

ANTITUMOR ACTIVITY OF ETHANOLIC EXTRACT OF *CNIDOSCOLUS CHAYAMANSA* MCVAUGH AGAINST DALTON'S ASCITIC LYMPHOMA IN MICE

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ABSTRACT

Aim: Traditional medicine has a long history of serving people all over the world. In recent years, the use of traditional medicine information in cancer research received considerable interest. Ethanolic Extract of *Cnidoscopus chayamansa* (EECC) has been used in traditional and folklore medicine for the treatment of cancer. The aim of the present study was to evaluate the effect of Ethanolic Extract of leaves of *Cnidoscopus chayamansa* (EECC) against intraperitoneally injected Dalton's Ascitic Lymphoma (DAL) cell lines in Swiss albino mice.

Materials and methods: DAL cells were injected intraperitoneally (1×10^6 cells/ml/mouse) to the mice. The EECC at a dose of 200 mg/kg and 400mg/kg body weight were administered orally for 14 consecutive days to the tumor bearing group of animals. Derived parameters, hematological parameters, serum enzyme and lipid parameters were measured and compared to the control group. 5-Fluorouracil (20 mg/kg) was used as a standard drug.

Results: Both doses of EECC decreased average increase in body weight, reduced the packed cell volume (PCV) viable tumor cell count and increased the life span of DAL treated mice and brought back the hematological parameters, serum enzyme and lipid profile near to normal values. All the values were found to be statistically significant with control group at $p < 0.01$. These observations are suggestive of the protective effect of extracts in Dalton's Ascitic Lymphoma (DAL)

Conclusion: All these findings enable to conclude that both doses of EECC possess a protective effect against DAL.

Keywords: Dalton's Ascitic Lymphoma, Chaya, *Cnidoscopus chayamansa*, 5-Fluorouracil, Tumor volume, Lifespan.

INTRODUCTION

Tumor is a mass of tissues which proliferate rapidly, spread throughout the body and may eventually cause death of the host. ⁽¹⁾ By 2050 over 20 million new cancer cases and over 17 million cancer deaths are probable to occur in the world. ⁽²⁾ 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. ^(3, 4)

C.Chayamansa, belonging to the family Euphorbiaceae, a shrub growing in central American and the hot tropics of southern Mexico ⁽⁵⁾. However, the plant shows great adaptability to milder climates and can be found growing in northern latitudes, under dry environments and in different soils. ⁽⁶⁾ The leafy vegetable was consumed by Mayan Indians and is traditionally incorporated in salad and regional dishes ⁽⁷⁾. *C.chayamansa* consumption has also become popular among Hispanic population in southern Texas Florida. The nutritional value of *C.chayamansa* is very attractive when compared with spinach of other common vegetables ^(8,9). The plant which is also called spinach tree, has a great potential to alleviate deficits in population of developing countries as it is rich in protein, vitamin and mineral ^(10,11,12). Unfortunately the leaves have undetermined amounts of cyanogenic glycosides ⁽⁶⁾. However, cooking as well as other heat treatment will hydrolyze these glycosides, minimizing the risk of poisoning ⁽⁶⁾. Although *C.chayamansa* is primarily as a food plant, it has been used therapeutically for a number of ailments such as diabetes ⁽¹³⁾, arteriosclerosis, gallstone and high cholesterol. It is also believed that *C.Chayamansa* cleans circulatory system, stimulates lactation, improves eyesight, strengthens nails, improves digestions and is a diuretic and laxative. ⁽¹⁴⁾

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 this study was planned to evaluate the effect of Ethanolic Extract of leaves of *C.chayamansa* (EECC) against Dalton's Ascitic Lymphoma (DAL).

MATERIALS AND METHODS

The leaves of *C.chayamansa* were collected from in and around Kanyakumari district, Tamilnadu. The plant material was taxonomically identified and authenticated by Dr.V. Chelladurai, research officer (Botany) CCRAS Govt. of India (Rtd), Tirunelveli, Tamilnadu and the voucher specimen (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 stored in a closed vessel for further use.

