

Antitumor activity of aerial parts of *vernonia cinerea*(L) Less. against Dalton's ascitic lymphoma

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Abstract: The aim of the present study is to evaluate the effect of various extracts of *vernonia cinerea* Linn. Against Dalton's Ascitic Lymphoma (DAL) in Swiss Albino mice. DAL cells were injected intraperitoneally (2×10^6 cells) to each mice. One day after, cells injected animals were treated with 500mg/kg of ethanolic extract of *vernonia cinerea* (EEVC), chloroform extract of *vernonia cinerea* (CEVC) and pet.ether extract of *vernonia cinerea* (PEVC) for 14 days. 5-fluorouracil (20mg/kg) was used as standard drug. The entire animals were evaluated for cancer cell count, Haematological parameters, serum enzyme and lipid profile, Body weight and Median Survival Time (MST) were compared with the same parameters of standard by collecting blood from retro orbital blood vessel of mice. These observations suggest that ethanolic extract and chloroform extract possess more significant antitumor effect than Pet.ether extract against Dalton's Ascitic Lymphoma(DAL).

Keywords: *vernonia cinerea*; 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 host¹. Chemotherapy is an effective treatment against various types of cancer either singly or in combination with surgery and/ or radiotherapy. However, chemotherapeutics 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 spices² and plants³ are quoted to be useful in different types of cancer.

The plants of the genus *Vernonia* (Asteraceae) are widely distributed in most tropical and subtropical countries, and have long been used in traditional medicine to treat various types of diseases. In recent years, the interest in the plant-based

medicine has increased noticeably worldwide. One of such plants belonging to genus *Vernonia* and known to have healing potential is *Vernonia cinerea* Less (Asteraceae). *V. cinerea* have many therapeutic uses in the practice of traditional medicine. Every part of the plant can be used medicinally. This herb has been used to treat a number of disorders including inflammation, malaria, fever, worms, pain, diuresis, cancer, abortion, and various gastro-intestinal disorders.⁴

A vast literature collection fails to produce a scientific evidence to prove the antitumor activity of *vernonia cinerea*. Hence this study was planned to evaluate the effect of ethanolic, chloroform and Pet.ether extracts of *vernonia cinerea* (aerial parts) against Dalton's Ascitic lymphoma(DAL).

Experimental

Materials and Methods

The aerial parts of *vernonia cinerea* Linn. was collected in and around Madurai, Tamilnadu. The plant was authenticated by Department of Botany, The American College, Madurai. The male Swiss albino mice weighing 25±5 were selected for this study. The mice's were housed in clean polynerlyc cages having 6 mice's per cage and maintained under standard condition (25±2°C) with photoperiod of 12±1h dark/ light cycle. The animals were fed with rat pellet and water ad libitum.

Preparation of Drug

The shade dried plants of *vernonia cinerea* Linn. was powdered coarsely and about 200g of this powder was extracted successively(Soxhlet) with pet.ether, chloroform and 70% ethanol for 72hrs. the extract was dried in vaccum and resuspended with 1%CMC and water before use. The phytochemical screening proves the presence of sterols, terpenoids, flavonoids, saponins, mucilage and tannins⁵.

Effect against DAL

Animals were divided into six groups viz. G₁, G₂, G₃, G₄, G₅ and G₆ of six each. For comparison, G₁ designated as normal control group was used which was neither inoculated with cancer cells nor treated with EEVC, CEVC and PEVC. Asicitic Lymphoma was induced according to Christina et

al⁶. DAL cells were obtained through the courtesy of Amala cancer Institute, Kerela, India and were injected intraperitoneally with 2X10⁶ DAL cells/mouse to all the mice of the G₂ G₃ G₄ and G₅ groups. As the group G₂ served as cancer control, it was not treated with any extract but only with saline. On the next day (24hrs after inoculation) the animals of G₃ were treated with 20mg/kg⁷ of 5-fluorouracil intraperitoneally while the mice of G₄ G₅ and G₆ treated with 500mg/kg of EEVC, CEVC and PEVC orally. The treatment was continued for 14 days. On the day 15, the following parameters were estimated.

