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Anti-Cancer Activity of Steroid of *Vitex Leucoxylon*

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Abstract

Objective: The aim of this study was to investigate the anti-tumour effect of the methanolic extract of the leaves of *Vitexleucoxylon* Linn (Verbenaceae).

Methods:The study sampled 30 mice, divided into 5 groups (6 in each). Ascites tumour was developed by administering Ehrlich ascites cells (0.1ml/10gm body wt of mouse,i.p) from $1x10^6$ cells. The mice were treated with steroid of *Vitexleucoxylon* and 5-flurouracil which serves as standard. The several changes of anti-tumour potential of steroidal extract of *Vitexleucoxylon* were accessed.

Results: Steroid of leaves of *Vitexleucoxylon* treated group showed significant decrease in total number of cells, percentage viability and tumour volume of Ehrlich Ascites Carcinoma in mice.

Conclusions: From results, it was concluded that steroid of leaves of *Vitex leucoxylon* showed anti-tumour activity.

Keywords

Anti-tumour, 5-flurouracil

Greentree Group

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INTRODUCTION

Cancer is one of the leading causes of mortality worldwide and the failure of conventional chemotherapy to affect major reduction in the mortality indicates that new approaches are critically needed. It is also 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. 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. Ehrlich Ascites Carcinoma (EAC) resembles human tumour which are the most sensitive to chemotherapy due to the fact that they are undifferentiated and that they have a rapid growth rate. A thorough and complete literature search on *Vitexleucoxylon* Linn was performed from the chemical abstracts, National and International journals, E-libraries, Internet and other research materials.

Vitex leucoxylon is an endemic tree found in India and Sri lanka. The pharmaceutically active extracts of vitex leucoxylon have shown hypoglycaemic and anti-inflammatory properties, Stem decoction to cure whooping cough, root bark to reduce fever, fresh twigs as insecticides dry leaf powder smoke as remedy for asthma[1]. It is used as a traditional medicine for relieving headache and catarrh however the detailed mechanism of pharmacological activities of this plant has not been thoroughly investigated. The major compound present in this plant is vitexin which is a flavonoid mainly responsible for the anti-cancer activity[2]. Based on our preliminary findings, it was of interest to find the in-vivo efficacy of *Vitexleucoxylon.* Hence in the present study, author reported first time, the effect of new steroidal molecule on *Vitexleucoxylon* in-vivo anti-tumour activity.

2. METHODS

2.1 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 was authenticated by Prof. Madhava Chetty, Botanist, Department of Pharmacognosy and Photochemistry (Padmavathi Mahila Kalasala, Tirupati). The collected plant material was washed thoroughly with water to remove the adhering soil, mud, and

debris. The leaves were dried in the shade at room temperature to a constant mass. The plant material was coarsely powdered into coarse powder and was stored in an airtight container and protect from light.

2.2 Preparation of Extract

Powdered parts of leaves (100gm) 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.

2.3 Isolation of Sterol:

Hundred grams of the dried powder of *Vitexleucoxylon* was extracted in Soxhlet extractor with 800 ml methanol. The marc 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.

2.4 Animals:

Adult male Albino mice (20-25) grams,were purchased from Indian institute of science (IISc) Bangalore. They were housed in microlon boxes in a controlled environment (temperature 25±2^{0}C and 12h dark /light cycle) with standard laboratory diet and water ad libitum. The study was conducted after obtaining institutional animal ethical committee (IAEC) clearance.

2.5 Cells:

EAC cells were obtained through the courtesy of Amala cancer research centre, Thrissur. They were maintained by weakly intra-peritoneal inoculation of 10^6cells[4].

2.6 Experimental Design:

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 Tumour cells (1×10^6 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[5].

The animals were divided in to five groups:

Group 1: Serves as normal control.

Group 2: Serves as Ehlrich Ascites control.

Group 3: Mice treated with steroid of *Vitexleucoxylon*(100mg/kg; orally;/day/12days).

Group 4: Mice treated with steroid of *Vitexleucoxylon*(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.

3. RESULTS

3.1 Effect on total number of cells:

Mice treated with low dose of steroid of *Vitexleucoxylon* (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 steroid of *Vitexleucoxylon* (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 steroid of*Vitexleucoxylon* (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.

3.2 Effect on Percentage viability:

Mice treated with low dose of steroid of *Vitexleucoxylon* (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 steroid of *Vitexleucoxylon*(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 steroid of *Vitexleucoxylon* (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.

