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RESEARCH PAPER

Jatamansi : A Source Against Lung and Colon Cancer Cells

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ABSTRACT **:** *In vitro* assay for cytotoxic activity of *Nardostachys jatamansi* has been carried out against eight human cancer cell lines from six different tissues *via* 95 per cent methanolic and aqueous extract at the concentration of 100 µg/ml using Sulphorhodamine blue (SRB) assay. Results revealed that methanolic extract from the stem leaves of *N. jatamansi* showed highest *in vitro* cytotoxic effect against four human cancer cell lines (A-549, COLO-205, SW-620, NCI-H322) from lung and colon origin. Based on *in vitro* data, it is suggested that further *in vivo* studies as well as identification of effective components from methanolic extract and their exact mechanism of action could be useful in designing new anticancer therapeutic agents.

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N. jatamansi, commonly known as jatamansi or muskroot and belonging to the Valerianaceae family, is distributed in the Himalayas from Pakistan, India, Nepal, Tibet and China upto high altitudes of 3000-5000 m (Airi *et al.*, 2000). Jatamansi possesses antiulcer action (Rucker *et al.*, 1978), hepatoprotective activity (Ali *et al.*, 2000) and antioxidant property (Salim *et al.*, 2003). Moreover, the rhizome of the plant possesses stress modulating antioxidant effect (Lyle *et al.*, 2009). *N. jatamansi* showed antidepressant like effect (Dhingra and Goyal, 2008) and has the potential to ameliorate the severity of acute pancreatitis (Bael *et al.*, 2012). In addition, the roots of plant showed *in vitro* cytotoxic effect against neuroblastoma cancer cells (Pandita *et al.*, 2012). Based on analysis of published literature, the stem-leaf part of the plant was selected to evaluate its *in vitro* anticancer potential against human cancer cells from different tissues by using SRB assay.

RESEARCH METHODOLOGY

Extracts, cell lines and positive controls :

Stem-leaves of jatamansi were collected in the month of July-August from IIIM (CSIR), Jammu, J&K, India and extraction of the plant material was carried out as per Kandil *et al*., 1994. The human cancer cells were obtained from National Centre for Cell Science, Pune, India and were further grown and maintained in RPMI-1640 medium. Positive controls like adriamycin and 5-fluorouracil were prepared in distilled water, while paclitaxel was prepared in DMSO. These were further diluted in gentamycin medium to obtain desired concentrations.

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

Extracts were subjected to *in vitro*

anticancer activity against various human cancer cell lines (Monks *et al.*, 1991). The anti-proliferative SRB assay which estimates cell number indirectly by staining total cellular protein with the dye SRB was performed to assess growth inhibition (Skehan *et al.*, 1990). The anticancer activity was determined by the cytotoxic potential of the test material at 100 µg/ml. Cells were allowed to grow for 24 h on 96 – well flat bottom tissue culture plates. Cells were further allowed to grow in the presence of test material for 48 h. Cell growth was terminated by addition of 50 per cent (w/v) trichloro acetic acid. Cells were stained with SRB dye. Excess dye was removed by washing with 1 per cent (v/v) acetic acid and bound dye was dissolved in Tris buffer. OD was taken at 540 nm and growth inhibition of 70 per cent or above was considered active for our bioassay purpose in case of extracts. Suitable blanks (growth medium and DMSO) and positive controls (prepared in DMSO and distilled water) were also included. Each test was done in triplicate and the values reported were mean values of three experiments.

The cell growth was determined by subtracting average absorbance value of respective blank from the average absorbance value of experimental set. Per cent growth in presence of test material was calculated as under:

–OD change in presence of control = Mean OD of control – Mean OD of blank

–OD change in presence of test sample = Mean OD of test sample – Mean OD of blank

– per cent growth in presence of control = 100/OD change in presence of control

–per cent growth in presence of test sample = per cent growth in presence of control × OD change in presence of test sample

–per cent inhibition by test sample = 100 – per cent growth in presence of test sample.

The growth inhibition of 70 per cent or above was considered active while testing extracts, but in testing of active ingredients at different molar concentrations, the growth inhibition of 50 per cent or above was the criteria of activity.

RESULTS AND DISCUSSION

The methanolic stem-leaf extract from *N. jatamansi* showed *in vitro* anticancer potential against four human cancer cell lines as 71 per cent growth inhibition was observed against A-549 and 82 per cent growth inhibition was observed against NCI-H322 (human cancer cell lines from lung origin). The extract also displayed 95 per cent growth inhibition of COLO-205 and 96 per cent growth inhibition of SW-620 (human cancer cell lines from colon origin). However, the aqueous extract from the stem-leaf part of the same plant did not exhibit *in vitro* cytotoxicity against any of the human cancer cell line. The growth inhibition by this aqueous extract was observed in the range of 05-31 per cent, which is not considered significant (Table 1). Cancer is a global public health problem and the leading cause of death in developed / developing countries. Cancer is becoming a big load on families and economies. A large number of plant species have been screened through bioassays for search of novel plant based anticancer drugs.

The Indian sub-continent has great botanical diversity and widespread use of traditional medicine

Table 1 : Growth inhibitory effect of *Nardostachys jatamansi* along with positive controls against human cancer cell lines

Plant part used	Extract	Conc. (µg/ml)	Human cancer cell lines from six different tissues							
			Lung	Colon	Colon	Breast	Lung	Prostate	Leukemia	Glioblastoma
			A-549	COLO-205	SW-620	MCF-7	NCI-H322	PC-3	THP-1	U-87-MG
			Growth inhibition (%)							
Stem -	Methanolic	100	71	95	95	14	82	23	27	04
Leaves	Aqueous	100	12	09	31	05	19	19	05	15
Positive controls		Conc.(Molar)								
5-Flurouracil		2×10^{-5}M	-	51	68	-	-	-	73	60
Paclitaxel		1×10^{-6}M	79	-	-	-	52	-	-	-
Adriamycin		1×10^{-6}M	-	-	-	60	-	59	-	-

Growth inhibition of 70 per cent or above has been indicated in bold numbers

The mark (-) indicates that particular human cancer cell line was not treated with that particular positive control

practice known as ayurvedic medicine, however, only a relatively small number of these plants have been subjected to accepted scientific evaluation for their potential anticancer effects. Natural products from a number of medicinal plants offer new sources of drugs, but there are still a large number of medicinal plants in which all the active constituents have not yet been fully investigated. Therefore, efforts are still being made for the search of effective naturally occurring anticarcinogen that would prevent, slow or reverse cancer development. Fortunately, medicinal plants have real significance and there is a need to screen them for their anticancer activity *in vitro* and the present work deals with the same. To conclude, active ingredients from the methanolic extract of muskroot can act as lead molecules for the development of anticancer drugs to provide a great service and promise to lung and colon cancer patients.

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