Anti-tumor activity of ethanolic extract of *Crataeva magna* Lour. (DC) against Dalton’s ascites lymphoma cell lines in mice

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**Keywords:** Dalton’s Ascitic Lymphoma, *Crataeva magna*Lour. DC, 5-Fluorouracil, Tumor volume, Lifespan

**ABSTRACT:** In recent years, the use of traditional medicine information in cancer research received considerable interest. Ethanolic Extract of *Crataeva magna*Lour. DC (EECM) has been used in traditional and folklore medicine for the treatment of cancer. The aim of the present investigation was to evaluate the effect of ethanolic extract root bark of *Crataeva magna*Lour. DC in swiss albino mice against intraperitoneally injected Dalton’s ascitic lymphoma (DAL) cell lines. DAL cells were injected intraperitoneally (1×10^6 cells/ml/mouse) to the mice. The EECM 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. Both doses of EECM 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). All these findings enable to conclude that both doses of EECM possess a protective effect against DAL.

**INTRODUCTION**

The traditional system of medicine became significantly more popular all over the globe because of the curative property, less toxic and has no side effects[1]. Indian system of medicine has been widely used for thousands of years in India. Now a day’s acceptance of traditional system of medicine in development world is sharply increasing [2-4]. Cancer is a group of more than 100 different diseases. Cancer occurs when cells become abnormal and keep dividing and forming cells without control or order. This uncontrolled cell division and growth ultimately results in cancer. World Health Organization (WHO) reported that there are 7.6 million deaths in 2008 and it is estimated up to 13.1 million deaths in 2030[5]. Treatment of cancer varies according to each type, has been facing large number of problems. Several ways in the treatment of cancer have been developed. Currently cancer is treated using surgery, radiation, and chemotherapy which are associated with severe side effects [6]. Herbal extracts with their proven potential and less side effects in therapeutics has replaced the synthetically derived drugs in modern allopathic medication system[7]. Dalton’s ascites lymphoma is transplantable, poorly differentiated malignant tumor which appeared originally as lymphocytes in a mouse[8,9].

*Crataeva magna* Lour DC (family Capparidaceae) is known as three leaved capers in English, Varuna in Sanskrit and Baruna in Hindi, a small tree with a much-branched head, found to be distributed mainly in the warmer (tropical) parts of the world. In folk medicine, its stem pith in the tribal peoples of Kandhamal district of Orissa known as Eastern Ghats of India that the bark is used for lactation after child birth, treat urinary disorders, kidney bladder stones, fever, vomiting and gastric irritation[10-12].
Leaves are deciduous three foliolate; petioles 3.8–7.6 cm long; leaflets 5–15 ovate, lanceolate or obovate, acute or acuminate, attenuate at the base, entire, glabrous on both surfaces, pale beneath, and reticulately veined [13]. The traditional plant used to treat various ailments to Urolithiasis [14], Hepatoprotective [15], Cardio protective [16], anti-arthritis and rubefacient[17-19].

Bark juice of this plant is given orally to prevent childhood diseases among the inhabitants of the Kanyakumari district [20]. The literature revealed that wide variety of medicinally important compounds including friedelin, diosgenin, sitosterol, butylic acid, dodecanoic anhydride, methyl pentacosanoate, kaemferol-3-O-α-D-glucoside and quercitin-3-O-α-D-glucoside have been reported from C. magna[21].

This present study was carried out to evaluate the anti-tumor activity of ethanolic extract of the root bark of Crataeva magnaLour DC belonging to family Capparidaceae against Dalton lymphoma ascites (DLA) in mice.

MATERIALS AND METHODS

Root bark of Crataeva magna Lour DC were collected in and around local forest area of Kanyakumari, Tamil Nadu and authenticated by the Botanist Prof.Chelladurai, Department of Botany, Govt. Siddha Medical College, Tirunelveli. A voucher herbarium specimen number KMCP/CM/01/2015 was also preserved in the K.M.College of Pharmacy, Madurai.

Preparation and Extraction of Plant material: The root bark is collected were subjected to dried in shade and then coarsely powdered. The 500 gms of powdered root bark of Crataeva magna Lour DC were defatted with petroleum ether and extracted successively with chloroform and ethanol using soxhlet apparatus. The extraction was carried out until the extractive becomes colorless. The extract was filtered through a cotton plug, followed by Whatmann filter paper (no.1). The extract was evaporated under reduced pressure using rotovac evaporator.