Selection Grouping and Acclimatization of Laboratory Animal

Male Swiss albino mice (20-25gm) were procured from central animal house, and used throughout the study. They were housed in micro nylon boxes in a control environment (temp $25 \pm 2^\circ\text{C}$) and 12 h dark/light cycle with standard laboratory diet and water *ad libitum*. The study was conducted after obtaining institutional animal ethical committee clearance kkp/Ph.D/pmu/2011. As per the standard practice, the mice were segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They were fed on healthy diet and maintained in hygienic environment in our animal house. ⁽¹⁵⁾

Induction of Tumor

Various techniques for induction of cancer in animals, viz. chemically induced (using DMBA/croton oil. etc) ⁽¹⁶⁾ virus induced, cell line induced (sarcoma - 180, ULCA fibro sarcoma and Jensen sarcoma, mouse lung fibroblast cells L-929, Dalton's Lymphoma Ascites

(DLA), Ehrlich Ascites Carcinoma (EAC) ^(17,18,19) methods have been used in experimental studies of anticancer activity.

Induction of cancer using DLA cells

Dalton's Lymphoma ascites (DLA) cells were supplied by Amala cancer research center, Trissur, Kerala, India. The cells maintained *in vivo* in Swiss albino mice by intraperitoneal transplantation. While transforming the tumor cells to the grouped animal the DLA cells were aspirated from peritoneal cavity of the mice using saline. The cell counts were done and further dilution were made so that total cell should be 1×10^6 , this dilution was given intraperitoneally. Allowed the tumor to grow in the mice for a minimum of seven days before starting the treatments.

Treatment Protocol

Swiss Albino mice were divided into five groups of six animals each. All the animals in four groups were injected with Dalton's Lymphoma Ascites (DLA) cells (1×10^6 cells per/ml/ mouse) ⁽²⁰⁾ intraperitoneally, and the remaining one group is normal control.

Group 1: The normal control.

Group 2: The tumor control

Group 3: The positive control was treated with injection fluorouracil at 20mg/kg body weight, i.p ⁽²¹⁾.

Group 4: Treatment control group and was administered EECC in a dose of 200mg/kg orally.

Group 5: Treatment control group and was administered EECC in a dose of 400mg/kg orally.

Preparation of solution

For the study against Dalton's Lymphoma Ascites (DLA) cell lines, the ethanolic extract of *C.chayamansa* was suspended in 2ml of sterile water for oral use.

Treatment

In this study, drug treatment was given after 24 h of inoculation, once daily for 14 days. On day 14th day after the last dose, all mice from each group were sacrificed by euthanasia. Blood was withdrawn from each mouse by retro orbital puncture method and the following parameters were checked. ⁽²²⁻²⁵⁾

1. Hematological parameters

- WBC count
- RBC count
- Hb content
- Platelet count
- Packed cell volume (PCV)

2. Serum enzyme and lipid profile

- Total Cholesterol (TC)
- Triglycerides (TGL)
- Aspartate amino Transferase (AST)
- Alanine amino Transferase (ALT)
- Alkaline Phosphatase (ALP)

3. Derived parameters

- Body weight
- Life span (%)
- Cancer Cell Count

Evaluation of Clinical Parameters

Cancer cell count

The fluid (0.1ml) from the peritoneal cavity of each mouse was withdrawn by sterile syringe and diluted with 0.8 ml of ice cold Normal saline or sterile Phosphate Buffer Solution and 0.1 ml of

trypan blue (0.1 mg/ml) and total numbers of the living cells were counted using hemacytometer. ⁽²⁶⁾

$$\text{Cell count} = \frac{\text{No of cells Dilution}}{\text{Area} \times \text{Thickness of liquid film}}$$

Hematological parameters

- WBC count
- RBC count
- Platelet count
- Hb
- PVC

i) WBC count

The WBC count was found to be increased in cancer control, when compared with normal and treated tumor-bearing mice. The WBC count were found to decrease significantly in animals treated with plant extract when compared with cancer control, indicating that the antitumor nature of the extract ⁽²⁷⁾.

ii) RBC and Hb

RBC and Hb content decreases with tumor bearing mice when compared with Normal control mice.

iii) Platelets

In Hodgkin lymphoma, increase in platelet count often reported in laboratory finding. Hence, this parameter in the study is investigated. ⁽²⁸⁾

iv) Packed cell volume

In the case of anemia the packed cell volume decreases.

