The Cytotoxic Activity of the Total Alkaloids Isolated from Different Parts of *Solanum pseudocapsicum*

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Received September 4, 2003; accepted December 8, 2003

The total alkaloid fractions of the methanolic extracts of the leaves, ripe fruits, roots, seeds and stem of *Solanum pseudocapsicum* were subjected to *in-vitro* cytotoxicity, short-term toxicity and long-term survival studies. All the five fractions exhibited potent activity. The total alkaloid fraction of leaves was found to be the most potent. The HT-29 cell line was the most sensitive to the fractions. The cytotoxic concentration (CTC₅₀) values for all these fractions ranged between 0.39—0.91, 0.68—2.8, 0.92—3.56, 4.05—8.2, 3.28—5.65 and 0.95—5.55 µg/ml, respectively for HT-29, RD-228, A-549, HEp-2, B₁₆F₁₀ and Vero cell lines. In short-term toxicity studies, the fractions showed 50% viability at 93—128 µg/ml for DLA cells and 141—189 µg/ml for human lymphocytes. In the long-term survival studies on the cell lines RD-228, HEp-2 and Vero, cells retained their regenerative capacities at concentrations below 8 µg/ml. The total alkaloids of the plant, especially from the leaves merit further investigations to identify the active constituents in animal models.

Key words *Solanum pseudocapsicum*; total alkaloid; cytotoxicity; anti-tumor

Solanum pseudocapsicum (Family: Solanaceae) is an erect, much branched, non-spiny bushy shrub and is distributed in the gardens of Simla, Mussoorie, Dun Valley, The Nilgiris and other higher altitude places in India. The plant, (commonly called Jerusalem cherry), grows to about 0.6—1.2 m in height and has lanceolate or narrowly elliptical leaves. Its berries are green when unripe and yellow, scarlet or bright red when ripe. Their seeds are ovoid, compressed at one end and rounded at the other end and yellowish in color.¹⁾ Its antimicrobial,²⁾ anti-viral,³⁾ anti-spasmodic⁴⁾ and anti-hypertensive⁴⁾ properties are known. The 50% methanolic extract of the entire plant of *Solanum pseudocapsicum* has also been reported to possess cytotoxic activity in a preliminary study of Dhar *et al.* (1973).⁴⁾ Earlier studies in our laboratories showed significant *in vitro* cytotoxic and anti-tumor properties of the total alkaloid fraction of unripe fruits of *Solanum pseudocapsicum*.⁵⁾ The present paper describes the *in vitro* cytotoxicity and anti-tumour properties on the total alkaloid fraction isolated from the methanolic extract of different parts of *Solanum pseudocapsicum* on malignant human rhabdomyosarcoma (RD-228) cells, human adenocarcinoma (HT-29) cells, Caucasian male larynx epithelium carcinoma (HEp-2) cells, Human small cell lung cancer (A-549) cells, Mouse melanoma (B₁₆F₁₀) cells and normal African green monkey kidney (Vero) cells, Dalton's lymphoma ascites (DLA) cells and freshly isolated human lymphocytes.

MATERIALS AND METHODS

Plant Material and Isolation of Total Alkaloids The shrub *Solanum pseudocapsicum* was collected from the grounds in and around Government Arts College, Ootacamund, India, during July 2000 and authenticated by Survey of Medicinal Plants and Collection Unit, Ootacamund, India (Voucher No: 7345). The leaves (240 g), ripe fruits (255 g), roots (200 g), seeds (180 g) and stems (175 g) were powdered and extracted in Soxhlet extractor using methanol for 18—

20 h. The extract was then concentrated to dryness under reduced pressure and controlled temperature. The total alkaloid fractions were isolated from the leaves (0.192%), ripe fruits (0.023%), roots (0.157%), seeds (0.148%) and stems (0.092%), respectively following a conventional procedure.⁶⁾ Each of the total alkaloid fractions was then dissolved in dimethyl sulphoxide (DMSO) separately and the volume was made upto 10 ml with DMEM/RPMI-1640 medium to obtain a stock solution of 1 mg/ml concentration, sterilized by filtration and stored at -20 °C till use.

Cell Lines and Culture Medium HEp-2, HT-29, RD-228, B₁₆F₁₀, A-549 and Vero cell cultures used in these experiments were obtained from National Centre for Cell Science, Pune, Pasteur Institute of India, Coonoor and Christian Medical College, Vellore, India. Stock cells of these cell lines were cultured in RPMI-1640 or DMEM supplemented with 10% inactivated sheep serum, penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphoterecin-B (5 µg/ml) in a humidified atmosphere of 5% CO₂ at 37 °C until confluent. The cells were dissociated with 0.2% trypsin, 0.02% EDTA in phosphate buffer saline solution. The stock cultures were grown in 25 cm² tissue culture flasks and all cytotoxicity experiments were carried out in 96 well microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India). DLA cells were obtained from Amala Cancer Institute, Trissur, India. DLA cells used were maintained in Swiss albino mice. Lymphocytes were isolated from an O +ve blood group healthy volunteer using histopaque. Studies for clonal growth were carried out in 24 well microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Cytotoxic Assay Cell lines in exponential growth phase were washed, trypsinized and re-suspended in complete culture media. Cells were plated at 10000 cells/well in 96 well microtitre plate and incubated for 24 h during which a partial monolayer forms. The cells were then exposed to various concentrations of the total alkaloid fractions in quadruplicate. Control wells received only maintenance medium.