- 1.Cancer cell count
- 2.Increase in life span
- 3.Body weight
- 4.Serum enzymes and lipid profiles

Determination of haematological parameters

Apart from above mentioned parameters, the effect of EEVC, CEVC and PEVC on hematological parameters was also studied in the mice of all groups. Blood was collected from all the mice in the groups by puncturing retro-orbital plexus and counted for RBC, WBC, Hb and platelets.

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. P<0.05 implied significance.

Table 1: Effect of EEVC, CEVC and PEVC on the life span, body weight and cell count.

Treatment	Number of animal with Tumor	Increase in Life span (%)	Increase in Body weight (gm)	Cell Count mlx10 ⁶
G ₁	0/6	100%	1.17±0.05	-
G ₂	6/6	34%	8.48±0.17 ^{a*}	1.42±0.06 ^{a*}
G ₃	6/6	75%	1.98±0.67	0.82±0.04
G ₄	6/6	40%	6.58±0.15 (b) n-s	1.28±0.12 (b) n-s
G ₅	6/6	50%	4.02±0.71 ^{b**}	1.08±0.18 ^{b**}
G ₆	6/6	65%	3.02±0.38 ^{b**}	0.93±0.22 ^{b**}

G₁- Normal Control, G₂- cancer control, G₃- Standard, G₄- Test (PEVC), G₅- Test (CEVC), and G₆- Test (EEVC).

All value are expressed as mean ±SEM for 6 animals in each groups

a - Values are significantly different from control (G₁)

b - Values are significantly different from cancer control (G₂)

n-s - Not - Significant, * P (<0.05), ** P (<0.01)

All values are found out by using one way ANOVA followed by students Newman Keul's Multiple range test, n=6

EEVC- ethanolic extract of *vernonia cinerea* Linn

CEVC- Chloroform extract of *vernonia cinerea* Linn

PEVC- petroleum Ether extract of *vernonia cinerea* Linn

Table 2: Effect of EEVC, CEVC and PEVC on Haematological parameters

Treatment	Total WBC cells/mlx10 ³	RBC Count Millions/cumm	Hb Gm/dl	Platelets Lakhs/cumm
G ₁	11.064±0.029	4.52±0.042	11.05±0.29	1.4±0.096
G ₂	15.280±0.018 ^{a***}	2.41±0.038 ^{a***}	6.98±0.18 ^{a***}	1.9±0.018 ^{a***}
G ₃	12.058±0.021	3.82±0.044	9.04±0.20	1.38±0.022
G ₄	14.019±0.038 (b) n-s	3.08±0.070 (b) n-s	7.12±0.36 (b) n-s	1.78±0.78 (b) n-s
G ₅	13.878±0.096 ^{b**}	3.42±0.099 ^{b**}	8.12±0.28 ^{b**}	1.50±0.48 ^{b***}
G ₆	12.268±0.010 ^{b***}	3.56±0.040 ^{b***}	8.48±0.96 ^{b***}	1.48±0.40 ^{b***}

G₁- Normal control , G₂- cancer control, G₃- Standard, G₄- Test (PEVC), G₅- Test (CEVC) , and G₆- Test (EEVC).

All value are expressed as mean ±SEM for 6 animals in each groups

a - Values are significantly different from control (G₁)

b - Values are significantly different from cancer control (G₂)

n-s - Not - Significant

** P (<0.01)

*** P (<0.001)

All values are found out by using one way ANOVA followed by students Newman Keul's Multiple range test, n=6

EEVC- ethanolic extract of *vernonia cinerea* Linn

CEVC- Chloroform extract of *vernonia cinerea* Linn

PEVC- petroleum ether extract of *vernonia cinerea* Linn

Table 3: Effect of EEVC, CEVC and PEVC on serum enzyme and lipid profile.

