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Cytotoxicity Effects and Apoptosis Induction by Isoquinoline Alkaloids from Argemone Mexicana

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Abstract
Argemone mexicana, a traditional medicinal plant, is known for its diverse pharmacological properties. This research investigates the cytotoxic effects and apoptosis induction of isoquinoline alkaloids isolated from *Argemone mexicana*. The study utilizes various assays to evaluate cell viability, morphological changes, and apoptotic mechanisms induced by these compounds. The findings demonstrate that isoquinoline alkaloids exhibit significant cytotoxicity and induce apoptosis, suggesting their potential as therapeutic agents in cancer treatment.

Keywords: Argemone Mexicana, Apoptosis, Cytotoxicity, Isoquinoline

Introduction

The ethno-pharmacological approach for the search of new anticancer agents from the plant sources has proved to be more predictive. There are many natural substances which exhibits its anti-tumor activities. Among these herbal sources have shown remarkable activities and proved them as excellent source of antitumor drug development.

Great advances have been made in the treatment of some cancer/tumor and new advances in surgery, radiotherapy and chemotherapy have lead to an increase in cure rates. But the price of conventional care is often out of reach for majority of cancer patients living in developing world (**Pal 2002**). Moreover in the post trade related intellectual property right (TRIPS) era many drugs have become extremely expensive. TRIPS now propagates monopoly, allowing multinationals to fix exhorbitant prices for patented medicines. Product patents reduce accessibility and affordability of drugs that generic drugs have not allowed to happen so far .Patients confronting the diagnosis of advanced (stage 4) cancer face the statistical reality that conventional chemotherapy can affect a cure for only a tiny minority of all such cases. More often, the reasonable impulse of these patients is to venture into various alternative options Like herbal medicines (**Treasure 2005**).

Argemone mexicana, commonly known as the Mexican poppy, has been utilized in traditional medicine for its analgesic, anti-inflammatory, and antitumor properties. The plant contains a variety of bioactive compounds, particularly isoquinoline alkaloids, which have garnered attention for their pharmacological effects. Isoquinoline alkaloids are a diverse class of compounds derived from the plant kingdom, known for their various biological activities, including antitumor, antimicrobial, and anti-inflammatory effects.

Materials and Methods

The plants of *Argemone mexicana*were collected from areas around Hoshangabad road, Bhopal, M.P. in India. Identification of plant was carried out at Department of Botany and a voucher specimen was procured in herbarium record maintained at the Laboratory, in the Department of Zoology, Career College,AutonomousBhopal (M.P.). The plant material was thoroughly washed with water and was kept for drying in the shade at room temperature for 20days. The thoroughly air dried plant material was grinded to powder to about 40-60 mesh size weighted and stored in large plastic bottles for future extraction and chemical testing.

Isolation of Isoquinoline Alkaloids

Isoquinoline alkaloids were extracted from the dried aerial parts of *Argemone mexicana* using solvent extraction techniques. The extracts were then subjected to chromatographic methods for isolation and purification. of **Isoquinoline Alkaloids** 300 mL of ethyl acetate was used for a solvent extraction at room temperature for 72 hours after 10 g of powdered plant material was moistened with 15 mL of NH4OH (25%, m/m). A rotary evaporator operating at 40 °C with reduced pressure was used to filter the extract and evaporate the solvent. To eliminate lipophilic, acidic, and neutral material, the residue was diluted in water and acidified with H2SO4 to a pH of 3–4. Petroleum ether and diethyl ether were then used for extraction. To extract crude alkaloids, the aqueous solution was first diluted with NH4OH (25%, m/m) to bring its pH down to 9–10, then it was extracted using chloroform, rinsed with distilled water to bring its pH down to neutral, dried with Na2SO4, and concentrated until it was dry under low pressure.

