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Research Article

IN VITRO CYTOTOXIC ACTIVITIES OF *CALOTROPIS PROCERA* LATEX AND FLOWER EXTRACTS AGAINST MCF-7 AND HeLa CELL LINE CULTURES

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ABSTRACT

Calotropis procera is a wild growing plant with multifarious medicinal properties. The present study was carried out to evaluate the effect of dried latex (DL) and flowers of *C.procera* and its ethanolic extracts against MCF-7 and HeLa cell line cultures. The MTT assay developed by Mosmann, it was modified and used to determine the inhibitory effects of test compounds on cell growth *in vitro.* Different concentrations of test compounds were used (Sample: 1 DL extract, Sample: 2 Flower extract) (4, 8, 16, 32, 64, 128, 256 and 512 µg/ml) in triplicates to achieve a final volume of 100 µl and then cultured for 48 hr. The standard (Tamoxifen) was prepared as 1.0 mg/ml concentration stock solutions in PBS. Culture medium and solvent were used as controls. The standard drug tamoxifen inhibits 60.46% breast cancer (MCF-7) cells when treated at 512 µg/ml concentration. At initial concentrations of the standard drug, the percent inhibition was reduced whereas both the samples: 1 and 2 displayed a dose-dependent inhibition at the tested concentrations. The ethanolic extract of DL and flowers showed cytotoxic properties against both MCF-7 and HeLa cells in a dose dependent manner.

Keywords: *Calotropis procera,* MCF-7 cell line, HeLa cell line, *In vitro* cytotoxicity

INTRODUCTION

Calotropis procera (Ait.)R.Br. (Asclepiadaceae) commonly known, as Arka' is a popular medicinal plant found throughout the tropics of Asia and Africa and is used in many traditional systems of medicine. Important factors of the various parts of this plant have been widely reported. In traditional medicinal system, it has been used for a variety of diseases condition that include leprosy, ulcers, tumors and piles[1].The plant has also been used as an emetic and purgative. Besides, it has been shown to exhibit spasmogenic and carminative properties[1, 2]. The flowers and every part of this plant contain latex in abundance[3]. Different parts of the *Calotropis procera* are used in treatment of various diseases in folk medicine, and their effects were confirmed by various scientific experiments[4]. Besides, the chloroform extract of the roots has been shown to possess anti-inflammatory and analgesic properties[5]. The anti-inflammatory and analgesic properties were exhibited by the latex of the plant which is abundant in the aerial parts[6, 7]. The latex also exhibits free radical scavenging and antioxidant properties[8, 9], in addition to its application as an antidote for snake poisoning[10]. It also showed antitumor activity tested in trials using different tumor cell lines and it was able to inhibit the development of cell lines HL-60 (leukemia), HCT-8 (colon cancer), MDA-MB-435 (Breast cancer) and F295 (cell cancer of the brain) when analyzed[11]. This study showed the *in vitro* cytotoxic activities of ethanolic extract of dry latex and flowers of *Calotropis procera* on MCF-7 and HeLa cell lines.

MATERIALS AND METHODS

Plant Material

The flowers and latex were collected in month of September from-2010 out fields of Agiripalli Mandal, Krishna district, identified and herbarium specimen was deposited in the department of Pharmacognosy with specimen No.NRI/COL/P.COG/1/PF (Flowers) and NRI/COL/P.COG/2/PL (Latex). The latex was collected from the aerial parts of the plant growing in wild, it was air dried under shade at ambient temperature and ground to small granules of dried latex (DL).

Preparation of extracts

The shade-dried powder of flowers was subjected to extraction in soxhlet extractor with 70% EtOH for 70 hours (extract yield: 9%) and Dry latex (DL) was macerated with 70% EtOH and extract (extract yield: 4.5%) was collected. Both extracts were evaporated to dryness and stored at 4 ◦ C until used.

In Vitro Cytotoxicity Studies

Maintenance of Cell Lines

Breast cancer (MCF-7) cells and carcinoma of cervix (HeLa) cells were maintained in Dulbecco's modified essential medium (DMEM) supplemented with 4.5 g/L glucose and 2 mM L-glutamine and 5% foetal bovine serum (FBS, Growth medium) at 37°C in 5% CO_2 incubator.

Cell culture

Carcinoma of cervix (HeLa), breast, melanoma and thyroid cells were maintained in Dulbecco's modified essential medium (DMEM) supplemented with 4.5 g/L glucose, 2 mM L-glutamine and 5% fetal bovine serum (FBS, Growth medium) at 37°C in 5% CO_2 incubator.

