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Antitumor Activity of *Vitex negundo* Linn. against Dalton's Ascitic Lymphoma

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Abstract: The aim of the present study is to evaluate the effect of ethanolic (EEVN) and aqueous (AEVN) extract of leaves of *Vitex Negundo* Linn. Against Dalton's Ascitic Lymphoma (DAL) in Swiss Albino mice. DAL cells were injected intraperitoneally (1×10^6 cells) to the each mice. Two days after cells injection the animals were treated with 200 mg/kg of ethanolic extract of *Vitex negundo* (EEVN) and aqueous extract of *Vitex negundo* (AEVN) for 14 days. 5 - fluorouracil (20 mg/kg) was used as standard drug. The entire animals were evaluated for Median Survival Time (MST), and Cancer cell count, packed cell volume, Hematological parameters was compared with the same parameters in standard by collecting blood from retro orbital blood vessel of mice. All the parameters were normalized in tumor-induced mice. These observations suggest that both the extract (EEVN and AEVN) posses antitumor effect against Dalton's Ascitic Lymphoma (DAL).
Keywords: *Vitex negundo*; Dalton's ascitic lymphoma; anticancer agents.

Introduction

Tumor is a mass of tissues which proliferate rapidly, spread throughout the body and may eventually cause death of the host[1]. 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 efficacies 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[2] and plants[3] are quoted to be useful in different types of cancer.

Vitex negundo Linn. belonging to the family Verbenaceae is distributed in all over India. *Vitex negundo* Linn. has very limited systematically carried out investigations. 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. It was found from the tribes of south India that the plant has been used for the suppression of tumor like syndrome among their own population.[4,5]

A vast literature collection fails to produce a scientific evidence to prove the anti tumor activity of *Vitex negundo* Hence this study was planned to evaluate the effect of ethanolic and aqueous extract of leaves of *Vitex negundo* against Dalton's ascitic lymphoma (DAL).

Experimental

Materials and Methods

The whole plant of *Vitex negundo* Linn. was collected from Algarkovil Temple, Madurai, Tamilnadu. This plant was authenticated by Department of Botany, The American College, Madurai. The male Swiss albino mice weighing 25 ± 5 g were selected for this study. [Approved by the institution animal ethical committee (Reg.No.KMCP/08/3-23)] The mice's were housed in clean polypropylene cages having 6 mice's per cage and maintained under temperature controlled room (27±2° C) with photoperiod of 12h light and 12h dark

cycle. The animals were fed with commercially available food pellet diet and water *ad libitum*.

Preparation of Drug

The shade dried plant leaves of *Vitex negundo* Linn. was powdered coarsely and about 200g of this powder was extracted (soxhlet) with 70% ethyl alcohol and aqueous for 72h. The yield was 22.4g w/w and 4.8g w/w respectively. The extract was dried in vacuum and resuspended in water before use. The Phytochemical screening proves the presence of carbohydrate, glycosides and flavonoids[4].

Effect against DAL

Animals were divided into five group's viz. G1, G2, G3, G4 and G5 of six each. For comparison, G1 designated as normal control group was used which was neither inoculated with cancer cells nor treated with EEVN and AEVN. Ascitic Lymphoma was induced according to Christina et al[6]. DAL cells were obtained through the courtesy of Amala Cancer Institute, Kerala, India and were injected intraperitoneally with 1 x 10^6 DAL cells/mouse to all the mice of the G2, G3, G4 and G5 groups. As the groups G2 was reserved as cancer control, it was not treated with any extract but only with saline. On the next day (24 h after inoculation) the animals of G3 were treated with 20mg/kg[7] of 5-fluorouracil intraperitoneally while the mice of G4 and G5 treated with 200mg/kg[8] of EEVN and AEVN orally. The treatment was continued for 10 days. On day 11, the following parameters were estimated.

1. Cancer cell count
2. Increase in life span(ILS)

Determination of hematological parameters

Apart from above mentioned parameters, the effect of EEVN and AEVN on hematological parameters was also studied in the mice of all groups. Blood was collected from the all mice in the groups by puncturing retro-orbital plexus and counted for RBC, WBC, Hb and Platelets and packed cell volume.

Statistical analysis

The results are expressed as mean ± SEM. The evaluation of the data was done using one way ANOVA followed by Newman – Keul's multiple range test. Difference below $P<0.05$ implied significance.