Isolation: This extract was concentrated in vacuum and subjected to flash column chromatography over TLC grade silica gel (60-120 mesh). Elution of the column first with petroleum ether, increasing amounts of EtOAc in petroleum ether and finally with methanol yielded several fractions. The proportion of solvent systems used to glubrable 1(10 mg) and 2(15 mg) were hexane-EtOAc (80: 20) and EtOAc–methanol (98:2) from fractions 5 and 8.

Selection Grouping and Acclimatization of Laboratory Animal: Male Swiss albino mice (20-25 gm) were produced from animal experimental laboratory, and used throughout the study. They were housed in micro nylon boxes in a control environment (temp 25±2°C) and 12 hrs dark /light cycle with standard laboratory diet and water ad libitum. The study was conducted after obtaining institutional animal ethical committee clearance. RM/PhD/MGR/2015. 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 [22].

Technique for inducing Tumor: Various technique for induction of cancer in animals, viz, chemically induced (using DMBA/croton oil, etc.). [23] virus induced, cell line induced (sarcoma – 180, ULCA fibro sarcoma and Jensen sarcoma, mouse lung fibroblast cells L-929, Dalton’s Ascites Lymphoma (DAL), Ehrlich Ascites Carcinoma (EAC)[24-26]methods have been used in experimental studies of anticancer activity. In the present study, cell lines induced cancer in mice was used to evaluate the anticancer activity of ethanolic extract of Crataeva magnaLourDC.

EVALUATION OF ANTICANCER ACTIVITY

Induction of cancer using DAL cells: Dalton’s Ascites Lymphoma (DAL) 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 DAL 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 1x10⁶, this dilution was given intraperitoneally. Let the tumor grow in the mice for minimum seven days before starting treatments.

Treatment Protocol: Swiss Albino mice were divided in to five group of six each. All the animals in four groups were injected with DAL cells (1x10⁶ cells per mouse) intraperitoneally, and the remaining one group is normal control group [27].

Group 1 served as the normal control.

Group 2 served as the tumor control. Group 1 and 2 receives normal diet and Water.

Group 3 served as the positive control, was treated with injection 5- fluorouracil at 20mg/kg body weight, Intra peritoneally[28].

Group-4 served as treatment control received 200 mg/kg EECM administrated through orally.

Group-5 servedas treatment control received 400 mg/kg of EECM administrated through orally.

Treatment: In this study, drug treatment was given after the 24 hrs of inoculation, once daily for 14 days. On day 14, after the last dose, all mice from each group were sacrificed by euthanasia. Blood was withdrawn from each mouse by retro orbital puncture bleeding and the following parameters were checked [29-31].

1. Hematological parameters
   - WBC count
   - RBC count
   - Hb content
   - Platelet count
   - Packed cell volume

2. Serum enzyme and lipid profile
   - Total Cholesterol (TC)
   - Triglycerides (TG)
   - Aspartate amino Transferase (AST)
   - Alanine amino Transferase (ALT)
3. Derived parameter

- 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 hemocytometer[32]:

\[
\text{No of cells dilution} = \frac{\text{Area} \times \text{Thickness of liquid film}}{\text{No of cells}}
\]

1) Hematological parameters

- WBC count
- RBC count and Hemoglobin
- Platelet count
- Packed Cell Volume

i) WBC count: The total WBC count was found to be increased in cancer control, when compared with normal and treated tumor-bearing mice [33].

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

iii) Platelets: In Hodgkin lymphoma, increased in platelet count often reported in laboratory finding. Hence, I investigated this parameter in the study [34].

iv) Packed cell volume: In any case of anemia the packed cell volume is 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)
- Triglyceride (TG)

1. TOTAL CHOLESTEROL AND TRIGLYCERIDE

(lipid profile): Abnormal blood lipid profile has been associated with cancer. In Hodgkin lymphoma, high cholesterol level and low triglyceride level has been reported. Hence, we investigated this parameter in the study [35].

2. LIVER ENZYMES (AST, ALT and ALP): Abnormal liver function seen in patient with Hodgkin lymphoma that these liver enzyme levels markedly increase in tumor bearing mice. ALP is an enzyme mainly derived from the liver, bones and in lesser amount from intestines, placenta, kidneys and leukocytes. An increase in ALP levels in the serum is frequently associated with the variety of disease [36]. ALP comprises a group of enzymes that catalyzes the phosphate esters in an alkaline environment, generating an organic radical and inorganic phosphate. Markedly elevated serum ALP, hyperalkaline-phosphatasemia, is seen predominantly with more specific disorders; including malignant biliary cirrhosis, hepatic lymphoma and sarcoidosis [37]. Hence, I investigated this parameter in this study.