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Table 1. Cytotoxic of the Total Alkaloid Fraction of Different Parts of *Solanum pseudocapsicum* on HEp-2, HT-29, RD-228, B₁₆F₁₀, A-549 and Vero Cell Lines

Sl. No.	Total alkaloid fraction	Cytotoxic concentration ^{a)} (CTC ₅₀) (μg/ml)											
		HEp-2		HT-29		RD-228		B ₁₆ F ₁₀		A-549		Vero	
		MTT	SRB	MTT	SRB	MTT	SRB	MTT	SRB	MTT	SRB	MTT	SRB
1.	Leaves	4.05±0.2	7.5±0.3	ND	0.39±0.02	0.68±0.03	0.82±0.03	3.28±0.18	3.55±0.2	0.92±0.04	1.05±0.04	0.95±0.03	2.58±0.12
2.	Ripe fruits	6.4±0.3	6.8±0.28	ND	0.47±0.02	0.97±0.04	1.6±0.04	5.26±0.26	5.65±0.3	2.26±0.1	3.12±0.17	2.6±0.1	3.45±0.2
3.	Roots	6.2±0.31	8.2±0.32	ND	0.91±0.04	1.8±0.08	2.8±0.12	3.32±0.13	4.24±0.16	2.14±0.09	3.09±0.1	5.2±0.22	5.55±0.21
4.	Seeds	6.6±0.32	7.6±0.31	ND	0.62±0.02	2.0±0.08	2.2±0.07	4.35±0.13	4.85±0.2	3.56±0.12	3.38±0.1	5.2±0.18	4.8±0.13
5.	Stem	4.03±0.16	6.3±0.21	ND	0.43±0.01	0.98±0.02	1.10±0.01	3.65±0.12	4.05±0.21	2.84±0.12	3.16±0.19	2.35±0.09	3.5±0.13

a) Average of 3 independent determinations, 4 replicates, values are mean CTC₅₀±S.E., ND=not done.

The plates were incubated at 37 °C in a humidified incubator with 5% CO₂ for a period of 72 h. Morphological changes of drug treated cells were examined using an inverted microscope at different time intervals and compared with the cells serving as control. At the end of 72 h, cellular viability was determined by using MTT⁷⁾ and SRB assays.⁸⁾

Short-Term Toxicity Studies These studies were carried out on DLA cells and freshly isolated lymphocytes⁹⁾ using Trypan blue dye exclusion technique.¹⁰⁾

Long-Term Survival Studies A series of cultures were prepared in 25 cm² tissue culture flasks (Tarsons India Pvt. Ltd., Kolkata, India), three for each concentration and three controls by seeding 5×10⁴ cells/ml and incubated for 48 h. Growth medium was then decanted and cultures were exposed to test agents, which have been diluted to 5 fold (200 μg/ml to 1.6 μg/ml) and incubated at 37 °C for a period of 2 h. The cultures were then washed, trypsinized and seeded at densities (5000 cells/ml) for clonal growth in 24 well microtitre plates in growth medium. Control groups received complete growth medium throughout the study. Cultures were incubated until colonies formed. The colonies were fixed and stained with 0.5% crystal violet in absolute ethanol. Colonies containing more than 32 cells were considered to have retained their regenerative capacity.¹⁰⁾

RESULTS AND DISCUSSION

The cytotoxic effect of the total alkaloid fraction of the methanolic extracts of various parts of *Solanum pseudocapsicum* against different cell lines is shown in Table 1. Among the five different total alkaloid fractions, the fraction from the leaves was found to be the most potent in all the cell lines. The CTC₅₀ values for the total alkaloid fraction of leaves were found to be 0.39 μg/ml for HT-29, 0.68–0.82 μg/ml for RD-228, 0.92–1.05 μg/ml for A-549, 0.95–2.58 μg/ml for Vero, 3.28–3.55 μg/ml for B₁₆F₁₀ and 4.05–7.5 μg/ml for HEp-2. HEp-2 cell line was found to be most sensitive to the total alkaloid fraction from the stem, which showed a CTC₅₀ value of 4.03–6.3 μg/ml. Among the six cell lines tested for cytotoxicity, HT-29 was found to be the most susceptible. The CTC₅₀ values for all the five fractions ranged between 0.39–0.91 μg/ml for HT-29. RD-228 was found to be the next sensitive cell line with CTC₅₀ values ranging between 0.68–2.8 μg/ml. The CTC₅₀ values for A-549, Vero, B₁₆F₁₀ and HEp-2 were between 0.92–3.56, 0.95–5.55, 3.28–5.65 and 4.05–8.2 μg/ml, respectively.