Using a capillary tube and their standards, one spot of the isolated alkaloids from preparative TLC was applied to a silica gel plate to compare its location with the standard senecionine and seneciphylline. The mobile phases utilized were listed in table 1. TLC plates with a 0.25 mm thick coating of silica gel applied to them were used for analytical TLC. chromatographic purification using solvents,Toluene: ethylacetate: diethylamine (70:20:1), dichloromethane: methanol: ammonia 25% (85:14:1), and chloroform: acetone: diethylamine (50:40:10) Using FT-IR spectra and senecionine and seneciphylline standards, the identification of the isolated compounds was further verified. FT-IR was performed using a KBr disc, and FT-IR spectra were recorded on a Shimadzu FT-IR-84005 Infrared Spectrometer.

Cytotoxicity Assays

Cell viability was assessed using the MTT assay. Cells were treated with varying concentrations of isoquinoline alkaloids for 24, 48, and 72 hours. The IC50 values were calculated to determine the effective concentration of the alkaloids.

Cell line culture:

We purchased human cancer cell lines from the National Center for Cell Science (NCCS) in Pune. The cells were cultured in tissue culture flasks in a carbon dioxide incubator (37°C, 5% CO2, 90% RH) using complete growth media (RPMI-1640 medium with 2 mM glutamine, pH 7.4) supplemented with 10% fetal calf serum, 100 µg/ml streptomycin, and 100 units/ml penicillin. Trypsin [0.05% in PBS (pH 7.4) containing 0.02% EDTA] was used to treat the cells at the subconfluent stage so they could be extracted from the tray. To assess cytotoxicity, cells with a vitality of greater than 98% as assessed by trypan blue exclusion were utilized. A 1 x 105 cell/ml cell suspension was made using full growth media.

Human cancer cell lines were tested for in vitro cytotoxicity (Skeham et al., 1990). using tissue culture plates with 96 wells. Each well of the 96-well tissue culture plate received 100µl of the cells suspension. For twenty-four hours, the cells were kept in a carbon dioxide incubator at 37°C, 5% CO2, and 90% relative humidity. Following a 24-hour incubation period, test materials in 100µl of complete growth media were introduced to the wells holding the cell suspension. In a carbon dioxide incubator, the plates were further incubated for 48 hours. Trichloroacetic acid (50%, 50µl) was carefully put on top of the medium in each well to inhibit the cell growth. To fix the cells adhering to the wells' bottoms, the plates were incubated for an hour at 4 °C. All of the wells' liquid wasAt 540 nm, the optical density (OD) was measured using an ELISA reader. The mean OD value of the experimental set was subtracted from the mean OD value of the corresponding blank to calculate the cell growth. The percentage growth in the presence of test material was computed by taking the growth in the absence of any test material as 100%. This was followed by the calculation of the percentage growth inhibition in the presence of test material

MTT(3-(4,5- dimethyl thiazolyl), 5–diphenyl tetrazolium bromide)Assay
Protocol: (Mosmann*et.al* 1983)

1. After cell incubation with the potential Extract for the desire time in a 24-cellculture plate (with 1ml of culture medium), add 100 µl of MTT stock solution(final concentration ~0.5 mg/ml) to each well.
2. Incubate at 37ºC for 2 hours in the humidified CO_2 incubator.
3. At the end of the incubation period, take off the medium and add 1 ml of acidicisopropanol to solubilise the purple formazan dye.
4. Carefully sonicate each well to solubilise completely the converted dye.
5. Transfer 200 µl of the dye solution of each well for a 96-well plate in duplicate.
6. Read the absorbance at 570 nm using 690 nm as reference in a plate readerspectrophotometer.
7. Express the results as % of sample absorbance in relation to the absorbance in the negative control.

Calculations

1. Subtract the blank absorbance value from all other values. This can bedone automatically in the SOFTmax Pro Software.For each well express the absorbance as:**Abs 570 nm – Abs 690 nm**
2. Express the results as percentage of cell survival:

(Absorbance treated wells / absorbance of control wells) × 100%

Dose response curves can be calculated for the compounds tested over a range ofconcentrations, enabling IC 50 values to be obtained (*i.e.* concentration ofchemical that reduces cell survival by 50%)

***In vivo* Antitumor activity Experimental Design (Nair *et al* 1991)**

The animals (mice) were divided into 2 groups. One group was be injected daily withthe extract 100 mg/kg i.p. for 10 consecutive days. The control group animals wereinjected with double distilled water. Three weeks after the last injection of the drug ,the animals were injected with 5 x 105 viable B16F10 melanoma cells into the dorsalskin.

