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Research Article

IN-VITRO CYTOTOXICITY AND ANTICANCER ACTIVITY OF SANSEVIERIA *ROXBURGHIANA*

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ABSTRACT

The anticancer activity of the methanol extracts of leaves of *Sansevieria roxburghiana* was evaluated on HepG2 liver cell line and it was compared with normal 3T3 cell line to find out the non toxic dose of methanol extract. Normal 3T3 cell line using MTT assay showed a percentage of cell viability of 92.2% at 125µg/ml which decreased with increase in concentration of leaf extract. Anticancer activity of leaf extracts of *S. roxburghiana* on HepG2 liver cancer cell line showed potent cytotoxic activity. The inhibition percentage with regard to cytotoxicity was found to be 81 % at 500 µg/ml which was comparable to positive control PC- cyclophosphamide, that showed a cytotoxicity of 85% followed by 250 µg/ml and 125 µg/ml which showed 70.8% and 57.3% respectively. Therefore the minimum effective concentration of methanol extract of leaves that was non-toxic to 3T3 cells but toxic to HepG2 cells (IC_{50}) was recorded at a concentration lesser than 100µg/ml of the plant extract.

Keywords: HepG2, Anticarcinogen, Cyclophosphamide, MTT, Cytotoxicity

INTRODUCTION

Cancer is one of the most life-threatening diseases with more than 100 different types. Due to lack of effective drugs, expensive cost of chemotherapeutic agents and side effects of anticancer drugs, cancer can be a cause of death. Therefore efforts are still being made for the search of effective naturally occurring anticarcinogen that would prevent, slow or reverse cancer development. Plants have a special place in the treatment of cancer. It is estimated that plant derived compounds one or the other way constitute more than 50% of anticancer agents [1,2]. *Sansevieria roxburghiana* belongs to the family *Dracanaceae*, commonly referred to as bowstring hemp, piles root[3] and Jaang Mattai in Tamil (Vernacular). The medicinal uses of *Sansevieria* species include treatment for abdominal pains, ear ache, diarrohea and hemorrhoids[4]. *Sansevieria* species are known for its anti-inflammatory activity[5], analgesic property[6], antioxidant and antimicrobial activity[7,8]. Antitumor activity of *S. roxburghiana* rhizome against Ehrlich ascites carcinoma in mice studied by Haldar[9] showed that *S. roxburghiana* rhizome extracts exhibited remarkable antitumor activity in Swiss mice that is attributed to its augmenting endogenous antioxidant mechanisms. Thus, the present study was done to evaluate the anticancer potential of the leaves of *S. roxburghiana*.

MATERIALS AND METHODS

Collection of plant material

Healthy, disease free leaves of *Sansevieria roxburghiana* were collected from the garden of Government Arts and Science College, Nandanam, Chennai, (India). The plants were authenticated at the Department of Botany. Washed and air dried fresh leaves were cut into small pieces and pulverized in a domestic blender.

Preparation of methanol extracts

10g of pulverized leaf material was mixed with 100 ml of methanol solvent and kept in rotary shaker at 100 rpm overnight and filtered with Whatman No.1 filter paper and concentrated to dryness at 40º C, lyophilized and stored at 4º C until further use. Different concentrations of the methanolic extracts (500, 250, 125 µg/ml) were prepared in 5% Dimethyl Sulfoxide (DMSO) for determining cytotoxicity.

Cell viability assay on 3T3 cells

3T3 cells were obtained from King Institute of Preventive Medicine, Chennai, India was used to determine the non-toxic dose of the plant extract[10,11]. The 3T3 cells were grown in a 96-well plate in Delbucco's Minimum essential medium (DMEM) (HiMedia, Mumbai) supplemented with 10% fetal bovine serum (Gibco Laboratories) and antibiotics (streptomycin, penicillin-G, kanamycin, amphotericin B). About 1 mL cell suspension (10^5 cells/mL) was seeded in each well and incubated at 37^0 C for 48 hour in 5% CO_2 for the formation of confluent monolayer. The monolayer of cells in the plate was exposed to various dilutions of the extract (500, 250 and 125µg). The cell viability was measured using MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Promega, USA) with MTT (5 mg/ml) and DMSO. This tetrazolium salt is metabolically reduced by viable cells to yield a blue insoluble Formosan product measured at 570nm spectrophotometrically. Controls were maintained throughout the experiment (untreated wells as negative control. The assay was performed in triplicate for each of the extracts. The mean of the cell viability values was compared to the control to determine the effect of the extract on cells and % cell viability was plotted against concentration of the plant extract.