3.3 Effect on volume:

Mice treated with low dose of steroid of *Vitexleucoxylon* (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 steroid of *Vitexleucoxylon* (200mg/kg; po;/day/12days) had volume significantly lower (P<0.001) when compared to volume in mice treated with low dose of steroid of *Vitexleucoxylon* (100mg/kg; po;/day/12days) and EAC control mice.

Mice treated with 5-FU (20mg/kg;ip; /day/12days) had volume significantly lower (P<0.001) when compared to volume in EAC control rats.

Table: 1 Effect of Steroid of *Vitexleucoxylon* and 5-flurouracil on Total number of cells, Percentage viability, Tumour volume of Ehrlich Ascites Carcinoma in mice

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

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

***P<0.001 compared to EAC control group

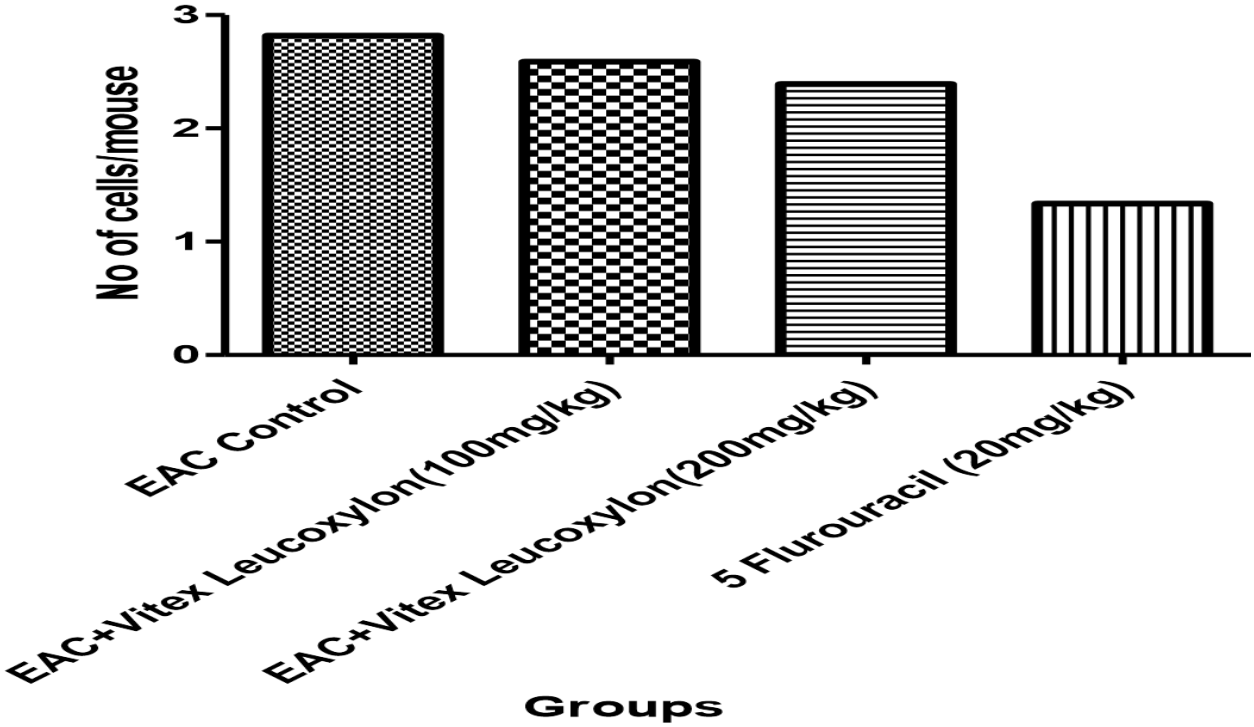

Fig.1 Effect of Steroid of Vitexleucoxylon and 5 flurouracil on Total number of cells