Animal Model

The C57BL and Swiss albino hybrid mice were chosen at random from a breed colony kept in the PBRI Bhopal department's animal house. The mice were kept in polypropylene cages with bedding made of sterile rice husk that was purchased locally. The temperature was kept at 23 to 20 degrees Celsius, the

humidity was kept at 50 to 5%, and the light was provided for 10 to 14 hours each day. The mice were given regular mice's food (a formula from the Cancer Research Institute in Mumbai) and unlimited access to filtered, acidified water. For the experiments, mice of either sex who weighed 22 ₱2g and were 6–8 weeks old were chosen from the above colony.

***Tumor model: B*16F10 Melanoma**

B16F10 melanoma originally obtained from National Cell Centre Pune, India,was maintained by serial transplantation in C57BL mouse.

Tumor propagation:

Mice with tumors were sacrificed by cervical dislocation, and the tumor was removed after the entire animal was soaked in 70% alcohol. Using mechanical dispersion, a single cell suspension was made in phosphate buffered saline (pH = 7.4). A 45-nylon mesh was used to filter the cell suspension. After that, the suspension of a single cell was run through a variety of gauze sizes. To eliminate cell clumping, the cells suspension was once more run through nylon mesh.

Cell viability by trepan blue exclusion test:

Suitable volume of the cell suspension and 0.1% trypan blue solution (dilution factor is 4) were mixed thoroughly. The diluted suspension was loaded into haemocytometer(Reichert, Buffalo, N.Y.,U.S.A.).The viable and dead cells were counted separately infour WBC chamber under the light microscope and the mean number of cells wascalculated.

Total number of cells was calculated as: Total no. of cells/4 X Dilution factor X 104 cells/ml

Percent viable cells: (Total no. of cells - Dead cells)/Total cells X 100

Tumor Growth Kinetics*:* The tumor size was measured every alternate day using vernier callipers, and the tumor volume were calculated (V = □/6 XD1XD2XD3 where D1 D2 and D3 are tumor diameters in there perpendicular planes). Tumor growth response was assessed from the following parameters.

Volume doubling time (VDT): The time, in days for the tumors size to reach double the treatment volume.

Growth delay (GD): difference in the time, in days, needed for the treated and untreated tumor to reach five times the treatment volume.

Results

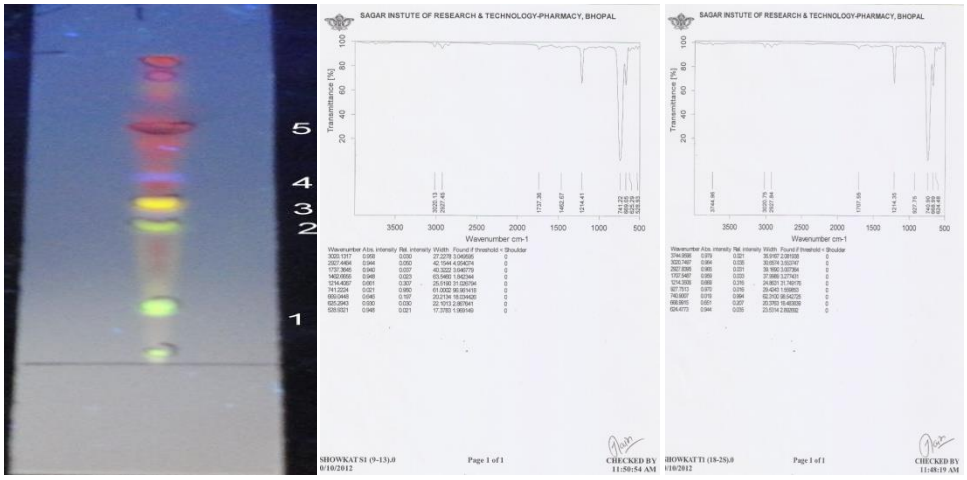
Argimone mexicana's qualitative phytochemical study.Biologically active phytochemicals, including flavonoids, phenols, steroids, tannins, terpenoid, alkaloids, and saponines, were detected in the methanol extract. Despite their toxicity when taken internally, the isoquinoline alkaloids have good antibacterial, antifungal, and antiviral activity, as demonstrated by numerous experimental studies. The plant's medicinal value is found in the bioactive phytochemical constituents that produce specific physiological actions on the human body. These phytochemicals are produced as secondary metabolites to protect the plant from the environment. When a particular medication is made from this plant, all of these ingredients will be quite beneficial.

