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In vitro cytotoxic and anticancer activities of *Asparagus racemosus* Willd. roots

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ABSTRACT

The root of the plant *Asparagus racemosus* Willd. was extracted with methanol by Soxhlet extraction. The extracts were vacuum dried and subjected to cytotoxic and anticancer activities on cultured cell line. From the results it becomes evident that *A. racemosus* acts as an anticancer agent at a narrow concentration of 400 µg / ml above which it becomes toxic to normal cell line and below that concentration, it has no significant anticancer activity on Hep_2 cell line.

KEYWORDS

Asparagus racemosus;
Anticancer;
Cytotoxic.

INTRODUCTION

Traditional medicinal plants and practices have remained as a component of health care system of many societies in spite of the availability of well established alternatives[1]. Apoptosis plays a central role in tumor development and it has been hypothesized that lack/ failure of apoptosis leads to the development of tumors, including colon tumors. Thus induction of apoptosis in tumor cells is an effective approach to the regulation of tumor growth[2]. Epidemiological and experimental studies indicate that the risk of developing cancer may be attributable to combined actions of environmental factors and endogenous promoting agents[3,4]. Apoptosis is a common protective mechanism by which individual cells containing unrepairable genetic lesions can be removed from the organism[5,6]. Several studies suggest that failure of apoptosis of cells with potentially malignant mutations plays a significant role in progression of normal epithelium to a malignant tumor[7,8].

Asparagus racemosus Wild, belonging to the family Asparagaceae is a well known Ayurvedic rasayana, and its root paste or root juice has been advocated in various ailments including the treatment of peptic ulcer[9], gastro duodenal ulcer[10], cough[11], immune adjuvant potential[12]. It has been reported to have potent adaptogenic activity[13]. Polysaccharide fraction of A. racemosus has been reported to possess significant antioxidant activity in vitro[14]. The present study involving in vitro estimation of Cytotoxic and anticancer activities were carried out to estimate the efficacy of *A. racemosus* as a potential anticancer agent.

MATERIALS AND METHODS

Plant material and extract preparation

The roots of *Asparagus racemosus* was collected from the outskirts of Chennai during April 2008 and

identified and authenticated by Dr. Sasikala Ethirajulu, Research Officer (Pharmacognosy), Central Research Institute for Siddha, Chennai, Tamil Nadu, India. The roots were washed in running water to remove the adherent soil particles and dried in shade. The powdered root was extracted with methanol (MEAR). The extract was reduced to a dark colored molten mass by removing the solvent in a rotary vacuum evaporator (Yield: 23.42 %) The coarse dry powder was used as such for nutritional analysis, the extract for antimicrobial activities.

CYTOTOXIC ACTIVITY[15]

Cell line culture medium

Vero cells (African green monkey kidney cells) obtained from the Dept. of Virology, King's Institute of Preventive Medicine, Chennai, India were used for the study. Vero cells were cultured in Minimum Essential Medium (MEM) supplemented with 10 % inactivated Foetal Calf Serum (FCS), Penicillin (100 IU / ml), Streptomycin (100 IU / ml) and Ampotericin –B (5µg / ml) in a humidified atmosphere of 5% CO_2 at 37°C until to reach the confluent monolayer.

Preparation of 24 well plates in Vero cells with monolayer

The culture of Vero cells was taken after 48 h to attain the complete confluency in the tissue culture flask. Thereafter the cells were taken separately and the medium was aspirated. Five ml of Phosphate buffer saline (PBS) was added to both of the cells for washing and the PBS was discarded. Trypsin Phosphate Versine Glucose (TPVG – 5 ml) was added to both and after 4 min TPVG was completely discarded. Thereafter, 5 ml of MEM (10%) was added. The cell suspension was mixed well with the help of pipette. To find out the cell count, 0.2 ml of the cell suspension was thoroughly mixed with 0.2 ml of trypan blue in the eppendroff. The cell count was done in the Naeubar counting chamber. Based on the cell count, the suspension was diluted to get 24 lakh cells/ml. One ml of the cell suspension was added to each well in the 24 well plates. After adding the cells, the plate was incubated at 37°C for 48 h in 5% CO_2 incubator. After 48 h all the wells were screened under the inverted microscope. Care was taken to obtain the monolayer and the medium was removed. The stock drugs were prepared from the test drug by dissolving in MEM W/O FCS individually (10 g in 10 ml of MEM W/O FCS) and in combination of equal concentration (each 10 g in 30 ml of MEM W/O FCS). From the stock solution, the serial dilutions were made (10 µg, 50 µg, 100 µg…..800 µg).

