In Vitro Anticancer Activity of the Root, Stem and Leaves of *Withania Somnifera* against Various Human Cancer Cell Lines

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Withania somnifera **Dunal know as *Ashwagandha* belong Solanaceae family. It is extensively used in most of the Indian herbal pharmaceuticals and nutraceuticals. The current study, evaluate *in vitro* cytotoxicity in 50% ethanol extract of root, stem and leaves of *Withania somnifera* against five human cancer cell lines of four different tissues i.e. PC-3, DU-145 (prostrate), HCT-15 (colon), A-549 (lung) and IMR-32 (neuroblastoma). Root, stem and leaves extracts showed cytotoxicity activity ranging 0-98% depending on the cell lines but maximum activity was found in 50% ethanol extract of leaves of *Withania somnifera*. Ethanol extract of leaves obtained from treatments T2, T3, T4 and T5 showed strong activity against PC-3 and HCT-15 with 80-98% growth inhibition, while the 50% ethanol extract of leaves from T1 treatment showed a minimum of 39% and T3 treatment showed a maximum of 98% growth inhibition against HCT-15. This investigation is the first report of the anticancer activity in various parts of *Withania somnifera* cultivated in fly ash amended soil.**

Key words: Anticancer, cytotoxicity, fly ash, PC-3, HCT-15, prostrate, *Withania somnifera*

Cancer is one of the major human diseases and causes large suffering and economic loss world-wide. Chemotherapy is one of the methods of treating cancer. However the chemotherapeutic drugs are highly toxic and have devastating side effects. Various new strategies are being developed to control and treat several human cancers[1]. Over 60% of anticancer drugs available in the market are of natural origin. Natural products are also the lead molecules for many of the drugs that are in use[2]. Therefore, the phytochemicals present in several herbal products and plants may have the potential to act as preventive or therapeutic agents against various human cancer[1]. The increased popularity of herbal remedies for cancer therapy perhaps can be attributed to the belief that herbal drugs provide benefit over that of allopathy medicines while being less toxic[3]. Since the conventional therapies have devastating side effects, there is a continuous need for search of new herbal cures of cancer[4].

Withania somnifera Dunal (*Ashwagandha*) is extensively used in most of the Indian herbal pharmaceuticals and nutraceuticals[5]. It is an annual herb growing in dry and arid soil as a wild plant[6] and well described in Ayurveda, the ancient Indian system of plant medicine for immunomodulation and antiageing[7-8]. Thus, *Withania somnifera* also have antiinflammatory[9] antitumour and radiosensitizing actions[10-11] and analgesic activity[12]. However no work was reported on *in vitro* studies of *Withania somnifera* cultivated in different concentration of fly ash amended soil. The present investigation was taken up for evaluating the antiproliferative potential possessed by the 50% ethanol extract of root, stem and leaves of *Withania somnifera* against various human cancer cell lines.

Ethanol GR Merck grade, RPMI-1640, fetal calf serum, trypsin, PBS, tryphan blue, penicillin, streptomycin, gentamycin, DMSO, sulphorhodamine, paclitaxel (taxol), 5-fluorouracil, were obtained from Sigma Chemical Co. USA and rest of the chemicals were of high purity and obtained locally. Tissue culture flasks and 96-Well cell culture plates were obtained from NUNC, Germany.

The *Withania somnifera* Dunal plant was cultivated in different concentration of fly ash amended soil at Advanced Materials and Processes Research Institute (CSIR), Bhopal, Madhya Pradesh, India. The experiments were designed to have five treatments, T1- control, T2- 5% fly ash, T3- 10% fly ash, T4- 15% fly ash and T5- 20% fly ash. After completion of cropping root, stem and leaves of *Withania somnifera* were collected separately for each treatment and then air dried.

The dried roots stem and leaves samples of *Withania somnifera* Dunal were powdered separately and about 50 g of each powder was extracted with 50% ethyl alcohol in 1:10 w/v ratio for 24 h. by separating funnel. After many cycles, the desired compound was extracted in flask. This extract was concentrated using a Heidolph Rota Evaporator (Laboratra 4001, Germany) and concentrate was oven dried at 50^0. The root, stem and leaves crude extract were utilized as test materials for *in vitro* anticancer activity testing.

