African Journal of Biotechnology Vol. 7 (22), pp. 3984-3988, 19 November, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Studies on cytotoxicity and antitumor screening of red and white forms of *Abrus precatorius* L.

R. Sivakumar[1] and C. Alagesaboopathi[2]*

[1]Department of Biotechnology, S.N.R. Sons College, (Autonomous), Coimbatore – 641 006, Tamilnadu, India.
[2]Department of Botany, Government Arts College (Autonomous), Salem – 636 007, Tamilnadu, India.

Accepted 29 May, 2008

The cytotoxicity study with cancer cell line A-549 indicated that the methanol insoluble fraction of crude red forms of *Abrus precatorius* is toxic (CTC_{50} 175,100 µg/ml) in microculture tetrazolium assay and sulphorhodamine B assays, whereas crude and fractions of the red and white forms exhibited toxicity at still higher concentrations. However, an antitumor screening by the short-term toxicity study with DLA cells showed the extracts to be comparatively less toxic.

Key words: *Abrus precatorius*, cytotoxicity, antitumor, medicinal plants.

INTRODUCTION

Abrus precatorius L. (Fabaceae) is a climbing shrub, widely distributed all over tropical India in hedges and among bushes on open lands (Gamble, 1984; Nair and Hendry, 1983; Matthew, 1999). Among variously coloured seed types (Singh et al., 1999), two common seed types namely red and black coloured seeds (red form) and the white coloured seeds (white form) were selected for this study.

The roots, leaves and seeds are used for medicinal purposes in Ayurveda, Siddha and Unani. The seeds are used in eye disease, jaundice, pain, poisoning, fainting, arthritis and leucoderma (Anonymous, 1959; Chopra et al., 1956; Kirtikar and Basu, 1980; Ahmad et al., 1993; Warrier et al., 1993; Yoganarasimhan, 2000; Gautam et al., 2001). The aerial parts extract were also used for treating certain infections like leucorrhoea, gonorrhea, diarrhoea and dysentery (Hemadri and Rao, 1983; Kirtikar and Basu, 1980; Vedavathy et al., 1997)

The antineoplastic effect of a protein extract isolated from the seeds of *A. precatorius* has been direct cytotoxic effect on the tumor cells (Subba and Sirsi, 1969). The seeds contain abrine, which suppresses Ehrlich ascites tumor growth in mice. Protein extract of the seeds possess antitumor activity against Yeshida sarcoma in rats and mice (Siddiqi et al., 2001).

Abrin was effective in reducing solid tumour mass development induced by Dalton's Lymphoma Ascites (DLA) (Ramnath et al., 2002). This investigation evaluates the cytotoxicity and antitumor properties of red and white forms of *A. precatorius*.

MATERIALS AND METHODS

Plant material

Fresh seeds of red and white forms of *A. precatorius* were collected from Maruthamalai Hills of Coimbatore district and Mettur, Salem district, Tamilnadu. India and identified at the Botanical Survey of India, Coimbatore. The voucher specimens are kept for reference (A.S. 1001 to 1008) in our department herbarium.

Preparation of extract

Shade-dried, seed powder of each form was extracted with 50% aqueous ethanol in cold maceration method (Harborne, 1984; Kokate et al., 1995) at room temperature, separately. After filtration, the marc was extracted twice in the same conditions. Ethanol was removed under vacuum and the aqueous residue was lyophilized to dryness. Extracts (crude 50% ethanolic extract) were fractioned in petroleum ether, chloroform and methanol. The crude (50% ethanolic extract of red form and white form) and methanol soluble and insoluble fractions of crude (red form and white form) were stored in desiccators for pharmacological experiments.

Cytotoxicity screening

Human cell line A – 549 from small cell lung carcinoma (morphology: epithelial) was used in the experiment. It is susceptible to

*Corresponding author. E-mail: alagesaboopathi@rediffMail.com.

adenovirus, polio virus, influenza virus. Properties: Fatty acid synthesis, tumor markers, factor dependent, replication, enzymology and virology. The cell line was obtained from Christian Medical College, Vellore, Tamilnadu, India.

