Internationally indexed journal

Indexed in Chemical Abstract Services (USA), Index copernicus, Ulrichs Directory of Periodicals, Google scholar, CABI, DOAJ, PSOAR, EBSCO, Open J gate, Proquest, SCOPUS, EMBASE, etc.

Rapid and Easy Publishing

The “International Journal of Pharma and Bio Sciences” (IJPBS) is an international journal in English published quarterly. The aim of IJPBS is to publish peer reviewed research and review articles rapidly without delay in the developing field of pharmaceutical and biological sciences.

Indexed in Elsevier Bibliographic Database (Scopus and EMBASE)

SCImago Journal Rank 0.288
Impact factor 2.958*
Elsevier Bibliographic databases
(Scopus & Embase)

SNIP value – 0.77
SJR – 0.288
IPP – 0.479

SNIP – Source normalised impact per paper
SJR – SCImago Journal rank
IPP – Impact per publication
Source – www.journalmetrics.com
(Powered by scopus (ELSEVIER)

International Journal of Pharma and Bio Sciences

<table>
<thead>
<tr>
<th>Indicator</th>
<th>2006-2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>SJR</td>
<td>0.29</td>
</tr>
<tr>
<td>Cites per doc</td>
<td>0.51</td>
</tr>
<tr>
<td>Total cites</td>
<td>852</td>
</tr>
</tbody>
</table>

And indexed/catalogued in many more university

*Instruction to Authors visit www.ijpbs.net
For any Queries, visit “contact” of www.ijpbs.net
AN INVITRO CYTOTOXICITY EFFECT OF **Cnidoscolus chayamansa** MCVAUGH ON EAC AND DAL CELLS

**KULATHURAN PILLAI K*¹, NARAYANAN N², CHIDAMBARANATHAN N³ AND JEGAN N³**

¹Periyar Maniammai University, Thanjavur, Tamil Nadu, India.
²Jaya College of Pharmacy, Thiruninravur, Chennai, India.
³K.M. College of Pharmacy, Madurai, Tamil Nadu, India.

**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 traditionally 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

---

*KULATHURAN PILLAI K*
Periyar Maniammai University, Thanjavur, Tamil Nadu, India.

*Corresponding author*
INTRODUCTION

Tumor is a mass of tissues which proliferate rapidly, spread throughout the body and may eventually cause death of the host. By 2050 over 20 million new cancer cases and over 17 million cancer deaths are probable to occur in the world. 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. 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, C. chayamansa traditionally has been recommended for a number of ailments including diabetes, obesity, kidney stones, hemorrhoids, acne and eye problems. The leaves contain mineral constituents like K, Ca, Mg, Na, Fe, Mn, Zn, Cu, flavonoids like Ametoflavone, Astragatin, Kaempferol-3O-Rutinoside 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, 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. 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 TRYSPAN 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.

\[
\% \text{Growth Inhibition} = 100 \times \left( \frac{\text{Total Cells} - \text{Dead Cells}}{\text{Total Cells}} \right)
\]

**RESULTS**

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

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Test drug</th>
<th>Test Conc. in µg/ml</th>
<th>Viable cell number</th>
<th>%Growth inhibition±SD</th>
<th>CTC&lt;sub&gt;50&lt;/sub&gt; in µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EECC</td>
<td>1000</td>
<td>69</td>
<td>18.82±0.8</td>
<td>&gt;1000.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>71</td>
<td>16.47±1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>77</td>
<td>9.41±0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>125</td>
<td>80</td>
<td>5.88±0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>62.5</td>
<td>82</td>
<td>3.53±0.4</td>
<td></td>
</tr>
</tbody>
</table>

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

This article can be downloaded from www.ijpbs.net
Table 2

Cytotoxicity of EECC against DAL cell lines

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Test drug</th>
<th>Test Conc. µg/ml</th>
<th>Viable cell number</th>
<th>% Growth inhibition±SD</th>
<th>CTC&lt;sub&gt;50&lt;/sub&gt; in µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EECC</td>
<td>1000</td>
<td>61</td>
<td>22.78±0.6</td>
<td>&gt;1000.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>67</td>
<td>15.19±1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>70</td>
<td>11.39±0.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>125</td>
<td>72</td>
<td>8.86±0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>62.5</td>
<td>77</td>
<td>2.53±0.3</td>
<td></td>
</tr>
</tbody>
</table>

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. 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 CTC50 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 effect12.

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.

REFERENCES