Human cancer cell lines were procured from National Cancer Institute, Frederick, USA. Cells were grown in tissue culture flasks in complete growth medium (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) in a carbon dioxide incubator (37°, 5% CO_2, 90% RH). The cells at sub confluent stage were harvested from the flask by treatment with trypsin (0.05% in PBS (pH 7.4) containing 0.02% EDTA). Cells with viability of more than 98% as determined by trypan blue exclusion were used for determination of cytotoxicity. The cell suspension of 1×10^5 cells/ml was prepared in complete growth medium. Stock solutions (2×10^{-2} M) of compounds were prepared in DMSO. The stock solutions were serially diluted with complete growth medium containing 50 µg/ml of gentamycin to obtain working test solutions of required concentrations.

In vitro cytotoxicity against four human cancer cell lines was determined[13] using 96-well tissue culture plates. One hundred microlitres of cell suspension was added to each well of the 96-well tissue culture plate. The cells were allowed to grow in a carbon dioxide incubator (37°, 5% CO_2, 90% RH) for 24 h. Test materials in complete growth medium (100 µl) were added after 24 h of incubation to the wells containing cell suspension. The plates were further incubated for 48 h. The cell growth was stopped by gently layering trichloroacetic acid (50%, 50 µl) on top of the medium in all the wells. The plates were incubated at 4° for one hour to fix the cells attached to the bottom of the wells. The liquid of all the wells was gently pipetted out and discarded. The plates were washed five times with distilled water to remove trichloroacetic acid, growth medium low molecular weight metabolites and serum proteins and then air-dried. The plates were stained with sulphorhodamine B dye (0.4 % in 1% acetic acid, 100 µl) for 30 min. The plates were washed five times with 1% acetic acid and then air-dried[14]. The adsorbed dye was dissolved in Tris-HCl Buffer (100 µl, 0.01M, pH 10.4) and plates were gently stirred for 10 min on a mechanical stirrer. The optical density (OD) was recorded on ELISA reader at 540 nm. The cell growth was determined by subtracting mean OD value of respective blank from the mean OD value of experimental set. Percent growth in presence of test material was calculated considering the growth in absence of any test material as 100% and in turn percent growth inhibition in presence of test material was statistically calculated.

Samples were evaluated against five cell lines representing four different tissues. These were PC-3 and DU-145 from prostrate, HCT-15 from colon, A-549 from lung and IMR-32 of neuroblastoma origin. All root samples showed cytotoxicity ranging between 0-46% depending on the cell lines but maximum cytotoxicity activity was found in T3 and T5, which showed more than 46% and 32% growth inhibition in A-549 and DU-145 (Table 1). Stem samples showed cytotoxicity ranging between 4-64%. Maximum cytotoxicity was found in T5 treatment i.e. more than 71% growth inhibition against HCT-15 (colon). Stem extract of *Withania somnifera* grown in fly ash-treated plot showed all most similar growth inhibition against all cell lines, PC-3 and DU-145, HCT-15, A-549 and IMR 032 (Table 2). The extract of leaves showed cytotoxicity ranging between 27-98%, which depended on the cell lines but maximum cytotoxicity activity was found in T3 and T2 i.e. more than 98% and 94% growth inhibition in HCT-15 (colon). Similarly the T4 and T5 treatments showed greater growth inhibition against PC-3 and HCT-15, while minimum cytotoxicity activity was found from T1 treatment. Almost remarkable growth inhibition was obtained from T3 treatment against all cell lines studied (Table 3). The anticancer activity has been previously reported in the *Withania somnifera* Dunal against various human cell lines, but according to the

TABLE 1: *IN VITRO* CYTOTOXICITY AGAINST VARIOUS HUMAN CANCER CELL LINES IN 50% ETHANOL EXTRACT OF ROOT

Samples	Concentration (µg/ml)	Cell line type				
		Prostrate		Colon	Lung	Neuroblastoma
		PC-3	DU-145	HCT-15	A-549	IMR-32
Root - T1	100	16	3	16	18	13
Root - T2	100	12	9	4	22	10
Root - T3	100	11	12	13	46	19
Root - T4	100	20	14	9	0	10
Root - T5	100	0	32	10	0	13
5- Fluorouracil	2×10^{-5}M	-	-	55	-	-
Adraimycin	1×10^{-5}M	-	-	-	-	-
Paclitaxel	1×10^{-5}M	52	60	-	-	-