Determination of mitochondrial synthesis by microculture tetrazolium (MTT) assay

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitrochondrial activity per cell. The cleavage of MTT to a blue formazan derivative by living cells is clearly a very effective principle on which the assay is based.

The principle involved is the cleavage of tetrazolium salt MTT (3-(4,5 dimethyl thiazole-2yl) –2,5-diphenyl tetrazolium bromide) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells is proportional to the extent of formazan production by the cells used.

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x10^5 cells/ml using the medium containing 10% new born calf serum. Each well of the 96 well microtitre plate, 0.1 ml of the cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different drug dilutions concentrations was added to the cells in microtitre plates. The plates were then incubated at 37°C for 72 h in 5% CO_2 atmosphere. Microscopical examination was carried out and observations were recorded every 24 h. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in MEM-PR was added to each well. Then plates were gently shaken and incubated for 3 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 50 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. (Ke et al., 1999). The percentage growth inhibition was calculated using the formula:

$$\text{Growth inhibition (\%)} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

Determination of total cell protein content by sulphorhodamine B (SRB) assay

Sulphorhodamine B (SRB) is a bright pink aminoxanthene dye with two sulfonic groups. Under mild acidic conditions, sulphorhodamine B binds to the proteins basic amino acid residues in TCA (trichloro acetic acid) fixed cells to provide a sensitive index and cellular protein content that is linear over a cell density range of at least two orders of magnitude.

Colour development in sulphorhodamine B assay is rapid, stable and visible. The developed colour can be measured over a broad range of visible wavelength in either a spectrophotometer or a 96 well plate reader. When TCA-fixed and sulphorhodamine B stained samples are air-dried, they can be stored indefinitely without deterioration.

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x10^5 cells /ml with medium containing 10% new born calf serum. Each of the 96 wells of microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed once with medium and 100 µl of different drug concentrations were added to the cells in microtitre plates. The plates were then incubated at 37°C for 72 h in 5% CO_2 atmosphere. Microscopical examination was carried out and observations are recorded every 24 h. After 72 h, 25 µl of 50% trichloro acetic acid was gently added to the wells such that it forms a thin layer over the drug dilutions to form a over all concentrations of 10%. The plates were incubated at 4°C for 1 h. The plates were flicked and washed five times with tap water to remove traces of medium, drug and serum, and were then air-dried. The air-dried plates were stained with sulphorhodamine B for 30 min. The unbound dye was then removed by rapidly washing four times with 1% acetic acid. The plates were then air-dried. 100 µl of 10 mM tris base was then added to the wells to solubilise the dye. The plates were shaken vigorously for 5 min. The absorbance was measured using a microplate reader at a wavelength of 540 nm (Philip et al., 1990). The percentage growth inhibition was calculated using the formula above.

Anti-tumor screening

Cells were obtained from Amala Cancer Institute, Trissur, India and was propagated and maintained in the peritoneal cavity of mice at J.S.S. College of Pharmacy, Ooty, Tamilnadu, India. The tests rely on a breakdown in membrane integrity determined by the uptake of a dye such as (Tryphan blue, erythrorisin and nigrosin) to which the cell is normally impermeable.

DLA cells were cultured in peritoneal cavity of mice by injecting a suspension of DLA cells (1.0 x10^5 cells/ml) intraperitoneally. The DLA cells were withdrawn from the peritoneal cavity of the mice between 15 - 20 days with the help of a sterile syringe. The cells were washed with HBSS and centrifuged for 10 - 15 min at 10,000 rpm. The procedure was repeated thrice. The cells were suspended in known quantity of HBSS and the cell count was adjusted to 2 x 10^6 cells /ml. The cell suspension was distributed into Eppendorf tubes (0.1 ml containing 2 lakhs cells). The cells were exposed to drug dilutions and incubated at 37°C for 3 h. After 3 h dye exclusion test, that is, equal quality of the drug treated cells were mixed with tryphan blue (0.4%) and left for 1 min. It was then loaded in a haemocytometer and viable and non-viable count was recorded within 2 min. Viable cells do not take up colour, whereas dead cells take up colour. However, if kept longer, live cells also generate and take up colour (Unnikrishnan and Ramadasan, 1998). The percentage of growth inhibition was calculated using the following formula:

$$\text{Growth inhibition (\%)} = 100 - \frac{\text{(Total cells – Dead cells)}}{\text{Total cells}} \times 100$$

Statistical analysis

Data from all experiments were statistically evaluated using analysis of variance (ANOVA) followed by Duncan's test; $p \leq 0.05$ were considered significant (Duncan, 1951).

