In vitro Cytotoxic Potential of Calotropis gigantea R.Br. Against Human Cancer Cell Lines

MADHULIKA BHAGAT AND VIKAS SHARMA

ABSTRACT

The goal of this research work was to evaluate the in vitro cytotoxic potential of the extracts from the traditional medicinal plant i.e., Calotropis gigantea R.Br. The extracts were screened for in vitro cytotoxicity by means of SRB assay on seven human cancer cell lines: colon cancer cells (COLO 205, 502713), liver cancer cells (HEP-2), prostate cancer cells (DU-145), lung cancer cells (HOP-62), ovary cancer cells (OVCAR-5) and cervical cancer cells (SiHa). Ethanol extracts and aqueous ethanolic extract did not show significant activity against any of the cancer cell lines at the concentrations of 10µg/ml and 30µg/ml, but at the concentration of 100µg/ml, both the extracts were found active against every human cancer cell line. Aqueous extract was found active against all the human cancer cell lines at the concentration of 100µg/ml, surprisingly this extract showed cytotoxic effect on two human cancer cell lines, viz., 502713 and HEP-2 at the concentration of 10 and 30 µg/ml. These results suggest that the aqueous extract possesses more cytotoxicity against 502713 and HEP-2 than ethanolic extract and aqueous extract.

C alotropis gigantea R.Br belongs to Asdepiadaceae family, commonly known as milkweed or swallowwort is a common wasteland weed and most abundant in the subtropics/tropics and rare in cold countries (Singh et al., 1996). Calotropis gigantea is a traditional medical plant (Rastogi and Mehrotra, 1991) with unique properties (Oudhia and Tripathi, 1998) and is used alone or with other medicinal plants (Caius, 1986) to treat common diseases such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting and diarrhea (Das, 1996). The plant is also a reputed Homoeopathic drug (Ferrington, 1990). The powdered root is used in asthma, bronchitis, dyspepsia and leaves are useful in the treatment of paralysis, swellings, intermittent fevers (Warrier et al., 1996). The plant possesses anti-diarrhoeal activity (Chitme et al., 2004) and the latex of the plant is a rich source of useful components that has medicinal properties and one of the main applications is in controlling bleeding. The plant is considered crude drug of Bangladesh and new oxipregnane-oligoglycosides named Calotropis A and B have been isolated from the root of C. gigantea (Isao et al., 1992). Cardenoloids glycosides calotropin frugoside and 4-O-Beta-D-glucopyranosyl frugoside were also obtained as the cytotoxic principles from the root of C. gigantea (Kiuchi et al., 1998). This study attempts to determine the in vitro cytotoxic effect of Calotropis gigantea R.Br. root extract on human cancer cell lines from six different origins. The results would enable more rational exploitation of the plant in both traditional and orthodox medicine.

MATERIALS AND METHODS

The plant was collected from Pounichak region of Jammu district, Jammu J&K, India in the month of June and authentication was done by Dr Yashpal Sharma at the herbarium of the Botany Department, University of Jammu, Jammu. The collected plant material (root part) was chopped, shade dried and ground into powder. Powdered dried plant material was then extracted with different solvents at room temperature.

Preparation of plant extracts:

For the ethanol extract, dried and powdered plant material (100g) was percolated with 95% ethanol (500ml) and evaporated to dryness under reduced pressure. Hydroethanolic extract was prepared by percolating another lot of dried powdered plant material (100g) with 50% ethanol with water (500ml) and then concentrating it to dryness under reduced pressure. The hot water extract was obtained by boiling powdered plant material...
(100g) for 30 min in distilled water (300ml). Stock solutions 20µg/ml were obtained by dissolving ethanolic extract in Dimethyl sulfoxide (DMSO), the hydro-ethanolic extract in 50% DMSO and the hot water extract in sterile water. The microbial contamination was controlled by the addition of 1% gentamycin in complete growth medium i.e., used for dilution of stock solution to prepare working test solutions 200 µg/ml. All extracts being freeze dried.

Preparation of positive controls:
Positive controls were prepared in distilled water (5-flourouracil, Mitomycin-C) and DMSO (Taxol) then diluted in gentamycin medium to obtain desired concentrations of $1 \times 10^{-4}$ M and $1 \times 10^{-4}$ M.

Human cancer cell lines:
The human cancer cell lines were obtained from National center for cell science, Pune, India. The cell lines namely colon (COLO 205, 502713), lung (HOP-62), ovary (OVCAR-5) and cervix (SiHa) were grown and maintained in RPMI-1640 medium, pH 7.4, whereas DMEM was used for liver (HEP-2) and prostate (DU-145). The media were supplemented with FCS (10%), penicillin (100 units/ml), streptomycin (100 µg/ml) and glutamine (2mM).