Serum Enzyme and Lipid Profile

The serum was analyzed for the following parameters

- Aspartate amino Transferase (AST)
- Alanine amino Transferase (ALT)
- Alkaline Phosphatase (ALP)
- Total Cholesterol (TC)
- Triglycerides (TGL)

1. Total Cholesterol and Triglycerides

Abnormal blood lipid profile has been associated with cancer. In Hodgkin lymphoma, high cholesterol level and low triglyceride level has been reported and investigated in this parameter study ⁽²⁹⁾.

2. Liver Enzymes (AST, ALT, ALP)

Abnormal liver function seen in patient with Hodgkin lymphoma ⁽³⁰⁾ that these liver enzyme levels markedly increase in tumor bearing mice. AST, ALT, ALP is an enzyme mainly derived from the liver, bones and in lesser amount from intestines, placenta, kidneys and leukocytes. An increase in AST, ALT and ALP levels in the serum are frequently associated with the variety of disease. ⁽³¹⁾ AST, ALT and ALP comprise a group of enzyme that catalyzes the phosphate esters in an alkaline environment, generating an organic radical and inorganic phosphate.

Markedly elevated serum AST, ALT and ALP, hyperalkaline-phosphatasemia, is seen predominantly with more specific disorders; including malignant biliary cirrhosis, hepatic lymphoma and sarcoidosis ⁽³²⁾.

Derived Parameters

1. Body weight:

All the mice were weighed, from the beginning to 15th day of the study. Average increase in body weight on the 15th day was determined.

2. Percentage increase in life span (ILS) ⁽²⁷⁾

% ILS was calculated by the following formulae:

$$\frac{\text{Life span of treated group}}{\text{Life span of control group}} - 1 \times 100$$

All biochemical investigations were done by using OBAS MIRA PLUS-S Auto analyzer from Roche Switzerland.

Hematological test are carried out in COBAS MICROS OT 18 from Roche.

Newly added Hi-Tech instruments MAX MAT used for an auto analyzer for all biochemical investigations in blood sample.

Table 1: Effect of EECC on Hematological Parameters

Treatment	Total WBC Cells /mlx10 ³	RBC Count Millon/cumm	Hb gm/dl	PCV in %	Platelets Lakhs/cumm
G1	9.90 ±1.32	4.33±0.87	12.35 ±1.08	14.63±2.21	3.12±0.66
G2	14.25 ±2.40a**	2.40±0.43a**	7.09 ±0.93a**	30.55±3.55a**	1.54±0.44a**
G3	11.30 ±1.40b**	4.12±0.85b**	11.0 ±1.42b**	18.40±1.33b**	2.63±0.68b**
G4	13.70±2.07b*	2.93±0.44b*	9.25±0.94b*	26.08±2.62b*	1.93±0.37b*
G5	12.69 ±1.29b**	3.53±0.64b**	10.65±1.20b**	22.07±1.43b**	2.22 ±0.71b**

G1 – Normal Control, G2 – Cancer Control, G3 – Positive control, G4 – Treatment control (EECC-200mg/kg), G5 – Treatment control (EECC-400mg/kg)

All values are expressed as mean ± SEM (n=6).

a** – Values are significantly different from control (G1) P < 0.001

b* – Values are significantly different from cancer control (G2) P < 0.01

b** – Values are significantly different from cancer control (G2) P < 0.001

Table 2: Effect of EECC on Serum Enzymes and Lipid Proteins

Treatment	Cholesterol (mg/dl)	TGL (mg /dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
G1	99.08±3.50	120.81±2.34	36.45 ±1.17	32.27 ±1.24	125.09 ±2.18
G2	140.86±4.54a**	206.14±4.63a**	85.0±2.69a**	60.18±2.55a**	240.18±4.26a**
G3	110.44±3.90b**	154.40±2.62b**	55.22 ±1.56b**	40.40±1.52b**	160.26±2.23b**
G4	135.39±3.53b*	185.83±2.27b*	78.67 ±2.19b*	55.25±1.92b*	230.28±2.31b*
G5	120.29±2.42b**	160.50±2.48b**	69.39±1.90b**	45.52 ±1.49b**	185.29±2.45b**