RESULTS

Cytotoxicity screening

The cytotoxicity study indicated that the methanol insoluble fraction of crude red forms is toxic (CTC_{50} 175, 100 µg/ml) to the cell in both assay (cancer cell line A-549 - small cell lung carcinoma; whereas all the other extracts showed toxicity at a higher concentration only (Tables 1 - 4).

Table 1. Determination of total cellular protein mitochondrial synthesis by microculture tetrazolium (MTT) assay in A-549 cell line in different extract of *A. precatorius* seeds.

Drug		Mean absorbance					
		Control	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml
Crude	Red form	0.188 a ± 0.0083	0.055 b ± 0.0015	0.094 b ± 0.0016	0.134 a ± 0.0018	0.138 b ± 0.0037	0.155 b ± 0.0071
	White form	0.188 a ± 0.0083	0.086 a ± 0.0055	0.106 b ± 0.0015	0.143 a ± 0.026	0.167 a ± 0.0067	0.185 a ± 0.0083
Methanol soluble fractions	Red form	0.165 b ± 0.0058	0.068 ab ± 0.001	0.099 c ± 0.0038	0.132 a ± 0.0035	0.138 b ± 0.0023	0.154 b ± 0.0059
	White form	0.165 b ± 0.0058	0.072 ab ± 0.0047	0.107 b ± 0.0041	0.137 a ± 0.0046	0.157 ab ± 0.0047	0.165 b ± 0.0051
Methanol insoluble fractions	Red form	0.165 b ± 0.0058	0.065 b ± 0.0078	0.069 c ± 0.0042	0.091 b ± 0.0063	0.098 c ± 0.0040	0.102 c ± 0.0047
	White form	0.165 b ± 0.0058	0.062 b ± 0.0033	0.125 a ± 0.0015	0.149 a ± 0.0033	0.170 a ± 0.0058	0.170 ab ± 0.0053

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT. Values are expressed in term of mean ± SE.

Table 2. Percentage of growth inhibition in determination of total cellular protein mitochondrial synthesis by microculture tetrazolium assay (MTT) in A-549 cell line in different extract of *A. precatorius* seeds.

Drug		Percentage of growth inhibition					CTC_{50}
		500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml	(µg/m)
Crude	Red form	70.74	50.50	28.72	26.59	17.55	250
	White form	54.25	44.14	23.93	11.17	01.59	400
Methanol soluble fractions	Red form	58.78	40.00	20.00	16.36	06.66	375
	White form	56.36	35.15	16.96	04.84	0.0	425
Methanol insoluble fractions	Red form	60.60	58.18	44.84	40.60	38.18	175
	White form	62.42	24.24	9.69	0.0	0.0	425

Table 3. Determination of total cellular protein by sulphorhodamine B (SRB) assay in A - 549 cell line in different extract of *A. precatorius*.

Drug		Mean absorbance					
		Control	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml
Crude	Red form	0.510 b ± 0.028	0.192 c ± 0.0034	0.463 a ± 0.009	0.525 a ± 0.0025	0.531 a ± 0.0025	0.520 c ± 0.0052
	White form	0.510 b ± 0.028	0.328 a ± 0.022	0.391 b ± 0.018	0.440 b ± 0.017	0.458 b ± 0.015	0.472 cd ± 0.010
Methanol soluble fractions	Red form	0.683 a ± 0.0082	0.225 bc ± 0.0108	0.431 ab ± 0.0122	0.536 a ± 0.0021	0.550 a ± 0.0191	0.594 b ± 0.0184
	White form	0.683 a ± 0.0082	0.271 b ± 0.0106	0.446 a ± 0.0195	0.543 a ± 0.0241	0.564 a ± 0.0387	0.631 ab ± 0.0105
Methanol insoluble fractions	Red form	0.683 a ± 0.0082	0.138 d ± 0.0087	0.241 c ± 0.0093	0.311 c ± 0.0187	0.370 c ± 0.0129	0.432 d ± 0.0187
	White form	0.683 a ± 0.0082	0.179 cd ± 0.0046	0.429 ab ± 0.0379	0.550 a ± 0.0109	0.567 a ± 0.0319	0.646 a ± 0.0057

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT. Values are expressed in term of mean ± SE.

