Evaluation of Anticancer Activity of Vitex Leucoxylon

Authors
Nagarathna PKM¹, Shemin S Dani², K Mekhana³, Rosey Sarraf⁴, Priya Soman⁵

Email id: sheminsdani93@gmail.com

ABSTRACT
This article presents a review of evaluation of anticancer effect of methanol extract of the leaves of vitex leucoxylon linn (verbenaceae) on EAC induced solid tumour. The study sampled 30 mice, divided into 5 groups (96 in each). Ascites tumour was developed by administering Ehrlich ascites cells (0.1ml/10gm body wt of mouse, i.p) from 1x10⁶ cells. The mice were treated with steroids of Vitex leucoxylon and 5-fluorouracil which serves as standard. The several changes of antitumor potential of steroidal extract of Vitex leucoxylon were accessed. Steroids of leaves of Vitex leucoxylon treated group showed significant decrease in Total number of cells, Percentage viability, Tumour volume of Ehrlich Ascites Carcinoma in mice. From result, it was concluded that steroids of leaves of Vitex leucoxylon shows anti-tumour activity.

Key-words: Anti-tumour, 5 flurouracil, Vitexleucoxylon,

INTRODUCTION
Cancer is a major public health burden in both developed and developing countries. It was estimated that there were 10.9 million new cases, 6.7 million deaths, and 24.6 million per-sons living with cancer around the world in 2002. Cancer is the second leading cause of death in the United States, where one in four deaths is due to cancer. Plants have long been used in the treatment of cancer. The National Cancer Institute collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity. Of the 92 anticancer drugs commercially available prior to 1983 in the US and among worldwide approved anticancer drugs between 1983 and 1994, 60% are of natural origin.¹

EAC resembles human tumors which are the most sensitive to chemotherapy due to the fact that they are undifferentiated and that they have a rapid growth rate. The ideal drug being ineffective or minimally effective for normal cells have been focused on, and at this point, the usage of natural sources as an alternative cancer therapy is thought to have a great value for cancer control and programs’ destruction.²,³

A thorough and complete literature search on Vitex leucoxylon Linn was performed from the chemical abstracts, National & International journals, E-library, Internet &other research materials. It also possess the Anti inflammatory, Antipyretic activity, However the detailed mechanism of pharmacological activities of this plant has not been thoroughly investigated. So far only few studies have been carried out on this plant. Based on our preliminary findings, it was of intrest to asses the in-vivo efficacy of Vitex leucoxylon. Hence in the present study, author reported first time, the effect of new steroidal molecule of Vitex leucoxylon on in-vivo anti tumour activity.

MATERIALS AND METHODS:
Collection of Plant Materials:
The leaves of Vitex leucoxylon was collected from Thirupati forest region, Thirupati District, Andhra Pradesh, INDIA in the month of July 2011. This plant species were authenticated by Prof. L. Ramesh

Botanist Department of Pharmacognosy and Photochemistry (Padmavathi Mahila Kalasala, Tirupati) The voucher specimen was deposited in the institutional museum. The collected plant material was washed thoroughly with water to remove the adhering soil, mud, and debris. All old insect damage or fungus infected leaves, and flowers were removed. The leaves were dried in the shade at room temperature to a constant mass. The plant material was coarsely powdered into coarse powder a warring blender. The powder was stored in an airtight container and protect from light.

**Preparation of Extract:**
100gm powdered leaves parts were subjected to successive extraction in a soxhlet extractor using methyl alcohol. The extract obtained was concentrated in a rotary shaker evaporator to dryness to get constant weight.

**Isolation, Purification, Characterisation of Sterols:**

![Fig: structure of sterol.](image)

**Isolation of Sterol:**
200g of the dried powder of Vitex leucoxylon was extacted in soxhlet extractor with 800 ml methanol. The mark was pressed and the expressed solvent was mixed with the main extract. The extract was concentrated to constant weight in a rotary shaker evaporator. A darkish obtained was pooled up with the further 10 batches of similar methanol extracts from the plant. Removal of fixed oil by saponification from the methanol extract:
The method is followed British Pharmacopeia (2005). 5g of the dried methanolic extract was taken in a 500ml conical flask containing 100ml Potassium hydroxide and boiled on a water bath under reflux condenser for one hour, with frequent swirling of the contents. The contents of the flask were washed in a separator by means of 100ml distilled water and while the liquid was slightly warm, was extracted by shaking vigorously with three successive quantities, each of 100ml solvent ether, washing on the flask with the first quantity of solvent ether. The ethereal solution was mixed and filtered to a separating funnel through the fat free filter paper to remove the solid particles. The separator was rotated carefully for few minutes, without violent shaking and allowed the liquids to separate, and run off the aqueous layer. The ethereal solution was washed by shaking vigorously with two successive quantities each of 40 ml of 0.5N aqueous layer was no longer alkaline to KOH solution. The ethereal solution was transferred to a weighed flask. The ether was distilled off and 6 ml acetone was added and shaken. By the aid of gentle current of air, the solvent was completely removed from the flask, which was held obliquely and rotated almost entirely immersed in boiling water. The residue was dried to a constant weight at a temperature not above 80oC to get the un saponifiable matter. Chemical tests were conducted on the un saponifiable matter to confirm the sterols[98].