Treatment	Cholestrol mg/dl	TGL mg/dl
G ₁	95.8±1.9	132.8±8.67
G ₂	122.9±2.36 ^{a***}	191.4±7.40 ^{a***}
G ₃	101.8±3.65	140.5±8.06
G ₄	118.4±3.10 (b) n-s	158.4±4.86 (b) n-s
G ₅	109.3±4.12 ^{b**}	149.4±9.76 ^{b**}
G ₆	104.5±3.96 ^{b***}	143.8±7.07 ^{b***}

G₁- Normal control , G₂- cancer control, G₃- Standard, G₄- Test (PEVC), G₅- Test (CEVC) , and G₆- Test (EEVC).

All value are expressed as mean ±SEM for 6 animals in each groups

a - Values are significantly different from control (G₁)

b - Values are significantly different from cancer control (G₂)

n-s - Not - Significant, ** P (<0.01), *** P (<0.001)

All values are found out by using one way ANOVA followed by students Newman Keul's Multiple range test, n=6

EEVC- ethanolic extract of *vernonia cinerea* Linn, CEVC- Chloroform extract of *vernonia cinerea* Linn

PEVC- petroleum ether extract of *vernonia cinerea* Linn

Result and Discussion

The intraperitoneal inoculation of DAL cells in the mice produces increased proliferation of cells. EEVC reduced the cancer cell count to $0.93 \pm 0.22 \times 10^6$ cells in the treated mice similarly CEVC and PEVC reduced the cancer cell count to $1.08 \pm 0.18 \times 10^6$ cells and $1.28 \pm 0.12 \times 10^6$ cells respectively. The percentage increase in life span of EEVC, CEVC and PEVC treated mice increased by 65%, 50% and 40% respectively (Table- 1). Extract treatment reduces the tumor weight and hence increased the life span of cancer induced mice⁸. Regarding the haematological parameters, cancer control mice showed reduced RBC count but increase in WBC count than normal group. The treatment with EEVC, CEVC and PEVC also raised the RBC count significantly to 3.56 ± 0.040 million/cumm P (<0.001), 3.42 ± 0.099 million/cumm P (<0.01) and 3.08 ± 0.070 million/cumm respectively. Similarly all the three extracts restored the WBC value to 12.26 ± 0.010 cells/mlx 10^3 P (<0.001), 13.878 ± 0.096 cells/mlx 10^3 P (<0.01) and 14.019 ± 0.038 cells/mlx 10^3 respectively⁹. Hb content in cancer control mice decreased significantly when compared with normal group. But, the EEVC, CEVC and PEVC increased the Hb content to 8.48 ± 0.96 gm/dl P (<0.001), 8.12 ± 0.28 gm/dl P (<0.01) and 7.12 ± 0.36 gm/dl. Similarly EEVC, CEVC and PEVC restored the normal platelet count in tumor induced extract treated mice (Table-2). Haematological studies exhibited an increase in WBC count in G₂ and this was reduced after treatment with the extracts. The another parameter is serum enzyme and lipid profile. Only EEVC and CEVC reduced the cholesterol rate to 104.5 ± 3.966 mg/dl P (<0.001) and 109.3 ± 4.12 mg/dl P (<0.01) than cancer

control. The EEVC and CEVC extracts also reduced the triglycerides to 143.8 ± 7.07 mg/dl, 149.4 ± 9.76 mg/dl (Table-3) respectively¹⁰. But EEVC showed more significance than CEVC and PEVC.

Lymphoma is a group of cancers that affect the cells that play a role in the immune system, and primarily represents cells involved in the lymphatic system of the body¹¹.

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 14 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 reported¹². 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. Haematological parameters also enable to conclude on the protective effect. A decrease in RBC count, increase in WBC count and increase in cholesterol, TGL and LDH 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 DAL¹³. A similar report was observed in the present study after treatment with the extracts. However from the above observations it was concluded that the plant possesses activity against DAL.

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