Table 4. Percentage of growth inhibition in the determination of total cellular protein by Sulphorhodamine B (SRB) assay in A-549 cell line in different extract of *A. precatorius* seeds

Drug		Percentage of growth inhibition					CTC_{50} (µg/ml)
		500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml	
Crude	Red form	62.35	05.21	0.0	0.0	0.0	450
	White form	35.68	23.33	13.72	10.19	07.45	>500
Methanol soluble fractions	Red form	67.05	36.89	21.52	19.47	13.03	325
	White form	60.32	34.69	20.49	17.42	07.61	400
Methanol insoluble fractions	Red form	79.79	64.71	54.46	45.82	36.74	100
	White form	73.79	37.18	19.47	16.98	05.41	350

Table 5. The antitumor screening (short term toxicity studies) used by DLA cells in different extract of *A. precatorius* seeds.

Drug		Percentage of growth inhibition				CTC_{50} (µg/ml)
		1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	
Crude	Red form	59.14	42.62	29.12	16.16	740
	White form	49.62	32.18	23.72	12.16	>1000
Methanol soluble fractions	Red form	58.16	32.34	18.18	19.23	860
	White form	51.41	40.16	33.16	11.01	940
Methanol insoluble fractions	Red form	62.32	50.70	34.17	16.18	490
	White form	64.46	49.78	31.26	23.26	510

Anti-tumor screening

In antitumor screening by the short term toxicity studies used by Dalton's lymphoma ascities (DLA) cells all the extracts showed less toxic (CTC_{50} 740, >1000, 860, 940, 490, 510 µg/ml) (Table 5).

DISCUSSION

The cytotoxicity study was performed by MTT and SRB assays with the crude and its fractions within the cancer cell line A-549 (small cell lung carcinoma). This indicates that the methanol insoluble fraction of crude red forms is toxic to the cell in both assays, whereas all the other extracts showed toxicity at a higher concentration only. In antitumor screening by short term toxicity studies used by Dalton's lymphoma ascities (DLA) cells all the extracts showed less toxic. The protein extract isolated from the seeds of *A. precatorius* had cytotoxic effect on the tumour cells, and exhibited activity against *Yoshida sarcoma* in rats and a fibrosarcoma in mice (Subba and Sirsi, 1969; Siddiqi et al., 2001).

A. precatorius seeds contain abrine, a poisonous principle similar to recin from castor seeds, which suppresses *Ehrlich ascites* tumor growth in mice (Siddiqi et al., 2001). Abrins are more toxtic to tumour cells than normal cells (Harborne et al., 1999). Abrin is effective in reducing solid tumour mass development induced by Dalton's Lymphoma Ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC) cells. DLA cell line was more sensitive to abrin than EAC (Ramnath et al., 2002). Abrine is a highly toxic protein (LD_{50}, 0.029 mg/kg body wt of mice) present to extent of 0.15% in the seed. It has been studied intensively for its antitumour activity (Anonymous, 1959). The result of the high performance thin layer chromatography (HPTLC) reports that presence of abrin (alkaloids) in both seed forms (Gautam et al., 2001; Sivakumar, 2002) may be responsible for this antitumour activity.

REFERENCES

Ahmad J, Amin KMY, Afaque SH, Khanna A (1993). A review of some U*nani* contraceptive drugs. Proc.I[st] National Seminar on Ilmul Advia, Beenapara. Abstr. 11: 23-25.

Anonymous (1959). The Wealth of India – Raw Materials*,* 1:CSIR, New Delhi, India.

