Anti-cancer Activity of Arka (Calotropis procera) on HCT-15 Cancer Cell Line

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ABSTRACT

In vitro assay for cytotoxic activity of the stem-leaves of Calotropis procera was carried out against human cancer cell lines at the concentration of 10, 30 and 100 μg/ml. Results revealed that the extracts of the plant possessed in vitro anticancer potential against HCT-15 (colon) cancer cell line at different concentrations. Further, the fractionation of the extracts was carried out and the fractions were tested on the same human cancer cell line. It was found that all the fractions inhibited the growth of HCT-15 at 100 μg/ml except water-soluble fractions, but the significant growth inhibition was shown by the chloroform-soluble fractions of the ethanolic extract and 50% ethanolic extract.

Calotropis procera (Ait.) R.Br., belongs to family Asclepiadaceae. It is a popular medicinal plant which possesses relevant medicinal properties especially lessening of inflammation, relief of pain, healing and reducing secondary bacterial infections (Fabiyi et al., 1993). The decoction of the aerial parts of the plant is commonly used in Saudi Arabia as traditional medicine for the treatment of variety of diseases including fever, joint pain, muscular spasm, constipation and its ethanolic extract have a significant antipyretic, analgesic, neuromuscular blocking activity (Mossa et al., 1991). The latex of Calotropis procera contains antinociceptive (Soares et al., 2005), anti-inflammatory (Kumar and Basu, 1994), antipyretic (Larhsini et al., 2002), antidiarrhoeal (Kumar et al., 2001), anthelmintic (Al-Qarawi et al., 2001) and analgesic (Dewan et al., 2000) properties. The present investigation is an attempt to identify novel anticancer agents from traditional herbal medicine by carrying out in vitro cytotoxicity of Calotropis procera stem-leaves extracts against human cancer cells.

Materials and Methods

Plant material:

The plant parts were collected in the month of July from Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu. The freshly collected plant parts were chopped, shade dried and ground into powder. The powdered dried plant material was then extracted with different solvents at room temperature to obtain extracts for bioevaluation.

Extraction of plant material:

The ethanolic extract was prepared by percolating the dried ground plant material (100g) with 95% ethanol and then concentrating it to dryness under reduced pressure. For aqueous ethanolic (50% ethanolic) extract, dried ground plant material (100g) was percolated with 50% ethanol and concentrated to dryness under reduced pressure. The aqueous extract was obtained by boiling dried ground plant material (100g) for 30 min. in distilled water (300ml).

Preparation of stock/working solutions and positive control:

Stock solutions of 20mg/ml were prepared by dissolving ethanolic extract in DMSO, 50% ethanolic extract in 50% DMSO and aqueous extract in sterile water. Stock solutions were prepared atleast one day in advance and were not filtered/sterilized, but the microbial...
contamination was controlled by addition of 1% gentamycin in complete growth medium i.e. used for dilution of stock solutions to make working test solutions of 200µg/ml. 5-Flourouracil was prepared in distilled water and diluted in gentamycin medium to obtain the concentration of 2 x 10^{-4} M.

**In vitro assay for cytotoxic activity:**

Test material was subjected to *in vitro* anticancer activity against human cancer cells (Monks *et al.*, 1991). The cells were grown in tissue culture flasks in RPMI 1640 growth medium at 37°C in an atmosphere of 5% CO₂ and 90% relative humidity in a CO₂ incubator. The cells at subconfluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in PBS containing 0.02% EDTA) 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 (10^5 cells/ml) was transferred to a well of 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.

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). The cell growth was stopped by gently layering 50µl of 50% (ice cold) trichloroacetic acid 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. Liquids of all the wells were then gently pipetted out and discarded. The plates were washed five times with distilled water and were air-dried. Sulphorhodamine B 100µl (0.4% in 1% acetic acid) was added to each well and the plates were incubated at room temperature for 30 min. The unbound SRB was quickly removed by washing the cells five times with 1% acetic acid. Plates were air-dried, tris buffer (100µl, 0.01M, pH 10.4) was added to all the wells to solubilise the dye. Plates were gently stirred for 5 min on a mechanical stirrer. The optical density was recorded on ELSIA reader 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 substracting 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:

\[
\text{Cell growth (%) = } \frac{\text{Growth in the presence of test material}}{\text{Growth in the absence of test material}} \times 100
\]

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

\[
100 - \text{per cent growth in the presence of test material}
\]

**RESULTS AND DISCUSSION**

In the first phase of experiment, extracts from the stem-leaves of *Calotropis procera* were evaluated against human cancer cells at the concentration of 10, 30 and 100µg/ml with appropriate positive controls. Results demonstrated that the ethanolic extract at the concentration of 100µg/ml showed 70% growth inhibition of colon cancer cell line *i.e.* HCT-15 (Fig. 1). At the concentration of 30 and 10µg/ml, the activity of the extract against HCT-15 was not up to the mark. In case of 50% ethanolic extract, 70% and 72% growth inhibition of HCT-15 was observed at 30 and 100µg/ml respectively, but at...
10µg/ml the extract was found inactive. In case of aqueous extract, 55%, 59% and 67% in vitro anticancer efficiency was produced at the concentration of 10, 30 and 100µg/ml, respectively against HCT-15. In the second phase of experiment, the fractions of the extracts were evaluated against HCT-15 (Fig. 2). Among the fractions from ethanolic extract, the most active fraction was observed to be chloroform, which showed activity against HCT-15 at 30µg (74%) and 100µg/ml (80%) followed by n-butanol (54% at 30µg and 78% at 100µg/ml) and hexane (62% at 100µg/ml). Water-soluble fraction was found inactive. In case of fractions from 50% ethanolic extract, the significant results were again produced by the chloroform fraction as it inhibited the growth of HCT-15 at three different concentrations, viz., 10µg/ml (70%), 30µg/ml (81%), 100µg/ml (83%) (Fig. 3). The n-butanol fraction showed activity at 30µg/ml (73%) and 100µg/ml (80%). The results produced by the water-soluble fraction were not much significant. In case of fractions from aqueous extract, 71% (at 30µg/ml) and 79% (at 100µg/ml) growth inhibition of HCT-15 was shown by n-butanol fraction (Fig. 4). The activity was negligible in case of water-soluble fraction. Thus, the chloroform fraction from 50% ethanolic extract was most active among all the fractions of ethanolic, 50% ethanolic and aqueous extracts as it showed 70% growth inhibition at the lowest concentration of 10µg/ml. This chloroform fraction from 50% ethanolic extract can be explored for probable anticancer lead molecules for the drug development in the management of cancer.

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