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Research Article

IN VITRO ANTICANCER ACTIVITY OF HYDRO-ALCOHOL EXTRACT OF LEAVES OF *ANDROGRAPHIS NEESIANA* AGAINST PC-3 AND MCF-7 CELL LINES

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ABSTRACT

Cancer is one of the most prominent human disease which have stimulated scientific and commercial interest in the discovery of new anticancer agents from natural sources. The present study has been formulated to understand the *in vitro* anticancer property elicited by *Andrographis neesiana* Wight. The cytotoxic activity of extract of *Andrographis neesiana* leaves was determined by MTT assay against cell lines of prostate (PC-3) and breast cancer (MCF-7). Cell proliferation was measured based on the ability of metabolic active cells to cleave the yellow tetrazolium salt MTT [3- (4, 5- dimethyl–thiazole-2-yl) -2, 5-diphenyl tetrazolium bromide] to form insoluble purple formazan crystals. The findings of the present study suggest that the hydro-alcoholic extract of leaves of *Andrographis neesiana* possess excellent anticancer potential that might be used for therapeutic purposes for cancer treatment with proper evaluation procedures.

Keywords: Anticancer, *Andrographis neesiana*, MTT assay, Hydro-alcohol, PC-3, MCF-7

INTRODUCTION

Cancer is a complex disease that is normally associated with a wide range of escalating effects both at the molecular and cellular levels[1]. It becomes the second major cause of death in the human after cardiovascular disease[2]. Every year, millions of people are diagnosed with cancer, leading to death. The major causes of cancer are smoking, dietary imbalances, hormones and chronic infections leading to chronic inflammation[3]. The number is believed to become 9 million in 2015 and 11.5 million in 2030[4]. The limited success of clinical therapies including radiation, chemotherapy, immunomodulation and surgery in treating cancer, as evident by the high morbidity and mortality rates, indicates that there is an imperative need of new cancer management. Chemoprevention involves the use of pharmacological, dietary bio-factors, phytochemicals and even whole plant extracts to prevent, arrest, or reverse the cellular and molecular processes of carcinogenesis due to its multiple intervention strategies[5].

Medicinal plants possess immunomodulatory and antioxidant properties, leading to anticancer activities. They are known to have versatile immunomodulatory activity by stimulating both non-specific and specific immunity[6,7]. Plants contain several phytochemicals, which possess strong antioxidant activity. The antioxidants may prevent and cure cancer and other diseases by protecting the cells from damage caused by 'free radicals' - the highly reactive oxygen compounds[8]. Many plant-derived products have been reported to exhibit potent antitumour activity against several rodent and human cancer cell lines[9]. Plant derived natural products such as flavonoids, terpenes, alkaloids etc. have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemopreventive effects[10].

Species of *Andrographis* Wallich ex Nees (Acanthaceae) are used in the Indian systems of medicine namely Siddha, Ayurvedha and Unani[11]. The genus exhibits antipyretic properties[12]. This genus consists of 40 species distributed in Tropical Asia[13]. About 21 species are distributed in India[14] and all of them available in Tamilnadu[15]. Among the 21 species, 18 species are reported to be endemic to India. *Andrographis neesiana* Wight (Acanthaceae) is an endemic medicinal herb found in wild in Kotagiri of Nilgiri district, Tamil Nadu[16]. It has been used as an herbal medicine by local communities. It is laxative, bitter and overcomes difficulty in breathing, burning sensation, cough, edema, thirst, skin diseases, syphilitic ulcers, worms, acidity and liver complaints[17].

In the present study, an attempt was made to evaluate the *in vitro* anticancer effects of hydro-alcoholic extract of leaves of *Andrographis neesiana* Wight.

MATERIALS AND METHODS

Chemicals

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine Serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, glucose and antibiotics were purchased from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and propanol were obtained from E.Merck Ltd., Mumbai, India. All other reagents and chemicals used in the study were of analytical grade.

Plant material

Andrographis neesiana plant was collected from the top hill of Yercaud, Salem, Tamil Nadu, India. The leaves of *Andrographis neesiana* Wight were separated, washed, shade dried, coarsely powdered and passed through sieve No.40 and was used for the extraction.

Preparation of hydro-alcohol extract

The shade dried coarsely powdered leaves of *Andrographis neesiana* (50g) was extracted with 500 ml of 80% aqueous ethanol by maceration at room temperature for 72 hours. After extraction, the extract was filtered, concentrated to dryness in rotavapour under reduced pressure and controlled temperature (40-50^{0}C). Dark yellowish brown colour residue was obtained and it was coded as HAAN. The residue was then stored in desiccators. The extractive value of hydro-alcohol extract of *Andrographis neesiana* was found to be 10.1%.

Cell lines and Culture medium

PC-3 (Human prostate cancer cell line), MCF-7 (Human breast adenocarcinoma cell line), and Vero (kidney epithelial cells, monkey) was cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 μg/ml) and amphotericin B (5 μg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test Solutions

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of

1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Cell viability by MTT assay

The ability of the cells to survive 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 mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used[18].

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37° C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 hours interval. After 72 hours, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 hours at 37° C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubulize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

$$\% \text{ Growth Inhibition} = 100 - \left(\frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} X100 \right)$$

RESULTS

The cytotoxic effects of hydro-alcohol extract of leaves of *Andrographis neesiana* are shown in Table 1 and Figure 1. *Andrographis neesiana* exhibits potent cytotoxicity against cancerous PC-3 and MCF-7 cell lines with the average CTC_{50} values of 163.25±26.51 and 137.50±8.85 µg/ml respectively. However, against normal Vero cell lines, the average CTC_{50} value was found to be 640±8.4 µg/ml. The results indicated that hydro-alcohol extract of leaves of *Andrographis neesiana* possesses strong cytotoxicity against cancerous cell lines, but is safe towards normal cells.