Table 2. Short-Term Toxicity Studies of the Total Alkaloid Fractions of Different Parts of *Solanum pseudocapsicum* on DLA Cells and Lymphocytes after 3 h of Exposure

Sl. No.	Total alkaloid fraction	Cytotoxic concentration ^{a)} (CTC ₅₀) μg/ml	
		DLA cells	Lymphocytes
1.	Leaves	128±3.38	141±3.50
2.	Ripe fruits	105±3.18	189±2.70
3.	Roots	108±3.56	156±3.04
4.	Seeds	115±3.06	168±3.12
5.	Stem	93±3.56	170±2.82

a) Average of 3 independent determinations, 6 replicates, values are mean CTC₅₀±S.E.

In the short-term anti-tumor studies, using DLA cells, treatment with the total alkaloid fractions resulted in 50% viability in the concentration range of 93–128 μg/ml. Among the five fractions, the stem fraction was found to be most active. A similar study on freshly isolated lymphocytes revealed CTC₅₀ values between 141–189 μg/ml. The total alkaloid fraction of the leaves was found to be the most potent with a CTC₅₀ value of 141 μg/ml.

In long-term survival studies carried out with RD-228, HEp-2 and Vero cells, the cells retained their regenerative capacities at concentrations below 8 μg/ml. Among the three cell lines tested for this activity, RD-228 cell line was found to be more sensitive to the total alkaloid fractions.

Plants with potential anti-cancer properties are known in the literature. *Catharanthus roseus*, *Taxus brevifolia* and *Podophyllum* species are the most successful higher plant materials used in cancer chemotherapy.¹¹⁾ Several plants belonging to the genus *Solanum* exhibited strong cytotoxic and anti-tumor properties. *Solanum trilobatum*,¹²⁾ *Solanum incanum*,¹³⁾ *Solanum capsicastrum*,¹⁴⁾ *Solanum gnera*,¹⁵⁾ *Solanum indicum*,¹⁶⁾ *Solanum sodomaeum*,¹⁷⁾ *Solanum nigrum*,⁷⁾ are a few plants with potential activities. In the present study, the total alkaloid fraction of different parts of *Solanum pseudocapsicum* exhibited potent cytotoxic and anti-tumor properties. The cytotoxic studies of these fractions were carried out in six different cell lines using MTT and SRB assays. Among the five total alkaloid fractions, the leaves fraction was the most potent. The maximum cytotoxicity was observed against human adenocarcinoma (HT-29) cells. The human rhabdomyosarcoma (RD-229) and human small cell lung cancer (A-549) were also susceptible to the

Table 3. Long-Term Test for Survival Method Studies of the Total Alkaloid Fractions of Different Parts of *Solanum pseudocapsicum* on HEp-2, RD-228 and Vero Cell Lines

Sl. No.	Total alkaloid fraction of	Number of cells per colony at different concentrations in cell lines ^{a,b)}											
		200 µg/ml			40 µg/ml			8 µg/ml			1.6 µg/ml		
		HEp-2	RD-228	Vero	HEp-2	RD-228	Vero	HEp-2	RD-228	Vero	HEp-2	RD-228	Vero
1	Leaves	0	0	0	16	6	0	43	35	>50	>50	>50	>50
2	Ripe fruits	0	0	0	8	6	8	>50	36	48	>50	>50	>50
3	Roots	0	0	0	10	8	4	48	38	42	>50	>50	>50
4	Seeds	0	0	0	5	9	5	>50	35	50	>50	>50	>50
5	Stems	0	0	0	12	7	8	41	33	48	>50	>50	>50

a) Average of 3 determinations, 4 replicates. b) All the cell lines in control group showed >50 cells/colony.

total alkaloid fraction of the leaves. In the short-term toxicity studies using DLA cells and lymphocytes, the CTC₅₀ values ranged between 93–128 µg/ml and 141–189 µg/ml, respectively. Long-term survival studies showed that the cells when treated with the total alkaloid fractions retained their proliferative capacities at concentrations below 8 µg/ml.

All these studies confirms the cytotoxic and anti-tumor nature of the plant *Solanum pseudocapsicum*. Similar results were obtained in earlier studies carried out in our laboratories using the total alkaloid fraction of unripe fruits of *Solanum pseudocapsicum*.⁵⁾ Reports indicate that steroidal alkaloids from *Solanum* species possess strong cytotoxic and anti-tumor properties. Solamargine from *Solanum nigrum*,⁷⁾ incanumine from *Solanum incanum*,¹³⁾ glycoalkaloids from *Solanum sodamaeum*¹⁷⁾ and several other steroidal alkaloidal glycosides,¹⁵⁾ are known to possess these properties.

Phytochemical tests indicated the steroidal nature of the total alkaloid of *Solanum pseudocapsicum*. Several steroidal alkaloids including solanocapsine, solacasine, solacapsine, episolacapsine, isosolacapsine and *o*-methyl solanocapsine have been isolated from the arboreal part of the plant.¹⁸⁾ Hence, the observed cytotoxic and anti-tumor principles may also be due to the presence of steroidal alkaloids. In conclusion, the total alkaloid fractions of the plant *Solanum pseudocapsicum* merit further investigations to identify the active principles responsible for the anti-tumor properties and to further prove the activity in animal models.

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