G1 – Normal Control, G2 – Cancer Control, G3 – Positive control, G4 – Treatment control (EECC low dose), G5 – Treatment control (EECC high dose)

All values are expressed as mean ± SEM (n=6).

a** – Values are significantly different from control (G1) P < 0.001

b* – Values are significantly different from cancer control (G2) P < 0.01

b** – Values are significantly different from cancer control (G2) P < 0.001

Table 3: Effect of EECC on the Life Span, Body Weight and Cancer Cell Count of Tumor Induced Mice

Treatment	Number of animals	% ILS Life span	Body weight in grams	Cancer cell count ml X 10 ⁶
G1	6	>>30 days	2.12±0.44	-
G2	6	48%	7.64±0.95a**	2.72±0.33a**
G3	6	92%	3.73±0.52b**	1.25±0.24b**
G4	6	66%	6.40±0.82b*	2.22±0.40b*
G5	6	72%	5.58±0.78b**	1.66±0.30b**

G1 – Normal Control, G2 – Cancer Control, G3 – Positive control, G4 – Treatment control (EECC low dose), G5 – Treatment control (EECC high dose)

All values are expressed as mean ± SEM (n=6).

a** – Values are significantly different from control (G1) at P < 0.001

b* – Values are significantly different from cancer control (G2) at P < 0.01

b** – Values are significantly different from cancer control (G2) at P < 0.001

RESULTS

Effect on Tumor Growth

In the DLA tumor control group, the average life span of animal was found to be 48% where as EECC at two dose of 200 and 400 mg/kg body weight increase the life span to 66% and 72% respectively. These values were significant. However the average life span of 5-FU treatment was found to be 92%, indicating its potent antitumor

nature. The antitumor nature of EECC was evidenced by the significant reduction in percent increase in body weight of animal treated with EECC at the dose of 200 and 400 mg/kg body weight when compared to DLA tumor bearing mice.

It was also supported by the significant reduction in packed cell volume and viable Tumor cell count in both the dose of treatment when compared to the DLA tumor control (Table: 3)

Effect on Hematological Parameters

As shown in (Table: 1) RBC, HB Platelets were decreased and WBC count was significantly increased in the DLA control group compared to the normal control group. Treatment with EECC at the dose of 200 and 400 mg/kg significantly increases the Hb content, RBC, Platelets and significantly decreased the WBC count to about normal level. All these results suggest the anticancer nature of the extract. However, the standard 5-FU at the dose of 20 mg/kg body weight produced better result in all these parameters.

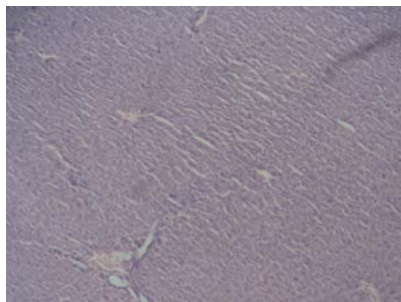


Fig. 1: Normal control section shows structure of liver of the mice.

Effect on Biochemical Parameters

The inoculation of DLA cells caused significantly increase in the level of Total Cholesterol, Aspartate amino Transferase, Alanine, amino Transferase, Alkaline Phosphatase in the tumor control animals(G2), when compared to the normal group. The treatment with EECC at the dose of 200 and 400 mg/kg body weight reversed these changes towards the normal level (Table-2) All the values were found to be significant. The treatment with standard 5- FU also gave similar results.

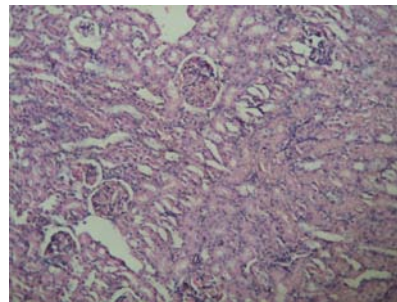


Fig. 2: Tumor Control section shows structure of liver of mice.