Table 1: Cytotoxic property of hydro-alcohol extract of *Andrographis neesiana* leaves against PC-3, MCF-7 and normal Vero cell lines

Name of cell lines	Concentration of extract (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
PC-3	1000	88.26	163.25 ± 26.51
	500	86.31	
	250	68.94	
	125	39.50	
MCF-7	1000	84.48	137.50± 8.85
	500	82.72	
	250	82.28	
	125	47.50	
Vero	1000	78.95	640± 8.4
	500	38.65	
	250	31.25	
	125	24.75	

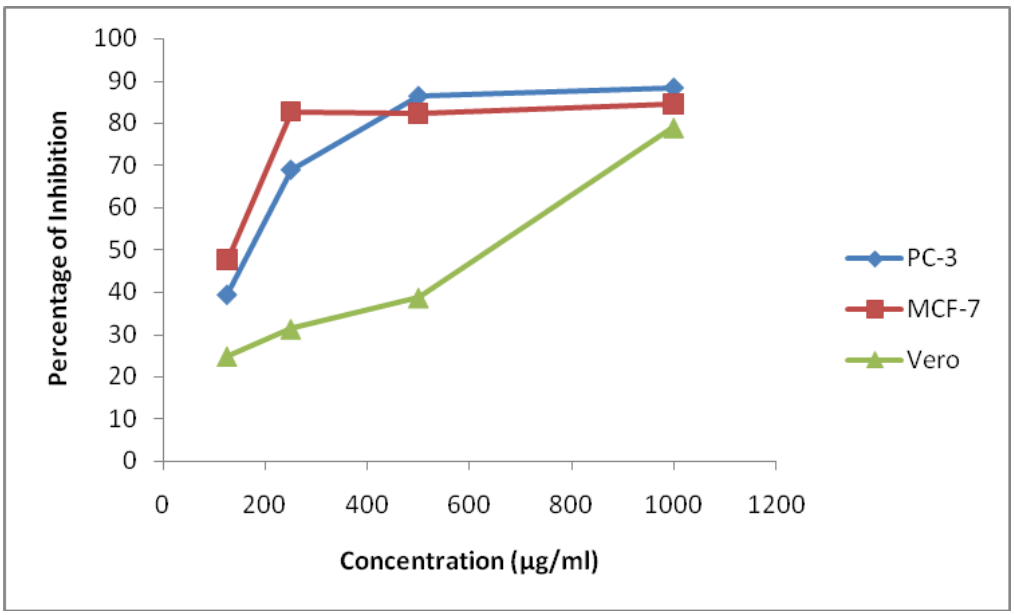

Fig. 1: Cytotoxicity of hydro-alcohol extract of *Andrographis neesiana* leaves against cell lines by MTT assay

DISCUSSION

Medicinal plants constitute a common alternative for cancer prevention and treatment in many countries around the world[19,20,21]. Approximately, 60% of the anticancer drugs currently used have been isolated from natural products from the plants. More than 3000 plants worldwide have been reported to possess anticancer properties. Extracts of these medicinal plants are believed to contain a wide array of polyphenolic compounds which might possess cancer preventive and/or therapeutic properties[22]. According to the US NCI plant screening program, a crude extract is generally considered to have *in vitro* cytotoxic activity if the IC_{50} value (concentration that causes reduction in cell viability to 50%) is less than 30µg/ml[23]. Time-and concentration-dependent manner of the extract activities reflects the logical pharmacokinetics and pharmacodynamics on the cancer cells[24,25]. This is normally indicated in the cellular uptake across membrane and the metabolic disturbance within the cells[26]. These cellular pathways of activities are concerned with necessary signaling transduction through cytosol and nucleoplasm. The study of drug response and development of drug response model using these cell lines is the key to determine safety and hazardous levels and dosages of the extracts to which the cells are exposed[27].

The biological selective activity of any compound might depend on the type of chemical composition and the concentration of active constituents as well as the types and developmental stages of the cancer[28]. The screening of plants for their anticancer properties use cell-based assays and established cell lines, in which the cytotoxic effects of plant extracts or isolated compounds could be measured. In the present study, we used MTT (3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. The result of our study revealed that hydro-alcohol extract of leaves of *Andrographis neesiana* has a potent cytotoxic effect on PC-3 (Human prostate cancer cell line) and MCF-7 (Human breast adenocarcinoma cell line) in a concentration dependent manner. The extract showed good therapeutic values against both PC-3 and MCF-7 cell lines with CTC_{50} values of 163.25±26.51 and 137.50±8.85 respectively. The present study coincide with[29] who reported that the acetone extract of *Tridax procumbens* leaves has shown potent anticancer activity aginst PC-3 cell line. The ethanolic extract of dried latex and flowers of *Calotropis procera* showed cytotoxic properties against MCF-7 cells in a dose dependent manner[30]. Morphological studies also confirmed that the hydro-alcohol extract of leaves of *Andrographis neesiana* has got potential cytotoxic effect.

CONCLUSION

Medicinal plants are an important resource to traditional society's health care systems. Most anticancer drugs have been discovered through random screening of plant materials. In today's world the percentage of people using chemicals and drugs are increasing with their side effects. "The boon given to our earth is the herbs", which needs to be utilized in sustainable manner. Many of today's drugs are derived from plant sources. The cytotoxicity assay indicated the potential of the hydro-alcohol extract of leaves of *Andrographis neesiana* could be a source of anticancer therapeutic agent against both PC-3 and MCF-7 cell lines. Hence, the need to exploit the potentials of these plants especially in areas of traditional medicine and pharmaceutical industries arises.

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