In vitro cytotoxic assay against human cancer cell lines:
Test material (extracts) was subjected to in vitro anticancer activity against various human cancer cell lines (Monks et al., 1991). For the assay (in brief) the cells were grown in tissue culture flask in growth medium at 37°C in an atmosphere of 5% CO$_2$ and 90% relative humidity, in a CO$_2$ incubator. The cells at subconfluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in Phosphate Buffer Saline containing 0.02% Ethylene diamine tetra acetic acid) and suspended in growth medium. Cells with more than 97% viability (Trypan blue exclusion) were used for determination of cytotoxicity. An aliquot of 100µl of cells (10’cells/ml) was transferred to a well 96 well tissue culture plate. The cells were allowed to grow for 24 h. Test material was then added to the wells and cells were further allowed to grow for another 48 h.

Sulphorhodamine B (SRB) assay:
The antiproliferative SRB assay was performed to assess growth inhibition which estimates cell number indirectly by staining total cellular protein with the dye SRB (Skehan et al., 1990). In brief, the cell growth was stopped by gently layering 50µl of 50% (ice cold) tri-chloro acetic acid (TCA) on the top of growth medium in all the wells. The plates were incubated at 4°C for an hour to fix the cells attached to the bottom of the wells. Liquid of all the wells were then gently pipetted out and discarded. The plates were washed five times with distilled water and air dried. SRB 100µl (0.4% in 1% acetic acid) was added to each well and the plates were incubated at room temperature for 30 min. Excess dye was removed by washing with 1% acetic acid and the bound dye was dissolved in tris buffer (100 µl, 0.01 M, pH 10.4). Plates were gently stirred on a mechanical shaker for 5 min and the optical density was recorded at 540nm. Suitable blanks and positive controls were also included. Each test was done in triplicate and the values reported herein are mean values of three experiments.

Calculations:
The cell growth was determined by subtracting average Absorbance (OD) value of respective blank from the average Absorbance (OD) value of experimental set.
Per cent growth in the presence of test material was calculated as under:

$$\text{Growth in the presence of test material} \times 100 \over \text{Growth in the absence of test material}$$

Per cent growth inhibition in the presence of test material was calculated as under:

100 – per cent growth in the presence of test material.
Criteria for activity:
The growth inhibition of 70% or above at 100µg/ml, 50% or above at 30µg/ml and 30% or above at 10µg/ml or above was considered active.

RESULTS AND DISCUSSION
In the first phase of experiment, ethanolic extract from the root part of *Calotrops gigantea* R.Br. was investigated at the concentration of 10, 30 and 100 µg/ml against seven human cancer cell lines from colon, liver, prostate, lung, ovary and cervical origin. Results showed that at the concentration of 10 and 30µg/ml, the extract showed no cytotoxic effect against any of the cancer cell lines. However, at the concentration of 100µg/ml, the per cent growth inhibition was observed in the range of 70-78% on OVCAR-5, HOP-62, COLO 205, SiHa , DU-145, 502713 and HEP-2.

In the second phase of experiment, aqueous ethanolic extract from the same plant was evaluated against same human cancer cell lines at the concentration of 10, 30 and 100 µg/ml. In this phase, the extract showed
In vitro CYTOTOXIC POTENTIAL OF Calotropis gigantea R.BR. AGAINST HUMAN CANCER CELL LINES

Table 1: In vitro cytotoxic effect of the extracts of the root part of Calotropis gigantea on human cancer cell lines with appropriate positive controls

<table>
<thead>
<tr>
<th>Generic name of the plant</th>
<th>Part used</th>
<th>Extract</th>
<th>Conc. (µg/mL)</th>
<th>Growth Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calotropis gigantea</td>
<td>Root</td>
<td>Ethanolic</td>
<td>10</td>
<td>07 00 01 07 01 02 04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td>09 00 03 11 04 03 09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>71 76 78 75 70 70 71</td>
</tr>
<tr>
<td></td>
<td>Aqueous ethanolic</td>
<td></td>
<td>10</td>
<td>01 00 01 03 00 00 00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td>03 00 07 11 00 00 00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>73 77 78 79 71 70 71</td>
</tr>
<tr>
<td></td>
<td>Aqueous</td>
<td></td>
<td>10</td>
<td>04 56 50 04 03 09 02</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td>12 78 84 07 05 11 05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>70 81 88 73 71 70 72</td>
</tr>
<tr>
<td>Positive controls</td>
<td>Conc. µM</td>
<td>5 - Flurouracil</td>
<td>1×10⁻⁴</td>
<td>81 74 - - - - 62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1×10⁻⁴</td>
<td>- - - - 86 73 -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1×10⁻⁴</td>
<td>- - 76 71 - -</td>
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</table>

Acknowledgment:
Authors are thankful to Indian Institute of Integrative Medicine (IIIM) for providing constant support.

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REFERENCES


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