MTT assay

The MTT assay developed by Mosmann was modified and used to determine the inhibitory effects of test compounds on cell growth *in vitro* [12]. In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate at a density of $5x10^3$ cells well in growth medium and cultured at 37°C in 5% CO_2 to adhere. After 48hr incubation, the supernatant was discarded and the cells pretreated with growth medium, subsequently mixed with different concentrations of test compounds (Sample: 1 DL extract, Sample: 2 Flower extract) (4, 8, 16, 32, 64, 128, 256 and 512 µg/ml) in triplicates to achieve a final volume of 100 µl and then cultured for 48 hr. The standard (Tamoxifen) was prepared as 1.0 mg/ml concentration stock solutions in PBS. Culture medium and solvent were used as controls.

Each well then received 5 µl of fresh MTT (0.5mg/ml in PBS) followed by incubation for 2hr at 37°C. The supernatant growth medium was removed from the wells and replaced with 100 µl of DMSO to solubilize the colored formazan product. After 30 min incubation, the absorbance (OD) of the culture plate was read at a wavelength of 570 nm on an ELISA reader, Anthos 2020 spectrophotometer.

RESULTS AND DISCUSSION

Carcinoma of breast

Results in table 1 showed that the standard drug tamoxifen inhibits 60.46% breast cancer (MCF-7) cells when treated at 512 µg/ml concentration. At initial concentrations of the standard drug, the

percent inhibition was reduced whereas both the samples: 1 and 2 displayed a dose-dependent inhibition (Table 2 and 3) at the tested concentrations. However, more than 50% inhibition was not observed in both the cases.

Carcinoma of cervix

It has been observed that the percentage inhibitory activity of the two samples 1 and 2 tested against carcinoma of cervix (HeLa cells) yielded much lower percentage inhibition than the standard drug tamoxifen. Results in table 4 showed that more than 50% inhibition was observed at 128 µg/ml concentration of the standard drug, whereas both the samples at highest tested dose displayed 41-43% inhibition only (Table 6 and 5).

The present study was carried out to evaluate the in vitro cytotoxic activities of ethanolic extract of both DL and flower of *C.procera* on MCF-7 and HeLa cells. However, from the graphs (Fig. 1, Fig. 2) it was evident that a dose-dependent relationship was observed for all the samples under study. This shows that the samples have some growth inhibitory characteristics against carcinoma of breast cancer and cervix cell line (Fig. 3). Inhibitory data as well as comparative graphs are also provided. Smit et al reported that *C. procera* flower extract showed the potent *in vitro* cytotoxic activity, which is comparable to standard anticancer drug cisplatin[13]. Another report explained that latex of *C. procera* exhibits cytotoxic properties like standard anticancer drugs podophyllotoxin and cyclophosphamide[14]. The latex has also showed cytotoxicity corroborated by morphological alterations in leukemia cells, such as chromatin condensation, DNA fragmentation and cell volume reduction[15, 16]. Another study reported that, X15-*myc* transgenic mice treated with DL (400mg/kg) for a period of 15 week, protected mice from malignant changes occurring in liver while sinusoidal architecture and cellular integrity were slightly disrupted as compared to normal and hydropic changes were observed. DL produced a significant decrease in the serum vascular endothelial growth factor (VEGF) levels of X15-*myc* transgenic mice. The methanolic extract (ME) of DL was induced cell death in cell lines viz., Huh-7 and COS-1 cells. It was evaluated by using tetrazolium (MTT) assay. The ME was subjected to silica gel G step column chromatography using a combination of non-polar and polar solvents and 11 fractions were obtained. Out of 11 fractions, fraction 8 exhibited potent cytotoxic effect on both the cell lines. However, a marginal effect on the killing of non-cancerous cell lines suggested a high degree of selectivity for transformed cells. Such differential killing of cancerous cells could relate to their altered metabolic status and/or membrane properties. The cytotoxic effect of DL was accompanied by intracellular fragmentation of target cell DNA[17].

Our study reported that ethanolic extract of DL and flowers showed cytotoxic properties against both MCF-7 and HeLa cells in a dose dependent manner.

As per our knowledge, our studies reported that both ethanolic extracts of DL and flower showed cytotoxic effects on estrogen positive cell line (i.e.MCF-7) and carcinoma of cervix (i.e. HeLa cells). The cytotoxic activities of *C. procera* may be due to the presence of various phytoconstituents such as cardenolides, flavonoids and other constituents[18-21]. However, the molecular mechanism for such an effect needs further evaluation.

