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AN INVITRO CYTOTOXICITY EFFECT OF *CNIDOSCOLUS CHAYAMANSA* MCVAUGH ON EAC AND DAL CELLS

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ABSTRACT

Traditional medicine has a long history of serving people all over the world, now a days the information regarding use of this medicine for cancer treatment received considerable interest.*Cnidoscolus chayamansa* also known as chaya has been used traditionaly for the treatment of cancer. The aim of the present study was to evaluate the effect of ethanolic extract of *cnidoscolus chayamansa* leaves (EECC) against EAC and DAL cell lines of their viability by trypan blue dye exclusion technique. The extracts showed moderate cytotoxic activity against both cancer and normal cell lines.

KEYWORDS: ***Cnidoscolus chayamansa*, cytotoxicity, *invitro,* Trypan blue, DAL, EAC**

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INTRODUCTION

Tumor is a mass of tissues which proliferate rapidly, spread throughout the body and may eventually cause death of the host. [1] By 2050 over 20 million new cancer cases and over 17 million cancer deaths are probable to occur in the world. [2] Chemotherapy is an effective treatment against various types of cancer either singly or in combination with surgery and/or radiotherapy. However, chemotherapeutic effects of most of the drugs showed limited efficacy due to the development of various side effects. This fostered our attempts to evaluate some plant products against cancer as they are less likely to cause serious side effects. Many Indian spices are quoted to be useful in different types of cancer .[3, 4] As per the indigenous system of medicine,The tree spinach *Cnidoscolus chayamansa* McVaugh, (Euphorbiaceae), called "*Chaya*" in south Texas, is popular in Mexico and central America and has been introduced into the United states (Mainly south Texas and Florida) and now presently available in and around southern part of India, for potential uses as a leafy vegetable and/ or as a medicinal plant.The edible parts of *C. chayamansa* plant which taste like spinach when cooked, provide important nutritional sources for proteins, vitamins (A and C), minerals (Calcium, iron,phosphorus), niacin, riboflavin and thiamine. Among populations that cannot afford expensive foods rich in these nutrients.[5] *C. chayamansa* traditionally has been recommended for a number of ailments including diabetes, obesity, kidney stones,hemorrhoids, acne and eye problems [6].The leaves contain mineral constituents like K, Ca, Mg, Na, Fe, Mn, Zn,Cu, flavonoids like Ametoflavone, Astragatin, Kaempferol-3O-Ruttinoside and Dihydromyricetin. Leaves also contain hydrocyanic glycosides, a toxic compound, easily destroyed by cooking, even though some people tend to eat raw *C. chayamansa* leaves, it is unwise to do so, while the nutritional value of *C. chayamansa* has been Demonstrated , although *C.chayamansa* is primarily as a food plant, it has been used therapeutically for a number of ailments such as diabetes[7] , arteriosclerosis, gallstone and high cholesterol. It is also believed that *C.chayamansa* cleans circulatory system, stimulate lactation, improve eyesight, strengthens nails, improve digestions and is a diuretic and laxative[8]. The traditional systems of Siddha and Ayurvedic medicine use this plant alone or in combination with other medicinal plants for the treatment of various diseases. A vast literature collection fails to produce a scientific evidence to prove the anti tumor activity of *C.chayamansa.* Hence the present study was taken up with intent to understand the cytotoxic nature of ethanolic extracts of *Cnidoscolus chayamansa* McVaugh on EAC and DAL cells.

MATERIALS AND METHODS

I) Plant material

The leaves of *C. chayamansa* McVaugh was collected from in and around Kanyakumari District, Tamilnadu. The plant material was taxonomically identified by Mr.V. Chelladurai research officer (Botany) CCRAS Govt. of India (Retd), Tirunelvelli, Tamil Nadu and the voucher spicemen (KMCP/kkp/CC-0288) were retained in the institute for future reference. The leaves of the plant *C. chayamansa* were dried in the shade, milled into coarse powder by a mechanical grinder and packed into soxhlet apparatus and extracted with 70% v/v ethanol in water at 75–79° C for 22 hrs. The extract obtained was evaporated at 45° C, then dried and stored in airtight container. The yield of the extract was 24.8% w/w.

II) Chemicals

Trypan blue, Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India.

III) Cells and Culture medium

EAC and DAL cells were separately propagated in the peritoneal cavity of mice by transplanting one million EAC and DAL cells per ml of Phosphate Buffered Saline (PBS).

For experimental purposes, the tumour cells were aspirated from tumour bearing mice aseptically.

IV) Preparation of Test Solutions

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

DETERMINATION OF CELL VIABILITY BY TRYPAN BLUE DYE EXCLUSION TECHNIQUE

Principle

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay was based on the assumption that the dead cells will take the dye and viable cells do not take up the dye.

