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RESEARCH ARTICLE

In vitro Anti-Cancer Study of *Vitis viniferae, Ixora coccinea* and *Piper longum* Extract on Human Prostate Carcinoma Cells

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ABSTRACT:
Because of their chemopreventive action, herbal constituents have got some attention and effectiveness in cost to reduce cancer. Cancer is one of the leading causes of death worldwide. Prostate cancer has become the second highest cause of cancer death and most common malignancy in Western world. The aim of present work is to find out the *invitro* cell line studies the polyherbal extract constituents on on LN-CAP human Prostate cancer cells. Here 3 drug extracts-*vitis viniferae*-Grape seed(GSE), *Ixora coccinea* flower and *Piper longum* root extract were investigated for their anti-cancer effects against prostate cancer treatment. LN-CAP cells were cultured in DMEM supplemented with1% penicillin, 10% inactivated Fetal Bovine Serum (FBS), humidified atmospheric condition of 5% CO2 at 37°C. By constructing a dose-response curve the IC_{50} of a drug can be determined. The percentage inhibition of, *Piper longum* (10–640 µg/ml), *vitis viniferae* (10–640 µg/ml)and *Ixora coccinea* (10–640 µg/ml) treatment of cells resulted in 10–58%, 3-53% and 1-55% growth inhibition respectively. In a concentration and time-dependent manner, cell proliferation decreased. In conclusion, results showed that all drug extracts exhibits cytotoxic effects to LN-CAP cells and it could be a potential anticancer drug against prostate cancer cells. Further studies are needed to carry out to confirm the action of drug extracts.

KEYWORDS: LN-CAP cells, *Vitis viniferae*, *Ixora coccinea* and *Piper longum, anticancer activity.*

INTRODUCTION:
Most frequently diagnosed malignancy in males and the second leading cause of cancer-related death in men is prostate cancer. American Cancer Society reported an total of 234, 000 newly diagnosed prostate cancers in 2006 and d 27,000 prostate cancer–related deaths[(1)]. Survival rate for advanced stages has not significantly improved during the past decade, despite significant advances in prostate cancer treatment[(2)]. Loss of androgen dependency for androgen therapy observed in prostate cancer patients leads to high mortality in advanced prostate cancer[(3)].

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Therefore slowing disease progression, to improve the general quality of life and to reduce side effects with standard medicines herbal medicines were adopted[(4)]. Thus herbal remedicines as a new drug search with less side effects is needed to control and treat the disease. All 3 plant(*Piper longum* root, *vitis viniferae*-Grape seed (GSE), *Ixora coccinea* flower and) extracts in earlier report showed anticancer activity[(5,6,7)] as because of alkaloid, phenolic components, flavonoids active constituents respectively. These some reports also proved effective in suppressing 58.77% tumour formation in not only in LNCAP cells but also in MCF-7 and PC-3 prostate cancer cell.

MATERIAL AND METHODS:
Roots of *piper longum*, flowers of *Ixora coccinea*, are collected in the month of May from Kerala and dried seeds of *Vitis viniferae* were procured as fresh grapes from Bangalore farmers and shade dried. Microscopica and morphological and characters of the plant was first identified in the different literature review with

description given[11,12,13]. Authentication of plants were done by Prof. M.D. Rajanna at Botonical garden, University of agricultural sciences, GKVK, Bangalore, Karnataka. All the plant materials were dried in shade and reduced to powder separately and stored in airtight containers at room temperature. All the plant parts were extracted with ethanol separately by soxhlet extraction.

Method[14,15]:

CO_2 incubator, 70%, Ethanol, MTT Powder[3-{4, 5-dimethylthiazol-2-yl}-2, 5-diphenyltetrazolium bromide], DMEM (Dulbecco's Modified Eagle's Medium), Microplate reader (Tecan). Cell lines and culture medium. Stock cells was cultured in 10% inactivated Fetal Bovine Serum (FBS), 1% penicillin (100IU/ml) with DMEM supplemented and streptomycin (100µg/ml) at 37°C in an humidified atmosphere of 5% CO_2. The cell was dissociated with TPVG (Trypsin Phosphate Versene Glucose)solution. Using trypan blue the viability of the cells are checked (dye) and centrifuged. Further, 50,000 cells/well of L929 (mouse fibroblast cell line: Adherent cells) was seeded in a 96 well plate and incubated for 24 hrs with 5% CO2at 37°C, incubator.