_Nagarathna PKM et al IJSRE Volume 4 Issue 2 February 2016_
**Thin Layer Chromatography of Sterols:**
Slurry of Silica gel G was prepared by mixing Silica gel G powder with water in the ratio 1:2 respectively. The slurry was spread (0.5mm) on a clean dry glass plates with help of Toshiba spreader. The plates were air dried and activated at 110oC for 30 minutes. The PRISMA system was used for the initial investigation of the separation of the mixture. Nine groups of solvents were considered, each having the solvent strength related to its polarity. The solvent giving the best resolution was then selected. The plant extract was dissolved in a small quantity of hexane and loaded on the activated TLC plates at 2.5cm from one of the ends of the plate. The spotted end of the plate was dipped in the developing chamber that was presaturated with the developing solvent for one hour. Plates were taken out of the developing chamber after the developing solvent has moved to 3/4th of the plate and solvent front was marked.

**Crystallisation of Sterol:**
Fractions showing the single pink spot of same Rf values on TLC (Fraction no. 46-53) were pooled up in a beaker and the solvent was evaporated. The residue left behind was dissolved in a small volume of methanol by gentle heating and kept aside for crystallisation at room temperature for two hours to get the white crystals.

Ehrlich ascites carcinoma (EAC) cells induced solid tumors:
Swiss albino mice were divided into five groups of 30 animals (n=6) each.
EAC cells were collected from the donor mice and were suspended in sterile isotonic saline. The viable EAC cells were counted (Trypan blue indicator) under the microscope and Tumor cells (1 × 106 cells/mouse) were injected into the right hind limb of all the animals intramuscularly to whole animals on day zero. A day of incubation was allowed for multiplication of the cells [100]. The animals were divided in to five groups:
- **Group 1**: Serves as normal control.
- **Group 2**: Serves as Ehrlich Ascites control.
- **Group 3**: Mice treated with steroid of Vitex leucoxylon (100mg/kg; orally;/day/12days).
- **Group 4**: Mice treated with steroid of Vitex leucoxylon (200mg/kg; orally;/day/12days).
- **Group 5**: Mice treated with 5Fluro-uracil serves as standard (20mg/kg; ip;/day/12days) 5Fluro-uracil was dissolved in normal saline and administered to mice (20mg/kg; ip;/day/12days). After the 12 days of treatment animals were sacrificed and in-vivo anti oxidant studies were performed.

**Results:**

**Effect on total number of cells:**
- Mice treated with low dose of steroids of Vitex leucoxylon (100mg/kg; po;/day/12days) had total number of cells significantly lower (P<0.001) when compared to total number of cells in EAC control rats.
- Mice treated with high dose of steroids of Vitex leucoxylon (200mg/kg; po;/day/12days) had total number of cells significantly lower (P<0.001) when compared to total number of cells in mice treated with low dose of steroids of Vitex leucoxylon (100mg/kg; po;/day/12days) and EAC control mice.
- Mice treated with 5FU (20mg/kg;ip;/day/12days) had total number of cells significantly lower (P<0.001) when compared to total number of cells in EAC control rats.

**Effect on Percentage viability:**
- Mice treated with low dose of steroids of Vitex leucoxylon (100mg/kg; po;/day/12days) had percentage viability cells significantly lower (P<0.001) when compared to percentage viability cells in EAC control rats.
- Mice treated with high dose of steroids of Vitex leucoxylon (200mg/kg; po;/day/12days) had percentage viability cells significantly lower (P<0.001) when compared to percentage viability cells in mice treated with low dose of steroids of Vitex leucoxylon (100mg/kg; po;/day/12days) and EAC control mice.
Mice treated with 5FU (20mg/kg; ip; /day/12days) had percentage viability cells significantly lower (P<0.001) when compared to percentage viability cells in EAC control rats.