Anticancer activity on liver cancer cell lines

The anticancer activity of methanol extracts of leaves of *S. roxburghiana* was performed on HepG2 cancer cell lines obtained from NCCLS, Pune, India. The cell viability was measured using MTT assay as described above. Controls were maintained throughout the experiment (untreated wells as negative control). The assay was performed in triplicate for each of the extracts. The mean of the cell viability values was compared to the control to determine the effect of the extract on cells and % cell viability was plotted against concentration of the plant extract. The minimum concentration of plant extract that was non-toxic to 3T3 cells but toxic to HepG2 cells was recorded as the effective drug concentration.

Statistical analysis

The experimental data were expressed as mean ± SEM. The significance of difference among the various treated groups and control group were analyzed by means of one-way ANOVA. The level of significance was set at $p < 0.05$. IC_{50} (inhibitory concentration which caused 50% inhibition) were estimated using linear regression method of plots of the percent of cell viability against the concentration of the tested compounds using Microsoft Excel Software Programme.

RESULTS AND DISCUSSION

Results of cell viability assay on normal 3T3 cell line are shown in Table1 and Fig 1. The non toxic dose of the methanol extract of leaves of *S. roxburghiana* on normal 3T3 cell line showed that the percentage with regard to viability of cells was found to be 92.2% at a concentration of 125 µg/ml which decreased with increase in concentration. Results of anticancer activity on HepG2 liver cell line is shown in Table 2. The extract showed a potent cytotoxic activity against HepG2 liver cancer cell line. (PC- cyclophosphamide served

as a positive control and 85% cancer inhibition was observed (Table 2 and Fig 2e). The concentration of leaf extract at 500 µg/ml showed inhibition percentage with regard to cytotoxicity of 81.6 %, that was comparable to the positive control. Leaf extracts at 250 µg/ml and 125 µg/ml showed cytotoxic activity of 70.8% and 57.3% respectively (Fig 2c, 2b).

Morphological changes of drug treated cells were examined using an inverted microscope and compared with the cells serving as control. (Fig 2a). These observations may be due to the presence of active biological compounds[12,13]. Therefore the minimum effective concentration of methanol extract of leaves that was non-toxic to 3T3 cells but toxic to 50% HepG2 cells was recorded (IC_{50}) at a concentration lesser than 100µg/ml of the plant extract (Fig 1). There are previous reports on the antitumor[9] as well as anticancer[14] activities of rhizome of *S. roxburghiana* .To the best of our knowledge no studies have been reported on the anticancer activity of leaves of *S. roxburghiana*. Therefore the present study has been undertaken to identify this plant leaves as a source of anticancer agent.

Table 1: Cell viability assay on 3T3 cell line

S.No	Concentration(µg)	O.D.	Percentage of cell viability	Percentage of cytototoxicity
1.	Control	0.901±0.32	100	0
2.	125	*0.837±0.14	92.89678	7.10322
3.	250	*0.717±0.07	79.57825	20.42175
4.	500	*0.636±0.39	70.58824	29.41176

Values are the mean three of replicates. * Experimental groups are compared with control group (p <0.05)

Table 2: Anticancer activity on HepG2 liver cell line

S.No	Concentration(µg)	O.D.	Percentage of cell viability	Percentage of cytotoxicity
1.	Negative Control	1.833±0.22	100	0
2.	125	*0.782±0.14	42.6623	57.3377
3.	250	*0.535±0.09	29.18712	70.81288
4.	500	*0.338±0.51	18.43972	81.56028
5.	Positive Control	*0.274±0.32	14.94817	85.05183

Values are the mean of three replicates. * Experimental groups are compared with control group (p <0.05)