Procedure

Short term cytotoxicity studies were done on EAC and DAL cells by Trypan blue exclusion method. Cells were aspirated from the peritoneal cavity of tumour bearing mice and washed in PBS twice and counted using a haemocytometer 2 X 10^5 cells/ml were taken for cell cytotoxicity studies. Different concentrations of the compound were added to the cells and then made up to 1 ml with PBS. Cells were incubated for 3 hours at 37^{o}C. After incubation, the cell death was evaluated using theTrypan blue exclusion method. To the cell suspension, 3 drops of Trypan Blue (0.5 % in PBS) were added and the cells were loaded immediately on to a haemocytometer. The number of dead cells was counted and the percentage of dead cells was calculated. Viable cells exclude the dye while non viable cells take up the dye and appear blue in colour. The percentage growth inhibition was calculated and CTC_{50} value is generated from the dose-response curves for each cell line[9].

$$\% \text{ Growth Inhibition} = 100 - \left(\frac{\text{Total Cells} - \text{Dead Cells}}{\text{Total Cells}} \right) X\,100$$

RESULTS [10]

The effect of ethanolic extluract of *Cnidoscolus chayamansa* (EECC) leaves on the growth of the two cell lines were examined by Trypan blue exclusion assay method.

Cytotoxic activity against EAC cell lines

The extracts were tested against a panel of normal and cancer cell (EAC) lines at a range of 62.5 to 1000 µg/ml. The CTC_{50} values were shown separately for normal and cancer cell lines as in table 1 and the CTC_{50} for short term study are depicted in fig.1. The EECC exhibited moderate cytotoxicity against cancer EAC cell lines, showing a higher affinity towards cytotoxicity as CTC_{50} was found below 1000 µg/ml. The photograph of the cytotoxicity of EECC against EAC cells were provided under fig. 2 (A- EAC control, B-EECC on EAC cell lines 1000 mcg/ml and C-EECC on EAC cell lines 500 mcg/ml).

Cytotoxic activity against DAL cell lines

The extracts were tested against a panel of normal and cancer cell (DAL) lines at a range of 62.5 to 1000 µg/ml. The CTC_{50} values were shown separately for normal and cancer cell lines as in table 2 and the CTC_{50} for short term study are depicted in fig.3. The EECC exhibited moderate cytotoxicity against DAL cancer cell lines, showing a higher affinity towards cytotoxicity as CTC_{50} was found below 1000 µg/ml. The photograph of the cytotoxicity of EECC against EAC cells were provided under fig. 4 (A- EAC control, B-EECC on EAC cell lines 1000 mcg/ml and C-EECC on EAC cell lines 500 mcg/ml).

Table 1
Cytotoxicity of EECC against EAC cell lines

Sl. No.	Test drug	Test Conc. in µg/ml	Viable cell number	%Growth inhibition±SD	CTC_{50} in µg/ml
1	EECC	1000	69	18.82±0.8	>1000.00
		500	71	16.47±1.5	
		250	77	9.41±0.6	
		125	80	5.88±0.3	
		62.5	82	3.53±0.4	

Figure. 1
Cytotoxic effect of EECC on EAC Cells

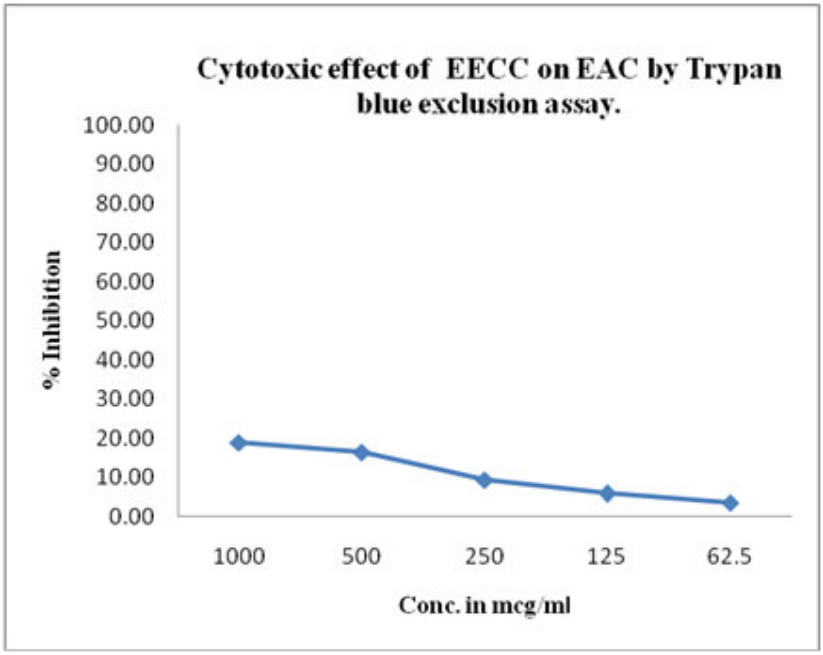


Figure 2
EAC cell lines treated with 1000C and 500C compared with EAC control
A)EAC Control B) EECC on EAC 1000C C) EECC on EAC 500C