**Effect on volume:**
Mice treated with low dose of steroids of Vitex leucoxylon (100mg/kg; po;/day/12days) had a volume significantly lower (P<0.001) when compared to volume in EAC control rats.
Mice treated with high dose of steroids of Vitex leucoxylon (200mg/kg; po;/day/12days) had volume significantly lower (P<0.001) when compared to volume in mice treated with low dose of steroids of Vitex leucoxylon (100mg/kg; po;/day/12days) and EAC control mice.
Mice treated with 5FU (20mg/kg; ip; /day/12days) had volume significantly lower (P<0.001) when compared to volume in EAC control rats.

Effect of Steroids of Vitex leucoxylon and 5 Flurouracil on Total number of cells, Percentage viability, Tumour volume of Ehrlich Ascites Carcinoma in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total no of cells 1x10^8 cells</th>
<th>Percentage Viability</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAC control(1X10^6) once. l.p</td>
<td>2.818 ±0.020</td>
<td>85.761 ± 0.451</td>
<td>6.85 ± 0.160</td>
</tr>
<tr>
<td>Steroids of Vitex leucoxylon (100 mg/kg; po/day/12days)</td>
<td>2.59 ± 0.041***</td>
<td>75.666 ± 0.921***</td>
<td>5.25 ± 0.140***</td>
</tr>
<tr>
<td>Steroids of Vitex leucoxylon (200 mg/kg; po/day/12days)</td>
<td>2.39±0.023***</td>
<td>65.662±0.625***</td>
<td>4.56±0.017***</td>
</tr>
<tr>
<td>5Flurouracil (20mg/kg;ip;/day/12days)</td>
<td>1.336±0.029***</td>
<td>12.55 ± 0.436***</td>
<td>0.566±0.055***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SEM, n = 6.

***P<0.001 compared to EAC control group

![Fig.1. Effect of Steroid of Vitex leucoxylon and 5 flurouracil on Total number of cells](image)

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*Nagarathna PKM et al IJSRE Volume 4 Issue 2 February 2016*
**DISCUSSION:**

Ehrlich Ascites cells are spontaneous murine adenocarcinoma cells adapted to Ascites form and causes increase growth of Ascites in ascitic fluid [6].

In EAC control group, a regular rapid increase in ascites was observed. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid was seen. The rate of tumor growth is dependent on the balance between the proliferative activity and death rate of the tumor cells.

The administration of steroids of Vitex leucoxylon and 5-Fluorouracil significantly decreased the Total number of cells, Tumour volume and Viable cell count, compared to EAC control group. The cell death is significantly increased in both steroids of Vitex leucoxylon at different doses (100mg/kg;200mg/kg) and 5-fluorouracil than EAC control.

In the morphology of EAC control cells, the cells and nucleolus were intact, where as steroids of Vitex leucoxylon and 5-fluorouracil treated group the cell membrane was disrupted and the nuclear material was
stained in purple colour indicating break down of chromatin and induction of cell death (cytotoxic effect) was observed. The studies on Amaranthus [7] Vitex nenuguda [8], and curcumin [9], reported the decrease in Total number of cells, Tumour volume and Viable cell count and cell death in Ehrlich ascites carcinoma. The steroids of Vitex leucoxylon induced high level of chromatin condensation, nuclear damage and cell death which may be linked to slower tumor growth.

EAC induces oxidative stress on tumour tissue by decreasing SOD, CAT antioxidant levels in tumour tissue and by increasing lipid peroxidation. Superoxide radicals may be reduced by the enzyme superoxide dismutase to form H$_2$O$_2$ and oxygen. Catalase is an enzyme which converts H$_2$O$_2$ to neutral products O$_2$ and H$_2$O$_2$[10,11]. The SOD, CAT were significantly reduced in EAC control group when compared to Normal control. Decline in the activities of SOD, CAT in EAC control in the present study, which may be due to the altered antioxidant defence system caused by enormous production of free Radicals in EAC induced carcinogens. In steroids of Vitex leucoxylon and 5 flurouracil treated group the activities of SOD, CAT were significantly increased when compared to EAC control.

The above parameters like improvement in the SOD, CAT, activity and decrease in the lipid peroxidation in solid tumour and decrease in tumor cell volume and number of viable tumor cells and total number of cells and induction of cell death in Ehrlich ascites carcinoma indicate that the effect of steroids of Vitex leucoxylon on the tumour may be due to its antioxidant and cytotoxic activities.

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