3. DERIVED PARAMETERS

A. 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.

B. Percentage increase in life span (ILS): % ILS was calculated by the following formulae

\[
\text{% ILS} = \frac{\text{Life span of treated group} - \text{Life span of control group}}{\text{Life span of control group}} \times 100
\]

All biochemical investigations were done by using COBAS MIRA PLUS-SAuto analyzer from Roche Switzerland. Hematological test is carried out in COBAS MICROS OT 18 from Roche. Newly added Hi-Tech instruments MAX MAT used for an auto analyzer for all biochemistry investigations in blood sample.

RESULTS

Effect on Tumor growth: In the DAL tumor control group, the average life span of animal was found to be 49% where as EECM at a dose of 200 mg and 400 mg/kg body weight increase the life span to 88%, and 87% 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 Cratavea magna Lour. DC were evidenced by the significant reduction in percent increase in body weight of animal treated with Cratavea magnaLou. DC when compared to DAL tumor bearing mice. It was also supported by the significant reduction in packed cell volume and viable Tumor cell count in EECM at a dose of 200mg and 400/kg body weight treatments when compared to the DLA tumor control (Table 1&2) Fig. (1-5).

Table 1: Effect of EECM on serum Enzymes and lipid proteins

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cholesterol(mg/dl)</th>
<th>TGL (mg/dl)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>125.30±3.70</td>
<td>138.60±2.55</td>
<td>43.65 ±1.35</td>
<td>34.50 ±1.55</td>
<td>121.40 ±2.45</td>
</tr>
<tr>
<td>G2</td>
<td>154.90±4.65**</td>
<td>223.40±4.85**</td>
<td>89.6±2.70**</td>
<td>64.45±2.70**</td>
<td>230.45±4.36**</td>
</tr>
<tr>
<td>G3</td>
<td>132.50±3.90**</td>
<td>168.65±2.45**</td>
<td>56.48 ±1.85**</td>
<td>45.50±1.85**</td>
<td>169.45±2.55**</td>
</tr>
<tr>
<td>G4</td>
<td>134.55±4.15**</td>
<td>172.30±2.85**</td>
<td>64.50±1.95**</td>
<td>47.36±1.98**</td>
<td>188.65±2.70**</td>
</tr>
</tbody>
</table>

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HISTOPATHOLOGICAL RESULTS

![Fig. 1: Normal control section shows structure of liver of the mice](image1)

![Fig. 2: Tumor Control section shows structure of liver of mice](image2)

![Fig. 3: Standard control section shows structure of liver of mice](image3)

![Fig. 4: Treatment control section shows structure of liver of mice](image4)

![Fig. 5: Treatment control section shows structure of liver of mice](image5)

**Effect on Hematological Parameters**

As shown in (Table 3) RBC, Hgb, Platelets were decreased and WBC count was significantly increased in the DAL control group compared to the normal control group. Treatment with *Cratavea magna* Lour. DC 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 *Cratavea magna* Lour. DC. However, the standard 5-FU at the dose of 20 mg/kg body weight produced better result in all these parameters.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals</th>
<th>% ILS</th>
<th>Life span</th>
<th>Increase in Body weight grams</th>
<th>Cancer cell count (ml X 10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>6</td>
<td></td>
<td>&gt;&gt;31 days</td>
<td>1.70±0.62</td>
<td>3.70±0.40**</td>
</tr>
<tr>
<td>G2</td>
<td>6</td>
<td>49%</td>
<td>2.78±0.25</td>
<td>6.80±0.96**</td>
<td>2.80±0.30**</td>
</tr>
<tr>
<td>G3</td>
<td>6</td>
<td>92%</td>
<td>4.85±0.70**</td>
<td>4.85±0.70**</td>
<td>2.59±0.22**</td>
</tr>
<tr>
<td>G4</td>
<td>6</td>
<td>88%</td>
<td>5.55±0.88**</td>
<td>5.55±0.88**</td>
<td>2.80±0.30**</td>
</tr>
<tr>
<td>G5</td>
<td>6</td>
<td>87%</td>
<td>5.70±0.86**</td>
<td>5.70±0.86**</td>
<td>2.78±0.25**</td>
</tr>
</tbody>
</table>
G₁ – Normal Control, G₂ – Cancer Control, G₃ – Positive control, G₄ – Treatment control 200mg/kg EECM, G₅ – Treatment control 400 mg/kg EECM; All values are expressed as mean ± SEM for 6 animals in each group. **a – Values are significantly different from control (G₁) at P < 0.01; **b – Values are significantly different from cancer control (G₂) at P < 0.01

