

***In vitro* cytotoxicity of *Argemone mexicana* Against Different Human Cancer Cell Lines**

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ABSTRACT

Cancer is a public health problem all over the world. Large number of plants and their isolated constituents has been shown to possess potential anticancer activity. Whole plant ethanolic extract of *Argemone mexicana* showed *in vitro* cytotoxicity against different human cancer cell lines. There was no growth of inhibition recorded against liver cancer cell line. Sulforhodamine B dye (SRB) assay was done for *in vitro* cytotoxicity test assay. The *in vitro* cytotoxicity was performed against five human cancer cell lines namely of lung (A-549), liver (Hep-2) colon (502713 HT-29) and neuroblastoma (IMR-32). Plant extract showed 83% growth of inhibition against lung (A-549) cell line. In case of liver (Hep-2), no activity was observed, where as plant extract showed maximum activity against colon 502713 cell line. It was found to be 99% and 96% respectively, in case of HT-29 liver human cancer line and IMR-32 neuroblastoma cell lines.

Keywords: Human cancer cell lines, *in vitro*, cytotoxicity test, SRB, *Argemone mexicana*

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INTRODUCTION

Argemone mexicana Linn. belongs to the family Papaveraceae. The plant is annually prickly herb. Its leaves are simple, alternate, cauline, sessile, exstipulate prickly deep cut, with spiny teeth and unicostate reticulate venation. The flowers are large, yellow, hermaphrodite and actinomorphic [14]. It is widely used in folk medicines to alleviate several ailments especially for its analgesic effects [1]. Chemical investigations of this plant have revealed the presence of alkaloids [9,13], amino acids [5], phenolics [8] and fatty acids [7]. Thus the authors set forth the objective of evaluating the *in vitro* cytotoxicity activity of *Argemone mexicana* against different human cancer cell lines.

MATERIALS AND METHODS

Plant material and Preparation of plant extracts

Plants were collected in February to March 2008 from eastern regions of Uttar Pradesh, India. The whole plant ethanolic extract was used for *in vitro* cytotoxicity assays. Plant material was dried at 37°C, powdered and extracted in ethanol. Extract was fine-filtered and freeze dried. For the preparation of the extracts, dried ground plant material was percolated with 95% ethanol and concentrated to dryness under reduced pressure. Extract was redissolved in Dimethylsulphoxide (DMSO) to form stock solutions, which were filter sterilized (0.2µm) before testing on cell lines.

Human cell lines

Human cancer cell lines namely of lung (A-549), liver (hep-2) colon (502713 HT-29) and neuroblastoma (IMR-32) were grown in RPMI-1640 with 2 mM L-glutamine medium pH 7.2. Penicillin was dissolved in PBS and sterilized by filtering through 0.2µ filter in laminar air flow hood. The media was stored at low temperature (2-8°C). Complete growth medium contained 10% FCS. The medium for cryopreservation contained 20% FCS and 10% DMSO in growth medium. The cell lines were maintained at 37°C in a 5% CO₂ atmosphere with 95% humidity.

***In vitro* assay for cytotoxic activity**

The anticancer activity was determined by evaluating the cytotoxic potential of the test material using human cancer cell lines that were allowed to grow on tissue culture plates in the presence of test material. The cell growth was measured using ELISA reader after staining with Sulforhodamine B dye (SRB) which binds to basic amino acid residues in the trichloroacetic acid (TCA) fixed cells.

Preparation of Cell suspension for assay

Human cancer cell lines were grown in multiple tri-conical flasks (TCFs) at 37°C in an atmosphere of 5% CO₂ and 90% relative humidity in complete growth medium to obtain enough number of cells. The flasks with cells at subconfluent stage were selected. Cells were harvested by treatment with Trypsin-EDTA. Cells were separated to single cell suspension by gentle pipetting action and the viable cells were counted in a hemocytometer using trypan blue. Cell viability at this stage should be >97%. Viable cell density was adjusted to 5,000 - 40,000 cells/100µl depending upon the cell line (Monks, 1991). Cell suspension (100µl) together with 100µl of complete growth medium was added into each well. The plates were incubated at 37°C for 24 hours in an atmosphere of 5% CO₂ and 90% relative humidity in a CO₂ incubator. After 24 hours, the test material, DMSO (vehicle control) and positive control were added.