Table 1: Percentage of inhibition of breast cancer (MCF-7) cells with respect to standard concentration of tamoxifen

Conc(µg/ml)	Observed OD	%Viability	%Inhibition
4	1.445	97.18	2.82
8	1.361	91.53	8.47
16	1.149	77.27	22.73
32	1.106	74.38	25.62
64	1.093	73.50	26.50
128	0.976	65.64	34.36
256	0.627	42.17	57.83
512	0.588	39.54	60.46

Control OD: 1.487
% Viability = Observed OD/Control OD * 100
% Inhibition = 100%viability

Table 2: Percentage of inhibition of breast cancer (MCF-7) cells with respect to test sample: 1(DL extract)

Conc. (µg/ml)	Observed OD	%Viability	%Inhibition
4	1.361	91.53	8.47
8	1.287	86.55	13.45
16	1.202	80.83	19.17
32	1.165	78.35	21.65
64	1.035	69.60	30.40
128	0.987	66.38	33.62
256	0.942	63.35	35.65
512	0.802	53.93	46.07

Control OD: 1.487
% Viability = Observed OD/Control OD * 100
% Inhibition = 100%viability

Table 3: Percentage of inhibition of breast cancer (MCF-7) cells with respect to test sample: 2 (Flower extract)

Conc. (µg/ml)	Observed OD	%Viability	%Inhibition
4	1.305	87.76	12.24
8	1.211	81.44	18.56
16	1.184	79.62	20.38
32	1.133	76.19	23.81
64	0.933	66.78	33.22
128	0.961	64.63	35.37
256	0.912	61.33	38.67
512	0.838	56.36	43.64

Control OD: 1.487
% Viability = Observed OD/Control OD * 100
% Inhibition = 100% viability

Table 4: Percentage of inhibition of carcinoma of cervix (HeLa cells) with respect to standard concentration of tamoxifen

Conc(µg/ml)	Observed OD	%Viability	%Inhibition
4	2.097	93.03	6.97
8	2.084	92.46	7.54
16	1.988	88.20	11.08
32	1.564	69.39	30.61
64	1.249	55.41	44.59
128	1.015	45.03	54.97
256	0.954	42.32	57.68
512	0.815	36.16	63.84

Control OD: 2.254
% Viability = Observed OD/Control OD * 100
% Inhibition = 100% viability

Table 5: Percentage of inhibition of carcinoma of cervix (HeLa cells) with respect to test sample: 1(DL extract)

Conc(µg/ml)	Observed OD	%Viability	%Inhibition
4	2.152	95.47	4.53
8	1.956	86.78	13.22
16	1.889	83.81	16.19
32	1.755	77.86	22.14
64	1.602	71.07	28.93
128	1.445	64.11	35.89
256	1.369	60.74	39.26
512	1.265	56.12	43.88

Control OD: 2.254
% Viability = Observed OD/Control OD * 100
% Inhibition = 100% viability

Table 6: Percentage of inhibition of carcinoma of cervix (HeLa cells) with respect to test sample: 2 (Flower extract)

Conc(µg/ml)	Observed OD	%Viability	%Inhibition
4	2.164	96.01	3.99
8	1.789	79.37	20.63
16	1.651	73.25	26.75
32	1.502	66.64	33.36
64	1.466	65.07	34.96
128	1.401	62.16	37.84
256	1.388	61.58	38.42
512	1.311	58.16	41.84

Control OD: 2.254
% Viability = Observed OD/Control OD * 100
% Inhibition = 100% viability

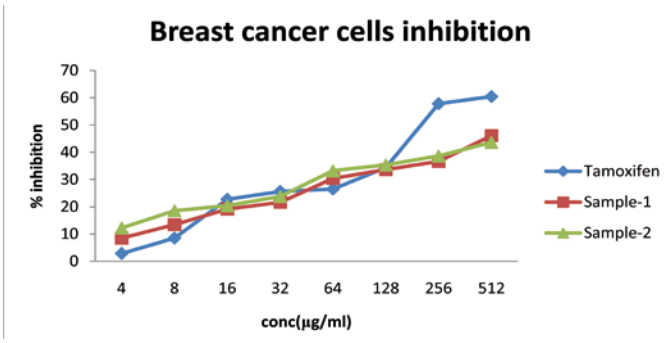

Fig. 1: Shows inhibition of breast cancer cells (MCF-7) by sample-1 and 2 with comparison to standard tamoxifen