Table 1: Showing percentage yield of crude extract of *Argemone mexicana*L. in different solvents

S. No.	Solvent	Weight of powdered material (gm)	Volume of solvent (ml)	Weight of crude extract (gm)	Characters of extract	Percentage yield %
1	Petroleum ether extract	200	500	1.62	dark green	0.81
2	Ethyl acetate extract	200	500	2.31	Green	1.15
3	Methenol extract	200	500	1.82	Brown	0.91

Table 2 Phytochemical Screening of crude extracts of Petroleum ether, Ethyl acetate and methanol from *Argemone maxicana*Linn

S.No.	Tests	Observation for extracts		
		Pet. Ether	**Ethyl acetate**	**Methanol**
1	**Test for carbohydrates**			
	Fehling's Test	_	+.	+
2	**Test for Alkaloid**	+	+	+
	Wagner's test	+	+	+
3	**Test for Flavonoids**			
	Shinoda test	_	+	+
	Alkaline reagent test	_	+	_
4	**Test for Terpenoids**			
	Salkowski test	+	_	_
5	**Test for Saponins**	_	+	_
	Foam test		+	
	Test for proteins	_	+	+



The cytotoxicity activity of compound Alkaloids from *Argimone Mexicana* was screened against human breast cancer cell lines (MCF-7), Lung cancer cell lines (A549) andnormal cancer cell lines (L929) with increasing concentration (2-10µg/ml)for 24hrs byMTT bioassay. Percent inhibition of compound C1 Alkaloids were calculated for MCF-7,A549 and L929 In case of compound C1 Alkaloids shows maximum inhibition87.43% against MCF-7 Breast cancer cell lines and for Lung cancer cell lines maximuminhibition 75.38% was achieved at the concentration of (10µg/ml) while on normal celllies compound C1 did not showed any marked cytotoxic activity.

In vivo* Antitumor activity of C1*Argemone mexicana

Tumor takes activity:

Statistical evaluation of the data was done by Student't' test. (Graph PAD In statsoftware, Kyplot).A value of $p<0.05$ was considered to be significant. The graphs were fitted using Microcal Origin, Version 2.8 on a Pentium Computer. From the growth curves of tumors, it is clear that control tumors showed an exponential l growth. The C1 (2mg/kg b.wt) produced slow growth response when comparedto control. The volume doubling time and growth delay were calculated from the growthcurves of individual bearing mice. The silent period (i.e. time taken for palpable growth) for the control group was found tobe 7.4 days while in case of C1treated group it was found to be 10.6 days which was very significant. ($p<0.0001$)

Cytotoxicity of Isoquinoline Alkaloids

The results indicate that isoquinoline alkaloids significantly reduced cell viability in all tested cancer cell lines in a dose- and time-dependent manner. The IC50 values ranged from 10 to 30 µM, highlighting their potent cytotoxic effects.

The cytotoxic effects and apoptosis induction by isoquinoline alkaloids from *Argemone mexicana* suggest their potential as a therapeutic option in cancer treatment. The ability to induce apoptosis through intrinsic pathways emphasizes their relevance in drug development. Future studies should focus on the in vivo efficacy and safety profiles of these compounds to support their clinical application.

Conclusion

Isoquinoline alkaloids from *Argemone mexicana* exhibit significant cytotoxicity against cancer cell lines and induce apoptosis through intrinsic mechanisms. These findings contribute to the understanding of

natural products in cancer therapy and encourage further exploration of isoquinoline alkaloids as potential anticancer agents.

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