTT assay. When the cells were treated for 72 hrs with various concentration of methanol extract (10mg-39µg/ml), the relative cell survival progressively decreased in dose dependantmanner.The total growth inhibition (TGI) of methanol extract was found to be >10 mg/ml on both cell line. The $LC_{50}$ of methanol extract was found to be > 625µg/ml for HEp 2 cell lines and >1.25mg/ml forHT29 Cell Lines. Based on Cytotoxicity results the extract produced potent cytotoxic effect on this Human cancer cell lines (Table 1).

### *Short Term Cytotoxicity studies*

Short term Cytotoxicity studies by Tryphan Blue exclusion method is a very simple method which can be carried out within a short time of 3hrs. It is a precise method, which takes in to account the viable and also the dead cells in addition to

**Table 1**Cytotoxicity effect of Vernoniacinerea L. on Human Cancer Cell Lines

| | | HEp 2 Cell Lines | | | HT29 Cell Lines | | |
|---|---|---|---|---|---|---|---|
| S.NO | Concentration (mg/ml) | Absorbance | % of cell viability | % of cell inhibition | Absorbance | % of cell viability | % of cell inhibition |
| 1. | 10 | 0.05 | 8.77 | 91.23 | 0.06 | 10.16 | 89.94 |
| 2. | 5 | 0.14 | 24.56 | 75.44 | 0.17 | 28.81 | 71.19 |
| 3. | 2.5 | 0.19 | 33.33 | 66.67 | 0.25 | 39.06 | 60.94 |
| 4. | 1.25 | 0.23 | 40.35 | 59.65 | 0.36 | 56.25 | 43.75 |
| 5. | 0.625 | 0.31 | 54.38 | 44.38 | 0.43 | 67.18 | 32.82 |
| 6. | 0.3125 | 0.35 | 61.40 | 38.6 | 0.49 | 76.56 | 23.44 |
| 7. | 0.156 | 0.42 | 73.68 | 26.32 | 0.55 | 85.93 | 14.07 |
| 8. | 0.078 | 0.47 | 82.45 | 17.55 | 0.58 | 90.62 | 9.38 |
| 9. | 0.039 | 0.52 | 91.22 | 8.78 | 0.63 | 98.43 | 1.57 |
| 10. | Cell control | 0.57 | 100 | - | 0.64 | 100 | - |

Estimation of $IC_{50}$ concentration. The $IC_{50}$ of MEVC was found to be > 500µg/ml against DAL (Table 2).

**Table 2** Short term Cytotoxicity Tryphan Blue Dye Exclusion technique

| DAL 1x10$^6$ cells | Drug conc. (µg) | % of Cytotoxicity |
|---|---|---|
| 1 | Control | - |
| 2 | 10 | 1 |
| 3 | 25 | 5 |
| 4 | 50 | 7 |
| 5 | 100 | 12 |
| 6 | 200 | 18 |
| 7 | 400 | 32 |
| 8 | 500 | 43 |
| 9 | 1000 | 77 |

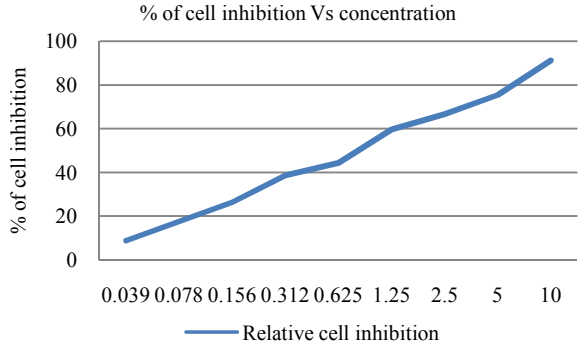



**Figure 1** on HEp 2 Cell Lines

Concentration mg/ml

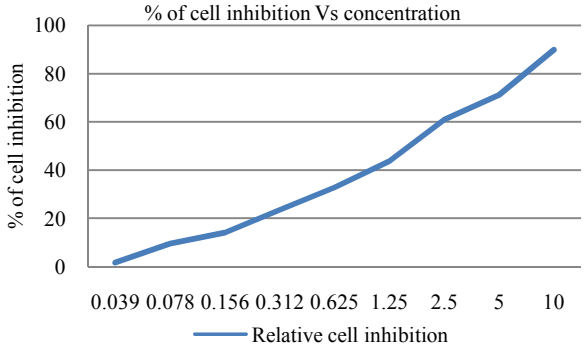
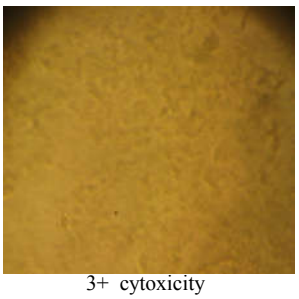
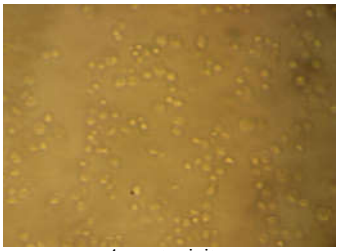