Cell line:

Human prostate cancer cell line (LNCAP) was procured from ATCC (American Type Culture Collection).

Cell proliferation by MTT assay[16,17]:

Test samples were placed in each well of the 96 well microtiter culture plate. The cell count was adjusted to 5.0 x 105 cells/ml using DMEM containing 10% FBS and the L929 monolayer cells were trypsinized.

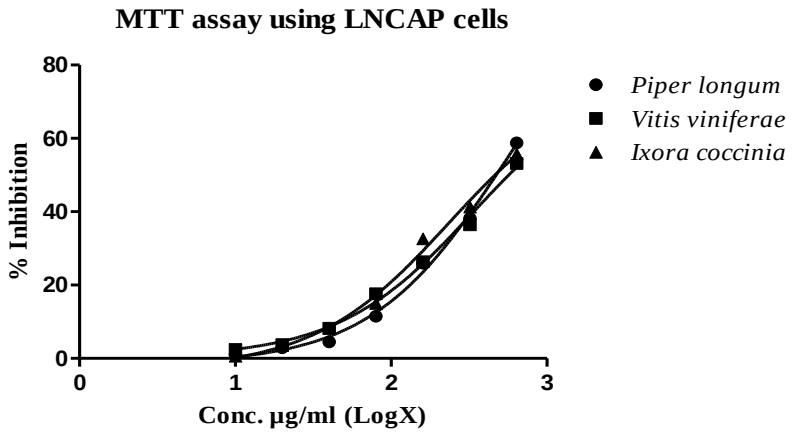
To each well of the 96 well microtiter plate, 100 µl of the diluted cell suspension (50, 000 cells/well) was seeded on each scaffold and cells seeded on cell culture plate. The plates were then incubated at 37°C for 1 day in 5% CO2 atmosphere. After 24 h the test solutions in the wells were discarded and 100 µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were gently shaken and incubated for 4 h at 37°C in 5% CO_2 atmosphere. Tetrazolium ring cleaved by the mitochondrial dehydrogenase enzymes of viable cells to an insoluble purple formazan. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the intracellular formed formazan and the absorbance was measured at a wavelength of 590 nm using a microplate reader. Inhibition was calculated using formula, Percent inhibition =(OD of control-OD of sample)X100.

RESULTS:

Cell proliferation by MTT assay: Using MTT, cytotoxic activity of all 3 extracts on human (LNCAP) prostate carcinoma cells was evaluated. By determining the concentration needed to inhibit half of the maximum biological response of the agonist IC50 values can be calculated for a given antagonist. Figure 1 shows that *Piper longum* having highest percentage inhibition (58.77%) than *Ixora coccinea* (55.76%) and *Vitis viniferae* (53.19%) at the dose of 120.5µg/ml, 139.6µg/ml and 110µg/ml, respectively.