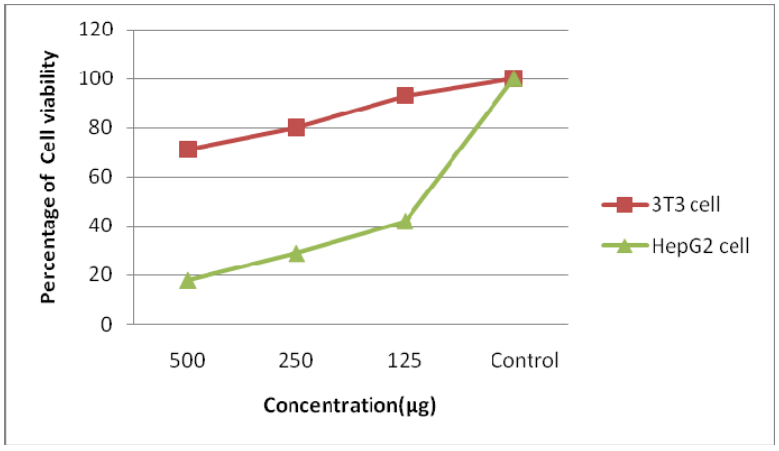
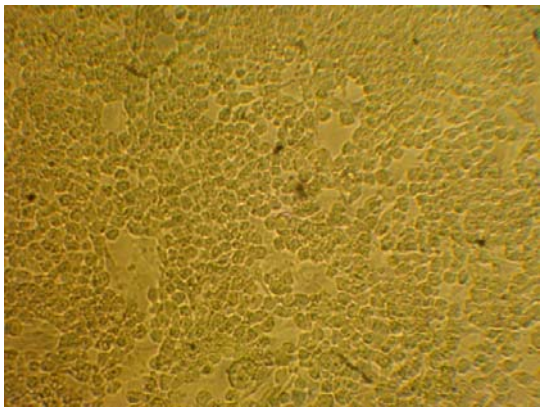
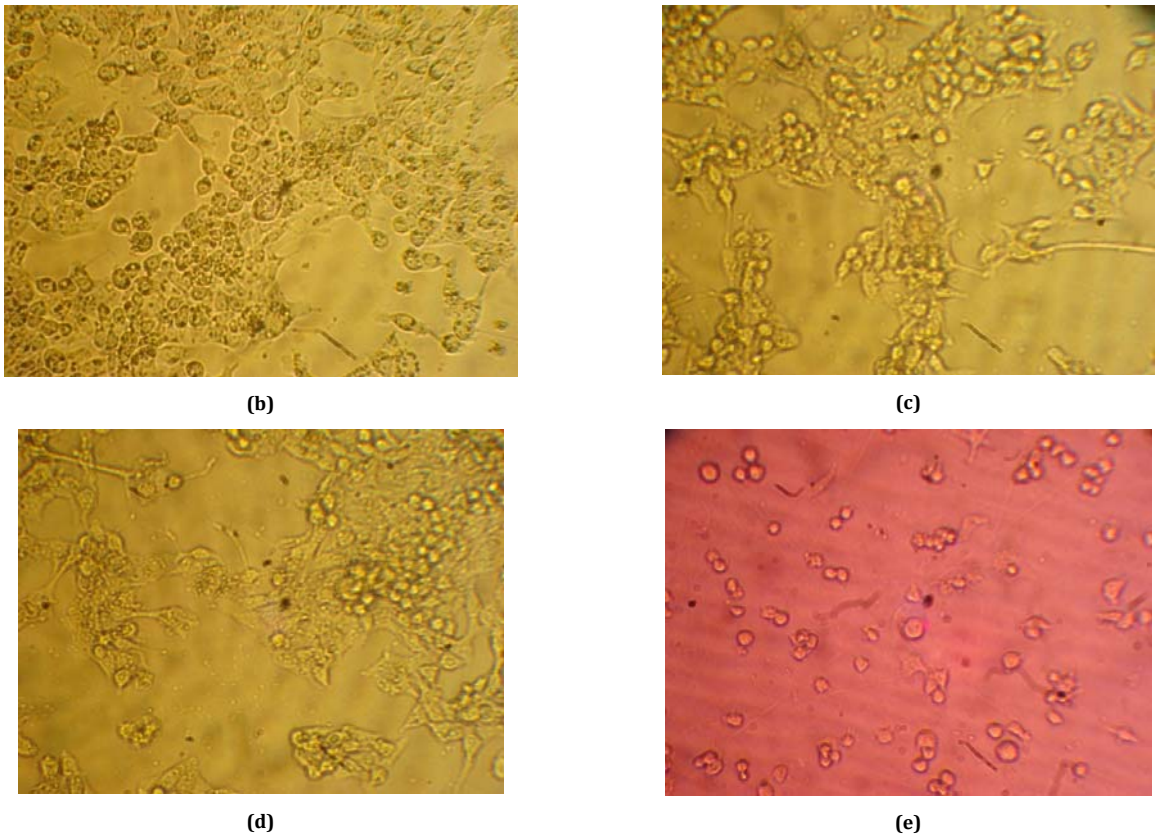


Fig. 1: The plot of percentage of cell viability Vs concentration shows the effective drug concentration as less than 100µg/ml, that is non toxic to 3T3 cell line but toxic to 50% of HepG2 cell line.



(a)



(b) **(c)**

(d) **(e)**

Fig. 2: Photomicrograph of HepG2 cell line a) negative control, b) Cells treated with 125µg/ml of methanol extract, c) Cells treated with 250 µg/ml of methanol extract, d) Cells treated with 500 µg/ml of methanol extract of leaves of *S. roxburghiana*, e) PC- cyclophosphamide-positive control.

CONCLUSION

The results of this study shows that the methanolic leaf extract of *S. roxburgiana* extract at 125 µg/ml was non-toxic to normal cells and also had both anticancer and anti-proliferative activities against cancerous cell. This study points to the probable anticancer potentials of methanol leaf extracts of *S.roxburghiana.* There is a need for further investigation of this plant in order to identify and isolate its active anticancer principle(s). The results of the study will also need to be confirmed using *in vivo* models.

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