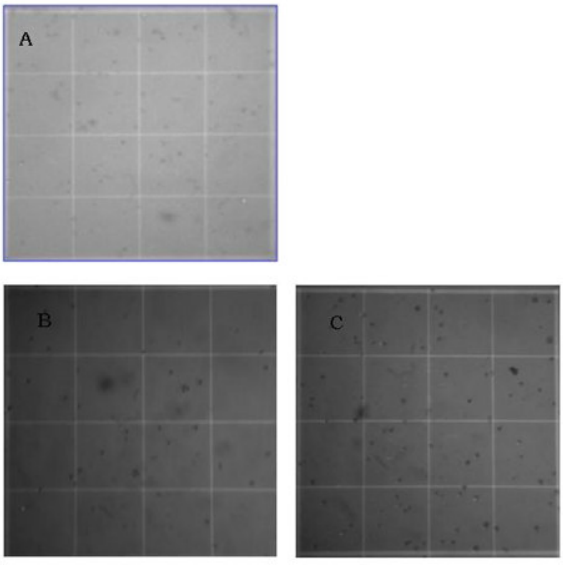


Table 2
Cytotoxicity of EECC against DAL cell lines

Sl No.	Test drug	TestConc. µg/ml	Viable cell number	% Growth inhibition±SD	CTC_{50} in µg/ml
1	EECC	1000 500 250 125 62.5	61 67 70 72 77	22.78±0.6 15.19±1.3 11.39±0.7 8.86±0.2 2.53±0.3	>1000.00

Figure 3
Cytotoxic effect of EECC on EAC Cells

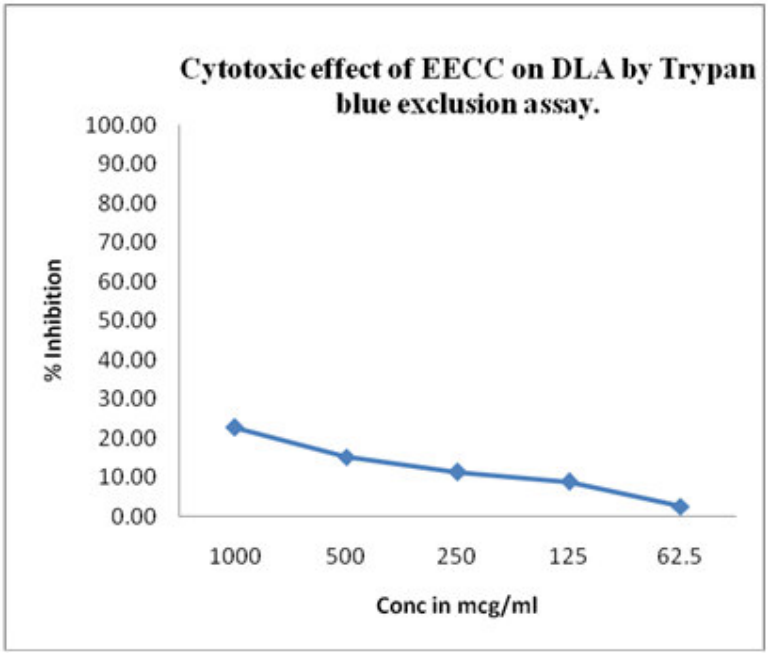
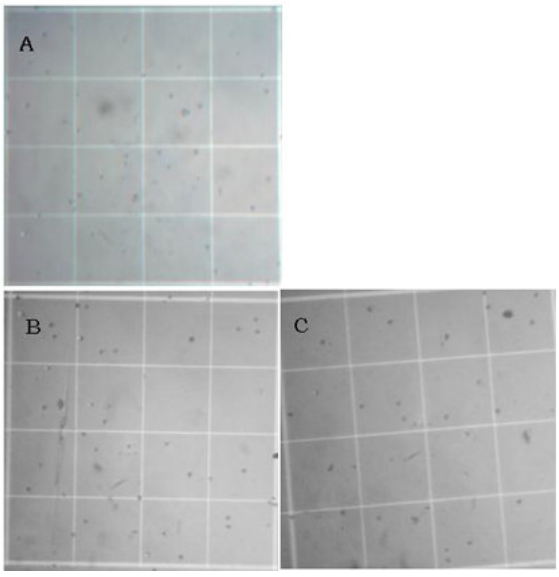


Figure 4
DAL cell lines treated with 1000C and 500C compared with DAL control
A)DAL Control B) EECC on DAL 1000C C) EECC on DAL 500C



DISCUSSION

The cytotoxic effect of the EECC was investigated using trypan blue exclusion methods. The trypan blue assay based on the assumption that the dead cells will take the dye and viable cells do not [11]. From the study, it was observed that extracts showed moderate cytotoxic against both cancer and normal cell lines. The cytotoxicity of the extract was found to be in a dose dependent and non selective as reflected by uniform CTC_{50} values independent of cell line origin. *C.chayamansa* rich in ascorbic acid and it is having a good antioxidant effect which may be due to this reason that it shows the cytotoxic effect[12].

CONCLUSION

From the above study, it was evaluated that the ethanolic extract of *cnidoscolus chayamansa* McVaugh had cytotoxic activity against EAC and DAL cell lines at the range of 62.5 to 1000 µg/ml .

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