Sulforhodamine B (SRB) assay

The antiproliferative SRB assay was performed to assess growth inhibition. This is a colorimetric assay which estimates cell number indirectly by staining total cellular protein with the SRB dye Skehan [17]. The microtiter plates were taken out after 48 hours incubation of the cells with test materials and gently layered with chilled 50% TCA in all the wells to produce a final concentration of 10%. The tissue culture plates were incubated at 4°C for one hour to fix the cells attached to the bottom of the wells. The supernatant was then discarded. The plates were washed five times with distilled water to remove TCA, growth medium, low molecular weight metabolites, serum proteins etc. Plates were air dried and stored until further use. SRB solution was added to each well of the plates and incubated at room temperature for 30 minutes. The unbound SRB was removed quickly by washing the wells five times with 1 % acetic acid and then air dried. 100µl of Tris buffer (0.01 M, pH 10.4) was added and shaken gently for 5 minutes on a mechanical shaker. Optical density was recorded on ELISA reader at 515 nm.

RESULTS AND DISCUSSION

Argemone mexicana is a storehouse of good variety of compounds. Chemically they could be alkaloids, flavonoids, coumarins, monoterpenes, sesquiterpenes, steroids, fatty acids, esters and phenolic acids etc. The flowers contain isorhamnein, isorham-nethin-3-glucoside and isorhamnetin-3, 7-diglucoside [4,16], Protopine, allocryptopine and berberine [15], Isoquinoline [2], argemexicaine A and argemexicaine, along with thirteen known alkaloids Chang *et al.*[3]. Thus the plant *Argemone mexicana* is one of the most valuable medicinal plants and attracted the attention of many workers.

Experimentally the test samples showing growth inhibition more than 70% at 100 µg/ml were considered to be active. The *in vitro* cytotoxicity was performed against lung (A-549) cell line showed 83% growth of inhibition. In case of liver (Hep-2), no activity was observed. Where as in case of colon 502713 cell line, it showed maximum growth inhibition. In case of HT-29 liver human cancer line and IMR-32 neuroblastoma cell line plant extract showed 99% and 96% activity respectively.

In the present study, we concluded that the plant extracts showed selective *in vitro* cytotoxicity against some human cancer cell lines. The activity might be depended upon the morphology of cell lines and mechanism of action of the plant extract. Many plant extract kill cancer cell lines through activating apoptosis and effecting growth regulators. The plant is also shown to accelerate the growth of the *Pestalotiopsis mangiferae* that causes a serious leaf-spot disease of *Mangifera indica*. It is widely used in Sudanese traditional medicine for the treatment of trypanosomiasis [10]. The Ethanolic and aqueous extracts were also observed to show antibacterial properties and immunomodulatory properties [6,11]. It is not possible at this juncture to single out the most effective *in vitro* cytotoxicity constituent of *Argemone mexicana*. However, based on the published studies flavonoids, alkaloids seem to be most likely candidates eliciting *in vitro* cytotoxicity effect. Its reported *in vitro* cytotoxicity effects warrant further investigation for its use in the cases of clinical anticancer activity.

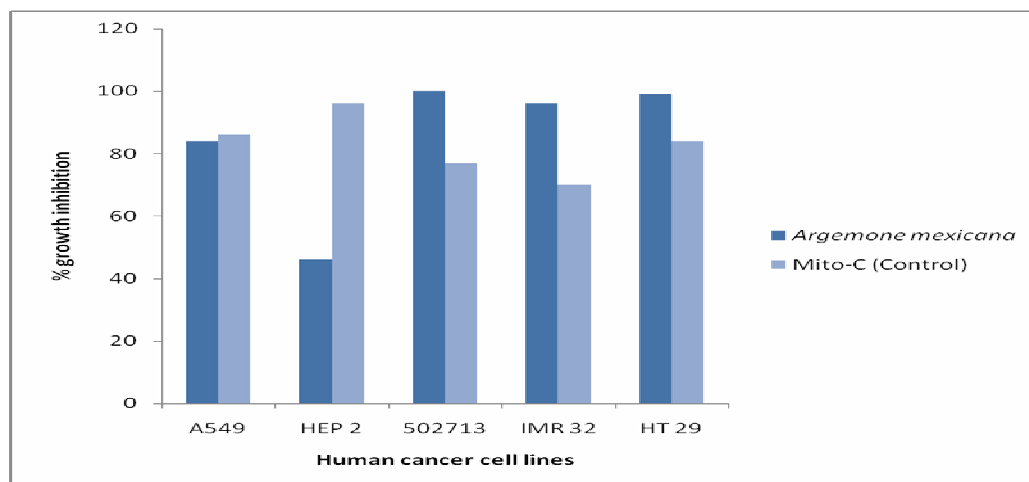


Fig-1: *In vitro* cytotoxic activity of plant extracts

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