



In vitro cytotoxic and anticancer activities of *Curculigo orchoides* Gaerten. rhizomes

N. Raaman¹, S. Selvarajan², C. P. Gunasekaran³, Varalakshmi Ilango³, Md Illiyas³, G. Balamurugan^{4*}

¹ Centre for Advanced studies in Botany, University of Madras, Guindy Campus, Chennai-25.

² Central Research Institute for Siddha, Arumbakkam, Chennai-106.

³ King's Institute of Preventive Medicine, Guindy, Chennai-25.

⁴ Dept. of Pharmacology, C. L. Baid Metha College of Pharmacy, Thoraipakkam, Chennai-97

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ABSTRACT

The rhizomes of the plant *Curculigo orchoides* Gaerten. was extracted with methanol by Soxhlet extraction. The extracts were vacuum dried and subjected to cytotoxic and anticancer activities on cultured cell line. Preliminary phytochemical screening was performed to identify the different phytoconstituents. From the results it was evident that the plant *C. orchoides* neither acts as an anti cancer or cytotoxic agent.

Keywords: *Curculigo orchoides*, 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. 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 growth¹. Epidemiological and experimental studies indicate that the risk of developing cancer may be attributable to combined actions of environmental factors and endogenous promoting agents^{2,3}. Apoptosis is a common protective mechanism by which individual cells containing unreparable genetic lesions can be removed from the organism^{4,5}. 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^{6,7}.

Curculigo orchoides Gaerten. belonging to the family Amaryllidaceae is a shrub found all over India, especially in sandy situations of hotter regions and in Ceylon. The rhizomes of this plant are extensively used as a demulcent, diuretic and aromatic tonic, aphrodisiac, in the treatment of leprosy and nervous disease⁸. The rhizomes also possess immune stimulant potential⁹, hepato protective¹⁰, antioxidant¹¹ and platelet regeneration effect¹² Since no scientific reports are available on the cytotoxic and anticancer activities, the present study was under taken using cell line cultures.

MATERIALS AND METHODS

Plant collection and Extraction

The rhizomes of *Curculigo orchoides* 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 rhizomes were washed in running water to remove the adherent soil

*Corresponding author.

Tel.: + 91-94454 52786

Telefax: +91-

E-mail: balamurugangunasekaran@gmail.com

particles and dried in shade. The powdered root was extracted with methanol (MECO). The extract was reduced to a dark colored molten mass by removing the solvent in a rotary vacuum evaporator (Yield: 23.42 %)

DETERMINATION OF PHYTOCONSTITUENTS

Preliminary phytochemical screening was performed as per standardized procedure^{13,14}. The various phytoconstituents in the methanol extract were identified.

CYTOTOXIC ACTIVITY¹⁵

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% CO₂ 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% CO₂ 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 concentra-

Table1: Cytotoxic activity of MECO on Vero Cell line ($\mu\text{g} / \text{ml}$)

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
NT	NT	NT	NT	NT	NT
CC	CC	Neat	Neat	800	800
		NT	NT	NT	NT

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

Table2: Anticancer activity of MECO on Hep₂ Cell line ($\mu\text{g} / \text{ml}$)

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
NT	NT	NT	NT	NT	NT
CC	CC	Neat	Neat	800	800
		NT	NT	NT	NT

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

tion (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 μg800 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 ACTIVITY¹⁶

Cell line culture medium

Hep₂ (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. Hep₂ 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 Amphotericin-B (5 μg / ml) in a humidified atmosphere of 5% CO₂ at 37°C until to reach the confluent monolayer.

Preparation of 24 well plates in Hep₂ cells with monolayer

The culture of Hep₂ 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% CO₂ 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 concentra-

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RESULTS AND DISCUSSION

Effect of *C. orchioides* on cytotoxic activity

The methanolic extract of *C orchioides* was found to be nontoxic up to a concentration of 800 $\mu\text{g} / \text{ml}$. 8 (Table 1).

Effect of *C. orchioides* on anticancer activity

The methanolic extract of *C orchioides* on Hep2 cell line indicated non toxicity up to 800 $\mu\text{g} / \text{ml}$ 8 (Table 2).

From the results it becomes evident that *C orchioides* neither is an anticancer agent nor a cytotoxic agent at concentrations up to 800 mg / kg.

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