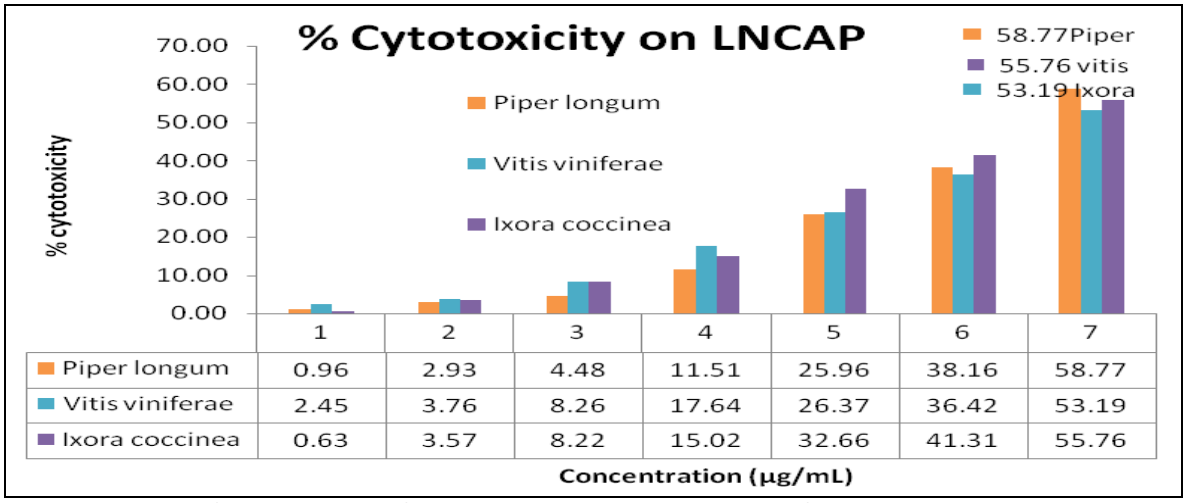
Table No:1 *In-vitro* cell line studies on LNCAP cells showing percentage inhibition and IC50 values of ethanolic extracts of *Piper longum, Ixora coccinea* and *Vitis viniferae.*

LNCAP		**Invitro cell line studies on LNCAP**		
Sample	**Conc. µg/ml**	**OD at 590nm**	**% Inhibition**	**IC50µg/ml**
Control	0	0.511	0.00	551.3
Ethanolic extract of *Piper longum*	10	0.506	0.96	
	20	0.496	2.93	
	40	0.488	4.48	
	80	0.452	11.51	
	160	0.378	25.96	
	320	0.316	38.16	
	640	0.211	58.77	
Ethanolic extract of *Vitis viniferae*	10	0.499	2.45	334
	20	0.492	3.76	
	40	0.469	8.26	
	80	0.421	17.64	
	160	0.376	26.37	
	320	0.325	36.42	
	640	0.239	53.19	
Ethanolic extract of *Ixora coccinea*	10	0.508	0.63	233.9
	20	0.493	3.57	
	40	0.469	8.22	
	80	0.434	15.02	
	160	0.344	32.66	
	320	0.300	41.31	
	640	0.226	55.76	



	Piper longum	Vitis viniferae	Ixora coccinia
IC50	551.3	334.0	233.9

Figure 1: Dose response curve to calculate IC50 value of ethanolic extracts of *Piper longum, Ixora coccinea* and *vitis viniferae* on LNCAP Cell lines.



	1	2	3	4	5	6	7
Piper longum	0.96	2.93	4.48	11.51	25.96	38.16	58.77
Vitis viniferae	2.45	3.76	8.26	17.64	26.37	36.42	53.19
Ixora coccinea	0.63	3.57	8.22	15.02	32.66	41.31	55.76

Figure 2:Percent toxicity of ethanolic extracts of Piper longum, Ixora coccinea and vitis viniferae on LNCAP Cell lines.

DISCUSSION:

In different parts of world, different types of herbal drugs used as therapeutic agent. Herb derived from plant may have therapeutic importance in illness[18,19]. Plant extract were reported for anticancer potential such as *Piper longum* root extract, *Vitis viniferae* seed extract, *Ixora coccinea* flower extract. Viability of LNCAP were reduced which was confirmed by cytotoxicity activity on LNCAP by treating with above mentioned 3 herbal exttracts.In literature review, it is found that normal cells were not affected by cyotoxic study. Anticancer activity was found because of constituents present in plant extrat such as antioxidants in *Ixora coccinea*, Phenolic compounds in *Vitis viniferae*, Alkaloid in *piper longum.*

CONCLUSION:

Herbal extracts shown significant effect on cancer cells (*in vitro*) on LNCAP observed . All 3 extracts will be potential candidate for cancer therapy. Constituents responsible for the activity further more studied with specific methods.

CONFLICT OF INTEREST:

The authors confirm that this article don't have conflict of interest.

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