Result and Discussion

The intraperitoneal inoculation of DAL cells in the mice produces increased proliferation of cells. EEVN reduced the cancer cell count to 0.92±0.38X 10^6 cells in the treated mice. Similarly AEVN reduced the cancer cell count to 1.02±0.18x 10^6 cell in the cancer treated mice[9]. The EEVN and AEVN treated mice survived upto 35 and 32 days respectively whereas the tumor control mice survived upto 20 days only. The percentage increase in lifespan of EEVN and AEVN treated mice increased by 78% and 65% respectively (Table 1). Extract treatment reduces the tumor weight and hence increased the life span of cancer induced mice. Regarding the hematological parameters, cancer control mice showed reduced RBC count but increase in WBC count than normal group. The treatment with EEVN, AEVN also raised the RBC count significantly to 3.7 ±0.19 million/cumm, 3.61 ± 0.15 million/cumm respectively. Similarly both extracts restored the WBC value to 11.5 ±714.36cells/ml x 10^3, 11.49 ±699.83 cells/ml x 10^3 respectively. Hb content in cancer control mice deceased significantly when compared with normal group. But, the EEVN and AEVN extracts increased Hb content to 10.16 ±0.53gm/dl, 10.36 ±0.44gm/dl. EEVN and AEVN restored the normal platelet count in tumor induced extract treated mice (Table 2). Hematological studies exhibited an increase in WBC count in G2 and this was reduced after treatment with the extracts[10]. PCV in cancer control mice increase significantly when compared with normal group. But, the EEVN and AEVN extract decrease PCV content to 22.3±0.57, 22.5±0.50.

Lymphoma is defined as malignant tumors of lymphoreticular origin i.e. from lymphocytes and histiocytes and their precursor cells. Many studies have reported the useful effects of plant products against DAL. When DAL is induced in animals, the cancer cell count in the peritoneal fluid has been used as the marker to confirm the proliferation of cells. For a similar observation, in this study a cancer control group was used. The increased cell count after 10 days confirmed the proliferation of cells in this group. A decrease in cancer cell count as a confirmatory evidence for protection against DAL has been reported[11]. In this study also a similar decrease was observed following the administration of the extracts. Consequently increased life span was observed with extract treated mice. Hematological parameters also enable to conclude on the protective effect. A decrease in RBC count and increase in WBC and PCV count following cancer cell proliferation. In the same study an increase in RBC count and a decrease in elevated WBC count were reported as confirmatory markers for the protection against DAL[12]. A similar report was observed in the present study after treatment with the extracts. However from the above observations on other parameters it was concluded that the plant possesses activity against DAL.

Table 1: Effect of EEVN, AEVN on the life span and cell count.

Treatment	Number of Animals with tumor	Increase in Life span (%)	Cell count mlx10^6
G_1	0/6	100 %	---
G_2	6/6	42 %	1.34 ± 0.26* [a]
G_3	6/6	90 %	0.70 ± 0.32
G_4	6/6	78 %	0.92 ± 0.38* [b]
G_5	6/6	65 %	1.02 ± 0.18* [b]

G_1 - Normal Control, G_2 – Cancer Control, G_3 – Standard, G_4- Test (EEVN), G_5- Test (AEVN).
All value are expressed as mean ±SEM for 6 animal in each groups.
a - Values are significantly different from control (G1)
b - Values are significantly different from cancer control (G2)
* - P (<0.05)
All values are found out by using one way ANOVA followed by students Newman Keul's Multiple range test, n = 6
EEVN- Ethanolic Extract of *Vitex negundo* (EEVN)
AEVN- Aqueous Extract of *Vitex negundo* (AEVN)

Table 2: Effect of EEVN, AEVN on Hematological parameters

Treatement	Total WBC Cells /mlx10^3	Rbc Count Mill/cumm	Hb Gm/dl	PCV	Platelets Lakhs/cumm
G1	10.93 ±316.65	4.4 ±0.19	11.31 ±0.21	15.26 ±0.87	2.65 ±0.14
G2	16.44 ±1082.2[a**]	2.11 ±0.08[a**]	7.89 ±0.62[a**]	29.76 ±1.89[a**]	1.65 ±0.13[a**]
G3	10.43 ±217.09[b**]	3.97±0.06[b**]	10.79 ±0.06[b**]	18.7±0.54[b**]	2.51 ±0.10[b**]
G4	11.5 ±714.36[b**]	3.7 ±10.19[b**]	10.16 ±0.53[b**]	22.3 ±0.57[b**]	2.21 ±0.09[b**]
G5	11.49 ±699.83[b**]	3.61 ±0.15[b**]	10.36 ±0.44[b**]	22.5 ±0.50[b**]	2.31 ±0.09[b**]

G_1 - Normal Control, G_2 – Cancer Control, G_3 – Standard, G_4- Test (EEVN), G_5- Test (AEVN)
All values are expressed as mean ±SEM for 6 animal in each groups.
a - Values are significantly different from control (G1)
b – Values are significantly different from cancer control (G2)
* P (<0.05)
** P (<0.01)
*** P (<0.001)
All values are found out by using one way ANOVA followed by Newman Keul's multiple range test.
EEVN- Ethanolic Extract of *Vitex negundo* (EEVN)
AEVN- Aqueous Extract of *Vitex negundo* (AEVN)

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