**Figure 2** on HT29 Cell Lines

Concentration mg/ml

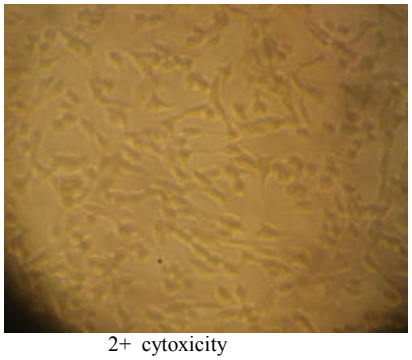


3+ cytoxicity

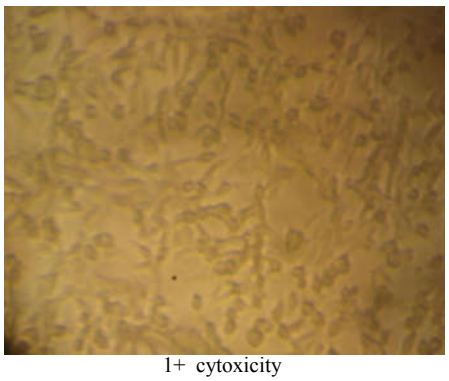


4+ cytoxicity

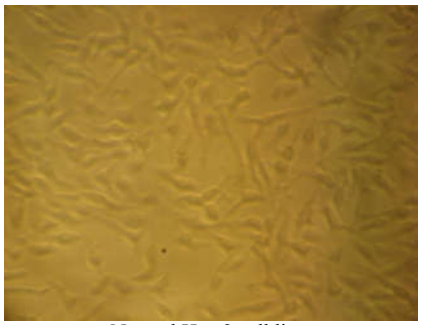
**Figure 3**



2+ cytoxicity



1+ cytoxicity



Normal Hep 2 cell line

**Figure 4**

| | |
|---|---|
| 1+ | For 25% dead cells |
| 2+ | For 50% dead cells |
| 3+ | For 75% dead cells |
| 4+ | For 100% dead cells |

## DISCUSSION

Cancer is basically a disease of cells characterized by a shift in the control mechanism that governs cell proliferation and differentiation. Cells that have undergone neoplastic transformation usually express cell surface antigens that appear to be normal foetal type and have other sign of apparent "immaturity" and may exhibit qualitative or quantitative chromosomal abnormalities, including various translocations and the appearance of amplified gene sequence. Such cells proliferate excessively and form local tumors that can compress or invade adjacent normal structures. Ideal characteristic of anticancer drug should eradicate cancer cells without harming normal tissues. Unfortunately, no current drug available agents meet this criterion and clinical use of this drug involves a weighing of benefits against toxicity in a search for favorable therapeutic index. Hence, the use of natural products now has been contempt of exceptional value in the control of cancer and its eradication programmer.

The results of the present study clearly demonstrate the tumor inhibitory activity of MEVC against DAL strain. The reliable

criteria for evaluating an anticancer drug are prolongation of lifespan of the animal and decrease in WBC count of bloodThe LC50 of methanol extract was found to be > 625µg/ml for HEp 2 cell lines and >1.25mg/ml for HT29 Cell Lines. Based on Cytotoxicity results the extract produced potent cytotoxic effect on this Human cancer cell lines.The IC50 of MEVC was found to be > 500µg/ml against DAL. These results clearly demonstrate the antitumour effect of MEVC against DAL. In cancer chemotherapy the major problems are of myelosuppression and anaemia [46][47]the anemia encountered in tumor bearing mice is mainly due to reduction in RBC and HB% and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions.[48][49][50].All these data point to the possibility of developing methanolic extract of *Vernoniacinerea*L. as a novel, potential agent in the area of cancer chemotherapy. Preliminary phytochemical screening indicated the presence of alkaloids and flavanoids in MEVC. Flavanoids, which have been shown to possess antimutagenic and anticarcinogenic activity.[51][52][53] Moreover, flavanoids have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation[54] and angiogenesis [55] The cytotoxic and antitumor properties of the extract may be due to these compounds.

## CONCLUSION

The present study points to the potential anticancer activity of *Vernoniacinerea*L. A further study to characterize the active principles and elucidate the mechanism of the action of MEVC is suggested.

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