**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][4][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.

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 study is 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 *Vernonia cinerea* Less has been procured from Kanyakumari district, Tamilnadu. The plant material was identified by a botanist, Prof. Dr. S. Jayaraman, plant anatomy Research Centre (PARC). Chennai.

**Preparation of plant extracts**

Freshly collected leaves were washed, shade dried under room temperature for a period of 3 weeks. The dried plant material was made into a coarse powder. A weighed quantity of the powder (750 g) 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°C before and after every extraction the marc was completely dried and weighed. The filtrate was evaporated to dryness at 40°C under reduced pressure in a rotary vacuum evaporator.

**Antitumor study- Human cell lines**

**Trypan Blue Dye Exclusion Technique**

Trypan Blue was a vital dye. The reactivity of Trypan blue was based on the fact that the chromophore was negatively charged and does not interact with the cell unless its membrane 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 medium without 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% Trypan Blue Stain added and mixed thoroughly. This mixture is allowed to stand for 5 minutes. This mixture is allowed to stand for 5 minutes. The mixture is then added to the cell suspension and observed under a microscope. The dye stains non-viable cells while viable cells remain unstained.

**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 microtitre plate, and then incubates with MTT solution for 2 hrs. 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 viability.

**RESULTS**

**Antitumor studies by Human cell lines**

In the present study the Cytotoxicity of methanol extract of *Vernonia cinerea* 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 a dose dependant manner. The total growth inhibition (TGI) of methanol extract was found to be >10 mg/ml on both cell lines. The 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 (Table 1).

**Short Term Cytotoxicity studies**

Short term Cytotoxicity studies by Trypan 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 viable and also the non-viable cells respectively.

**Table 1:** Cytotoxicity effect of *Vernonia cinerea* L. on Human Cancer Cell Lines

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

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Estimation of IC₅₀ concentration. The IC₅₀ of MEVC was found to be > 500µg/ml against DAL (Table 2).

Table 2: Short term Cytotoxicity Trypan Blue Dye Exclusion technique

<table>
<thead>
<tr>
<th>Drug conc. (µg)</th>
<th>% of Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>50</td>
<td>7</td>
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<td>100</td>
<td>12</td>
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<tr>
<td>200</td>
<td>18</td>
</tr>
<tr>
<td>400</td>
<td>32</td>
</tr>
<tr>
<td>500</td>
<td>43</td>
</tr>
<tr>
<td>1000</td>
<td>77</td>
</tr>
</tbody>
</table>

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 blood. The 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. 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. 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 antimitagenic and anticarcinogenic activity. Moreover, flavanoids have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis. 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.

Reference

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