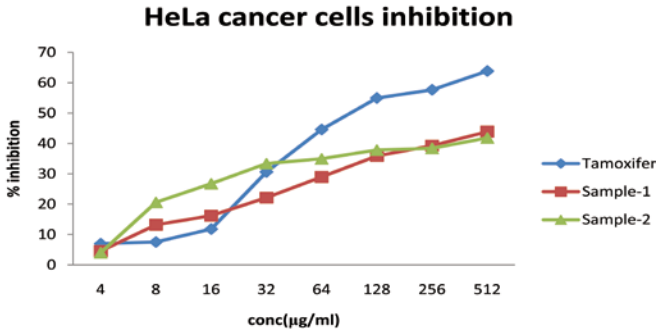

Fig. 2: Shows inhibition of carcinoma of cervix (HeLa cells) by sample-1 and 2 with comparison to standard tamoxifen

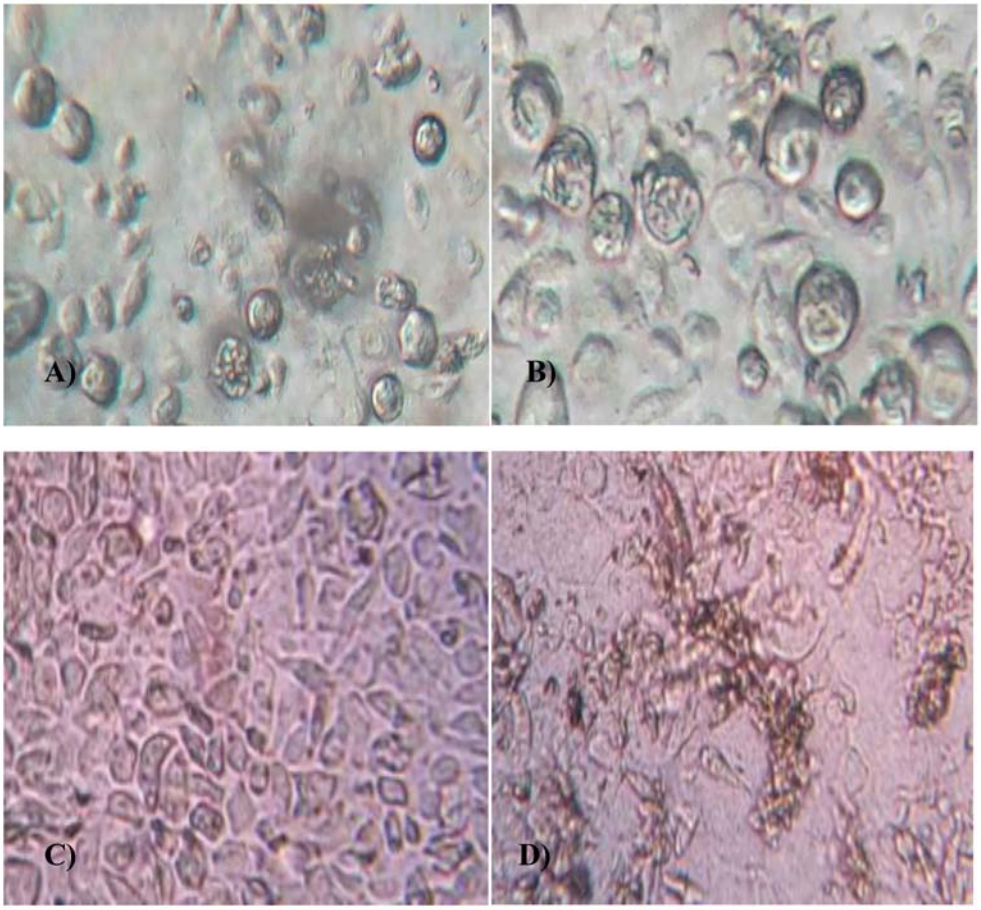

Fig. 3: Shows A) MCF-7 cells before treatment showing actively dividing cells, B) MCF-7 cells after treatment with sample-1 at 512µg/ml concentration incubated for 24hrs showing uncharacterized bodies, C) HeLa cells before treatment with samples or drug showing active cells grown in DMEM in a 96 well plate, D) HeLa cells after treatment with sample-1 at highest tested dose (512 µg/ml) showing unchracterized bodies. (All photographed viewed under inverted microscope)

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