Chopra RN, Nayar SL, Chopra IC (1956). Glossary of Indian Medicinal Plants, Publication and information Directorate, Council of Scientific and Industrial Research (CSIR), New Delhi.

Duncan D (1951). A significance test for differences between ranked treatments in analysis of variance. Virginia J. Sci. 2: 171-189.

Gamble JS (1984). Flora of the Presidency of Madras.1: 349-350. London. Gautam, DNS., Dixit, SK., Benerji,R. and Malhrotra, S., (2001). A HPTLC study for assessment of *sodhana* effect on alkaloids of gunja (*Abrus precatorius*). *Aryavaidyan*, 14: 146-153.

Gautam DNS, Dixit SK, Benerji R, Malhrotra S (2001). A HPTLC study for assessment of *sodhana* effect on alkaloids of gunja (Abrus precatorius). Aryavaidyan, 14: 146-153.

Harborne JB (1984). Phytochemical methods, 2[nd] edition, Chapman and

Hall, New York.
Harborne JB, Herbert B, Gerard P, Moss (1999). Phytochemical dictionary. Taylor and Francis Ltd. London.
Hemadri K, Rao SS (1983). Leucorrhoea and menorrhagia; Tribal medicine. Ancient Sci. Life. 3: 40-41.
Ke H, Hisayoshi K, Aijun D, Yongkui J, Shingeo I, Xinsheng Y (1999). Antineoplastic Agents III: Steroidal from *Solanum nigrum,* Planta Med. 65: 35-38.
Kirtikar KR, Basu BD (1980). Indian medicinal Plants. Vol.1: Bishen Singh Mahendra Pal Singh, Dehradun, India.
Kokate CK, Purohit AP, Gokhale SB (1995). Pharmacognosy, 3rd edition, Nirali Prakashan, Pune.
Matthew KM (1999). The Flora of the Palni Hills, Part 1:282.The Rapinat Herharium St. Joseph's College, Tiruchirapalli, India.
Nair NC, Hendry AN (1983). Flora of Tamil Nadu, India, Series I; Botanical Survey of India, Southern Circle, Coimbatore. 1: 90.
Philip S, Rista S, Dominic S, Anne M, James M, David V, Jonathan TW, Heidi B, Susan K, Michale RB (1990). New colorimetric cytotoxic assay for anticancer –Drug Screening. J. Nat. Cancer Inst. 82: 1107-1112.
Ramnath GK, Ramadasan K (2002). Antitumour effect of abrin on trans-planted tumours in mice. Indian J. Physiol. Pharmacol. 46: 69-67.
Siddiqi TO, Bhupinder D, Peerzada SHK, Iqbal M (2001). *Angiospermous* seeds of medicinal importance. J. Econ. Tax. Bot. 25: 101.
Singh G, Singh DNPN, Shanta M (1999). Comparative study of processed (Shodhit) and unprocessed seeds of 'Gunja' - *Abrus precatorius* L. Nat. Prod. Sci. 5(3): 127-133.
Sivakumar R (2002). Pharmacognostical, Phytochemical and Pharmacological studies on *Abrus precatorius* L. Ph.D. Thesis Bharathiar University, Coimbatore, Tamilnadu, India.
Subba RVV, Sirsi M (1969). Effect of *Abrus precatorius* L. on experimental tumors. Cancer Res. 29: 1447- 1451.
Unnikrishnan MC, Ramadasan K (1998). Cytotoxicity of extracts of Spices to Cultured Cells. Nutr. Cancer 11(4): 251-257.
Vedavathy S, Mrudula V, Sudhakar A (1997). Tribal Medicine of Chittoor District, A.P. (India). Published by Herbal Folklore Research Centre, with the aid from IDRC, Ottawa, Canada, pp. 13-14.
Warrier PK, Nambiar VPK, Ramankutty (1993). Indian medicinal plants a compendium of 500 species 1: Orient Logman Ltd., Madras, India.
Yoganarasimhan SN (2000). Medicinal Plants of India. 2 Tamil Nadu, Reg. Res. Inst. (Ay), Bangalore, India.