Table 3: Effect of EECM on Hematological Parameters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total WBC (Cells/mlx10³)</th>
<th>RBC Count (Mill/cumm)</th>
<th>Hb (Gm/dl)</th>
<th>PCV %</th>
<th>Platelets (Lakhs/cumm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁</td>
<td>13.85 ±1.80</td>
<td>5.60±0.86</td>
<td>13.65 ±1.30</td>
<td>16.40±2.45</td>
<td>5.46±0.90</td>
</tr>
<tr>
<td>G₂</td>
<td>15.65 ±2.60**</td>
<td>4.48±0.20**</td>
<td>8.36 ±0.92**</td>
<td>32.40±3.25**</td>
<td>3.75±0.62**</td>
</tr>
<tr>
<td>G₃</td>
<td>12.60±1.75**</td>
<td>5.20±0.78**</td>
<td>12.30±1.45**</td>
<td>18.40±1.50**</td>
<td>4.80±0.96**</td>
</tr>
<tr>
<td>G₄</td>
<td>11.42±1.90**</td>
<td>5.45±0.58**</td>
<td>11.40±1.32**</td>
<td>22.40±1.70**</td>
<td>4.15±0.80**</td>
</tr>
<tr>
<td>G₅</td>
<td>13.40±1.75**</td>
<td>5.25±0.50**</td>
<td>11.5±1.05**</td>
<td>23.26±1.85**</td>
<td>4.30±0.92**</td>
</tr>
</tbody>
</table>

G₁ – Normal Control, G₂ – Cancer Control, G₃ – Positive control, G₄ – Treatment control 200mg/kg EECM, G₅ – Treatment control 400 mg/kg EECM; All values are expressed as mean ± SEM for 6 animals in each group. **a – Values are significantly different from control (G₁) at P < 0.01; **b – Values are significantly different from cancer control (G₂) at P < 0.01

Effect on Biochemical Parameters

The inoculation of DAL cells caused significantly increase in the level of Total Cholesterol, Aspartate amino Transferase, Alanine amino Transferase, Alkaline Phosphatase in the tumor control animals (G₃), when compared to the normal group. The treatment with cratavea magna Lour. DC weight reversed these changes towards the normal level (Table 2). All the value was found to be significant. The treatment with standard 5-FU also gave similar results.

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 anticancer agents. Several studies have been conducted on traditional medicine by a multitude of ethanol botanical grounds. Many plants possess anticancer properties have been documented[38-43]. Plants of ethanolic extract of Cratavea magna Lour. DC was traditionally used in the treatment of tumors. The present investigation was carried out to evaluate the antitumor activity of ethanolic extract of Cratavea magna Lour. DC in DAL tumor bearing mice. The ethanolic extract of Cratavea magna Lour. DC treated animals at the doses of 200 & 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 DAL tumor bearing, 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 [44].

Treatment with Cratavea magna Lour. DC 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[45]. It may be concluded that by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of DAL bearing mice. Thus, Cratavea magna Lour. DC at a dose of 200 mg and 400 mg/kg body weight have antitumor activity against DAL bearing mice.

Usually, in cancer chemotherapy the major problems that are being encountered are of myelo suppression and anemia [46,47]. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency or due to hemolytic or myeloplastic conditions. Treatment with Ethanolic extract of Cratavea magna Lour. DC brought back the hemoglobin (Hb) content, RBC and WBC count to normal levels significantly. This clearly indicates Ethanolic extract of Cratavea magna Lour. DC 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 the function of the liver. The significantly elevated level of total cholesterol, TG, AST, ALT and ALP in serum of tumor inoculated animal indicated liver damage and loss of functional integrity of cell membrane. The significant reversals of these changes towards the normal by Ethanolic extract of Cratavea magna Lour. DC treatments.

In the present study, the biochemical examination of DAL inoculated animals showed marked changes indicating the toxic effect of the tumour. The normalization of these effects observed in the serum treated Ethanolic extract of Cratavea magna Lour. DC weight supported the potent antitumor effect of the Ethanolic extract of Cratavea magna Lour. DC.

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