TABLE 2: *IN VITRO* CYTOTOXICITY AGAINST VARIOUS HUMAN CANCER CELL LINES IN 50% ETHANOL EXTRACT OF STEM

Samples	Conc. µg/ml	Cell line type				
		Prostrate		Colon	Lung	Neuroblastoma
		PC-3	DU-145	HCT-15	A-549	IMR-32
Stem - T1	100	17	63	62	55	36
Stem - T2	100	22	29	54	14	38
Stem - T3	100	26	29	33	4	35
Stem - T4	100	28	48	71	58	52
Stem - T5	100	29	33	54	64	40
5- Fluorouracil	2×10^{-5}M	-	-	55	-	-
Adraimycin	1×10^{-5}M	-	-	-	-	-
Paclitaxel	1×10^{-5}M	52	60	-	-	-

TABLE 3: *IN VITRO* CYTOTOXICITY AGAINST VARIOUS HUMAN CANCER CELL LINES IN 50% ETHANOL EXTRACT OF LEAVES

Samples	Conc. µg/ml	Cell line type				
		Prostrate		Colon	Lung	Neuroblastoma
		PC-3	DU-145	HCT-15	A-549	IMR-32
Leaves - T1	100	32	28	39	49	50
Leaves - T2	100	88	70	94	53	77
Leaves - T3	100	80	70	98	47	87
Leaves - T4	100	85	70	86	42	77
Leaves - T5	100	86	27	83	54	74
5- Fluorouracil	2×10^{-5}M	-	-	55	-	-
Adraimycin	1×10^{-5}M	-	-	-	-	-
Paclitaxel	1×10^{-5}M	52	60	-	-	-

literature anticancer activity in root, stem and leaves of *Withania somnifera* cultivated in fly ash amended soil has been reported probably for the first time.

In this study, growth inhibitory activity of root, stem and leaves of *Withania somnifera* using anticancer drugs paclitaxel, adriamycin and 5-fluorouracil (5-Fu) as positive controls was investigated against five cell lines representing four different tissues, PC-3, DU-145, HCT-15, A-549 and IMR-32. Although its root is well known for its importance in Ayurveda but reports on the medicinal activities of stem and leaves are very few. Thus this study revealed the enormous medicinal potential of stem and leaves of this plant. This study gives support that *Withania somnifera* 50% ethanol extract were highly cytotoxic to the human cell lines studied.

Earlier studies indicated that only the root extract of *Withania somnifera* as a potential source of new molecules that can curtail cancer growth[15]. *Withania somnifera* leaves have also been shown to inhibit the growth of human cancer cell lines comparable to that produced by adriamycin. The leaf extract produced antiproliferative activity on NCI-H460 (lung), HCT-116 (colon), SF-268 (central nervous system) and MCF-7 (breast) human tumor cell lines. The inhibitory concentrations obtained was 25.1±0.91 against colon cell line HCT-116[11], but in this study leaf extracts from T2, T3, T4 and T5 treatments of *Withania somnifera* cultivated in fly ash containing soil had shown more than 80% inhibition against PC-3 and HCT-15 cell lines. Further more this study has reported growth inhibitory importance in *Withania somnifera* against various human cancer cell lines i.e. PC-3, DU-145, HCT-15, A-549 and IMR-32. Hence, this study has revealed remarkable anticancer potential in the leaves of *Withania somnifera*.

ACKNOWLEDGEMENTS

The authors thank Dr. Navin Chandra, Acting Director, Advanced Materials and Processes Research Institute (CSIR), Bhopal, India for the support and permission to publish this paper. They also wish to thank Ms. Monika Verma, IIIM, Jammu for samples analysis.

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Accepted 30 September 2010
Revised 21 June 2010
Received 20 November 2009
Indian J. Pharm. Sci., 2010, 72 (5): 659-663