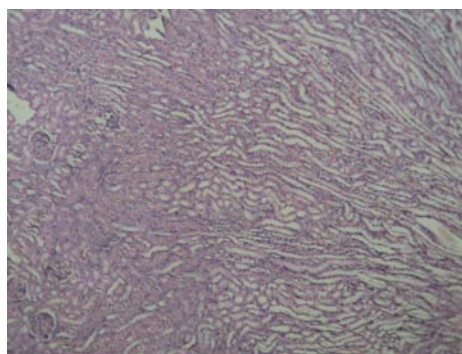


Fig. 3: Standard control section shows structure of liver of mice

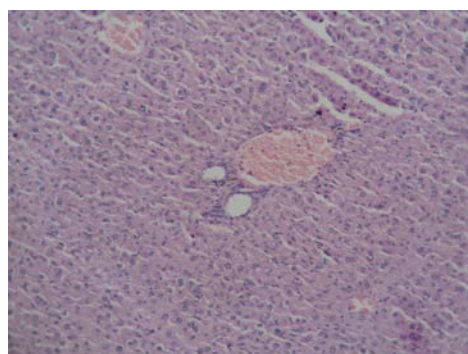


Fig. 4: Treatment control section shows structure of liver of mice.

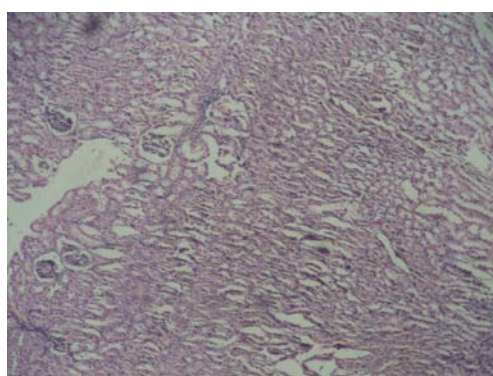


Fig. 5: Treatment control section shows structure of liver of mice.

DISCUSSION AND CONCLUSION

The alternative system of medicines like Ayurvedic, Siddha, Unani and other tribal folklore medicines have significantly contributed to the health care of the population of India. Today these systems are not only complementary but also competitive in the treatment of various diseases. Plants have served as a good source of antitumor agents. Several studies have been conducted on herbs under a multitude of Ethanobotanical grounds. A large number of plants possessing anticancer properties have been documented. (33-38)

Plants of *C.chayamansa* were traditionally used in the treatment of tumors. (39) The present investigation was carried out to evaluate the antitumor activity of Ethanolic extracts of *C.chayamansa* in DLA tumor bearing mice. The EECC treated animals at the doses of 200 and 400 mg/kg significantly inhibited the tumor volume, packed cell volume, tumor (viable) cell count and brought back the hematological parameters to more or less normal levels.

In DLA tumor bearing animals a regular rapid increase in ascitic tumor volume was observed. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with

tumor growth would be a means to meet the nutritional requirement of tumor cells⁽⁴⁰⁾. Treatment with EECC inhibited the tumor volume, viable tumor cell count and increased the life span of the tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the lifespan of animals⁽⁴¹⁾. It may be concluded that EECC by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of DLA bearing mice. Thus EECC have antitumor activity against DLA bearing mice.

Usually, in cancer chemotherapy the major problems that are being encountered are myelo suppression and anemia^(42, 43). The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or Hb and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions⁽⁴⁴⁾. Treatment with both doses of EECC brought back the (Hb) content; RBC and WBC count more or less to normal levels significantly. This clearly indicates that EECC possess protective action on the haemopoietic system.

It was reported that the presence of tumor in the human body or in the experimental animals is known to affect many functions of the liver. The significantly elevated levels of total cholesterol, TGL, AST, ALT, ALP in serum of tumor inoculated animal indicated liver damage and loss of functional integrity of cell membrane. The significant reversal of these changes towards the normal by EECC treatments.

In the present study, the biochemical examination of DLA inoculated animals showed marked changes indicating the toxic effect of the tumor. The normalization of these effects observed in the serum treated with EECC possesses significant antitumor and hepatoprotective effect of the extracts.

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