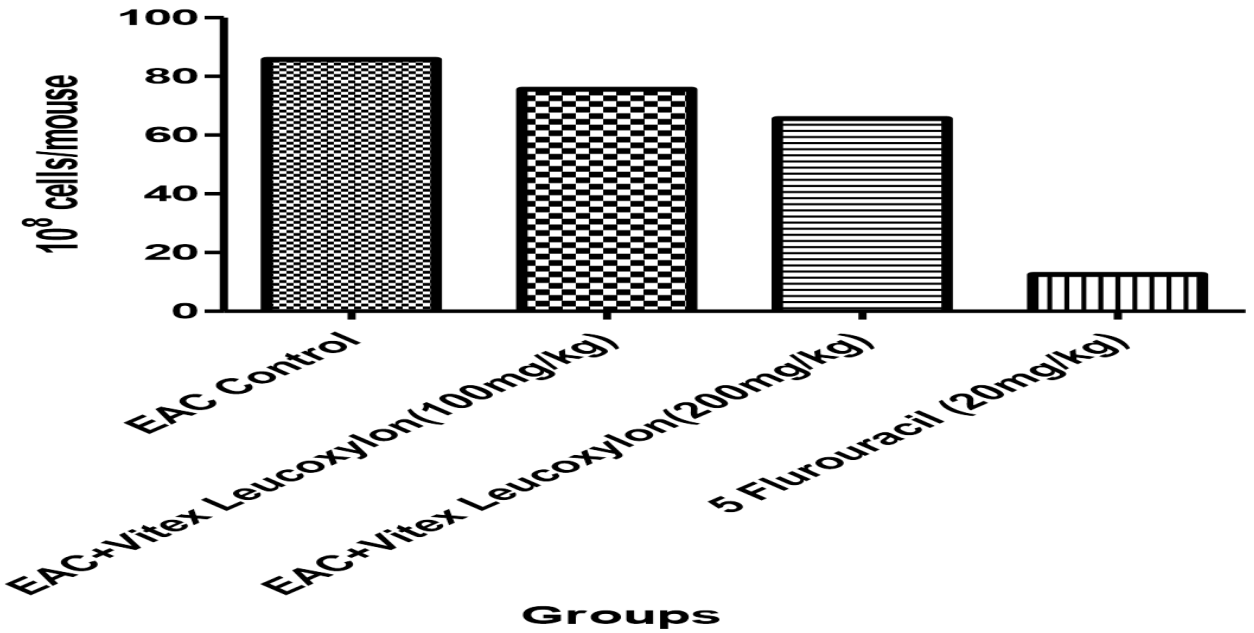

Fig.2 Effect of Steroid of Vitexleucoxylon and 5 flurouracil on Percentage Viability

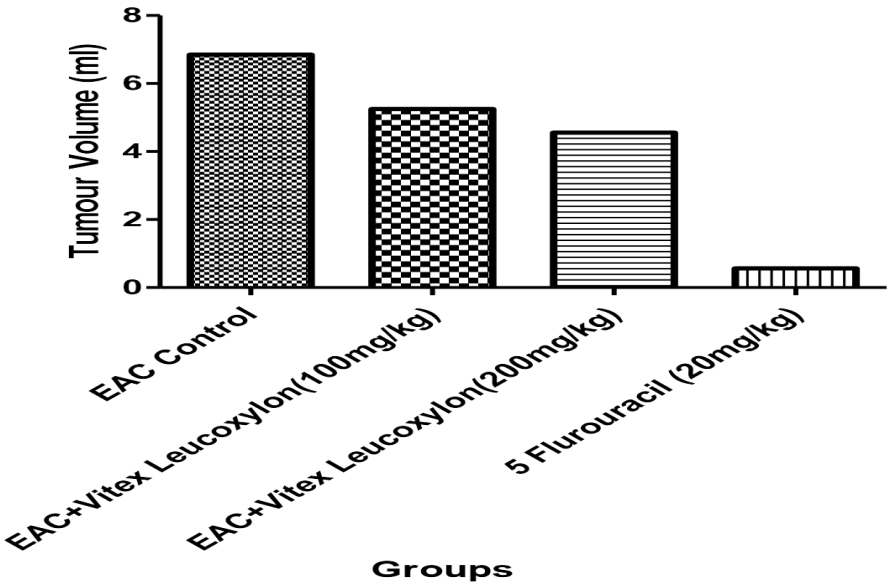

Fig.3 Effect of steroid of vitexleucoxylon and 5 flurouracil on tumour volume

DISCUSSION

Ehrlich Ascites cells are spontaneous murine adenocarcinoma cells adapted to Ascites form and causes increase growth of Ascites in ascites fluid [6]. In EAC control group, a regular rapid increase in ascites was observed. Ascitic fluid is the direct nutritional source for tumour cells and a rapid increase in ascites fluid was seen. The rate of tumour growth is dependent on the balance between the proliferative activity and death rate of the tumour cells.

The administration of steroid of Vitexleucoxylon 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 steroid of *Vitexleucoxylon* at different doses (100mg/kg;200mg/kg) and 5-flurouracil than EAC control.

The studies on Amaranthus[7] Vitexnenuguda [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 steroid of *Vitexleucoxylon* induced high level of chromatin condensation, nuclear damage and cell death which may be linked to slower tumour growth.

The SOD, CAT were significantly reduced in EAC control group when compared to Normal control. In steroid of Vitexleucoxylon and 5-flurouracil treated group the activities of SOD, CAT were

significantly increased when compared to EAC control.

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