For the cell control purpose, the MEM W/O FCS was added separately into 2 wells (1 ml cell with 1 ml MEM). For the drug control purpose, 1 ml of drug was added separately into 2 wells (1ml cell with 1 ml drug), which were labeled as neat. The culture preparation was performed in 24 well plates with confluent monolayer. After 24 h, the cells were screened under the inverted microscope to find out the cellular morphology.

ANTICANCER ACTIVITY[16]

Cell line culture medium

Hep_2 (Human epithelial carcinoma cell line of larynx) cells were obtained from the Dept. of Virology, King's Institute of Preventive Medicine, Chennai, India were used for the study. Hep_2 cells were cultured in Minimum Essential Medium (MEM) supplemented with 10 % inactivated Foetal Calf Serum (FCS), Penicillin (100 IU / ml), Streptomycin (100 IU / ml) and Ampotericin –B (5µg / ml) in a humidified atmosphere of 5% CO_2 at 37°C until to reach the confluent monolayer.

Preparation of 24 well plates in Hep_2 cells with monolayer

The culture of Hep_2 cells was taken after 48 h to attain the complete confluency in the tissue culture flask. Thereafter the cells were taken separately and the medium was aspirated. Five ml of Phosphate buffer saline (PBS) was added to both of the cells for washing and the PBS was discarded. Trypsin Phosphate Versine Glucose (TPVG – 5 ml) was added to both and after 4 min TPVG was completely discarded. Thereafter, 5 ml of MEM (10%) was added. The cell suspension was mixed well with the help of pipette. To find out the cell count, 0.2 ml of the cell suspension was thoroughly mixed with 0.2 ml of trypan blue in the eppendroff. The cell count was done in the Naeubar counting chamber.

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RESULTS AND DISCUSSION

Effect of A. racemosus on cytotoxic activity

The methanolic extract of A. racemosus was found to be nontoxic up to a concentration of 400 µg / ml. Toxicity was exhibited thereafter the concentration ranging from 500 µg / ml onwards (TABLE 1).

TABLE 1 : Cytotoxic activity of MEAR on Vero Cell line (µg / ml)

10	10	50	50	100	100
NT	NT	NT	NT	NT	NT
400	400	300	300	200	200
NT	NT	NT	NT	NT	NT
500	500	600	600	700	700
T	T	T	T	T	T
CC	CC	Neat	Neat	800	800
		T	T	T	T

NT: Non Toxic; T: Toxic; CC: Cell Control; Neat: Drug Control

Effect of A. racemosus on anticancer activity

The methanolic extract of A. racemosus on Hep2 cell line indicated non toxicity up to 300 µg / ml and thereafter exhibited toxicity (TABLE 2).

TABLE 2 : Anticancer activity of MEAR on Hep_2 Cell line (µg / ml)

10	10	50	50	100	100
NT	NT	NT	NT	NT	NT
400	400	300	300	200	200
T	T	T	NT	NT	NT
500	500	600	600	700	700
T	T	T	T	T	T
CC	CC	Neat	Neat	800	800
		T	T	T	T

NT: Non Toxic; T: Toxic; CC: Cell Control; Neat: Drug Control

From the results it becomes evident that A. racemosus acts as an anticancer agent at a narrow concentration of 400 µg / ml above which it becomes toxic to normal cell line and below that concentration, it has no significant anticancer activity on Hep_2 cell line.

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