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Inhibition of Ehrlich Ascites Carcinoma by *Crotalaria verrucosa* L. Leaf in Swiss Albino Mice

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Abstract

Objective: *The objective of the present study was to evaluate the antitumor activity of Crotalaria verrucosa L. leaf against Ehrlich ascites carcinoma (EAC) in Swiss albino mice.* ***Methods:*** *The in vivo antitumour activity of the ethanol extract of leaf of C. verrucosa L. (ELCV) was evaluated at 50, 100 and 200 mg/kg bw against EAC, using mean survival time. After administration of the extract of C. verrucosa, viable EAC cell count and body weight in the EAC tumour hosts were observed. The animal was also observed for improvement in the haematological parameters (e.g., heamoglobin content, red and white blood cells count) after ELCV treatment.* ***Results:*** *Intraperitoneal administration of ELCV reduced viable EAC cells, increased the survival time, and restored altered haematological parameters. Significant efficacy was observed for ELCV at 100 mg/kg dose (P<0.05).* ***Conclusions:*** *It can be concluded that the ethanol extract of leaf of C. verrucosa L. possesses significant antitumour activity.*

Keywords: *Crotalaria verrucosa L., Ehrlich ascites carcinoma, antitumour property, ethanol extract*

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INTRODUCTION

The majority of the world's people in emerging nations still trusts on herbal remedies to meet their health needs in cases when artificial drug could not relieve patients who suffer from painful diseases such as cancer [1]. In the modern system of medicine, numerous chemotherapeutic agents have been developed as a result of screening of the medicinal plants in various parts of the world [2]. So there is a mounting attention in the pharmacological estimation of medicinal plants.

Crotalaria verrucosa (Fabacea) is well known as blue rattlesnake. It is an erect shrub which nurtures to 80-100 cm high with angular branches. It has oviate to triangular leaves. The extract of its leaves is used in scabies and impetigo, and is considered effective in weakening salivation. In Nigeria it is used for various skin infections, colic and flatulence. It is widely spread in tropics and is found in waste places of native India. The leaf extract is given orally to cure jaundice. Aqueous and ethanolic extracts of aerial parts of *C. verrucosa* were effective for fertility and estrogenic implantation in Albino rats and also has exhibited very substantial hepato protective property against paracetamol induced hepatotoxicity study models in Wistar rats [3]. However to the best of our knowledge, a systematic study on anticancer activity of *C. verrucosa* has not been commenced to estimate the anticancer properties of ethanol extract of *C. verrucosa* on animal models in Swiss albino mice [4].

MATERIALS AND METHODS

Plant Materials

Leaf *Crotalaria verrucosa* (Fabacea) were collected in December 2015, from Kolli Hills district of Namakkal, Tamil Nadu, India. The plant was identified with the help of flora of Karnataka and authenticated by Rabinet Herbarium, St. Joseph's College, Tiruchirappalli, Tamil Nadu. The leaves of the plant were dried, powdered and passed through 40 mesh sieve and stored in an airtight container for further use.

Extraction
The collected leaves were cleaned and shade-dried. The dried leaves were then pulverized into a coarse powder by a grinding machine. The powdered leaves (500 g) were extracted with ethanol at room temperature. The extract was then filtered through filter papers and filtrates were evaporated under reduced pressure at 40°C using a rotary evaporator to get 5.7 g ELCV.

Animals
Male Swiss albino mice (25-30 g) were procured from the BioGen Animal's Supplier, Hoshur. They were used throughout the study and housed in iron cages in a controlled environment (temperature (25±2)°C and 12 h dark/light cycle) with standard laboratory diet and water *ad libitum.* Experiments were carried out in accordance with the Ethical Committee Guidelines laid down by the local committee regarding the care and use of animals for experimental procedures.

Tumour Cells
EAC cells were obtained from the Courtesy of Amala Institute of Medical Sciences, Amala Nagar, Thrissur, Kerala, India, and maintained by weekly intraperitoneal (ip) inoculation of 10^6 cells/mouse in the laboratory.

Acute Toxicity Studies
An acute toxicity study concerning to the determination of median lethal dose (LD_{50}) was performed by the method of Lorke [5]. Mice were randomly divided into ELCV-treated 'test' groups and vehicle-treated 'control' group consisting of seven groups with six mice per cage. ELCV (500, 1000, 2000, 3000, 4000, and 5000 mg/kg) was separately administered intraperitonealy to the mice in each of the test groups. Each mouse in the control group was treated with vehicle alone (2% dimethylsulfoxide (DMSO)). Then after 24 h, the mortality number caused by the extract was observed from which the LD_{50} of ELCV was determined.

Cell Growth Inhibition
In vivo tumour cell growth inhibition was carried out by the method as described by Sur *et al.* [6]. 1×10^6 EAC cells were inoculated into five groups (Groups 2 to 6) of mice (six in each) on day 0. The control Group 2 was treated with vehicle (2% DMSO). Mice in Group 3, 4 and 5 were administered (ip) with ELCV at 100, 200 and 300 mg/kg bw doses and Group 6 received 5-fluorouracil (20 mg/kg bw). Treatment was continued for 13 days and on the 14th day after tumour transplantation, animals were sacrificed. Tumour cells were collected by repeated washing with 0.9% saline and viable tumour cells in the treated groups were compared with those of the control.

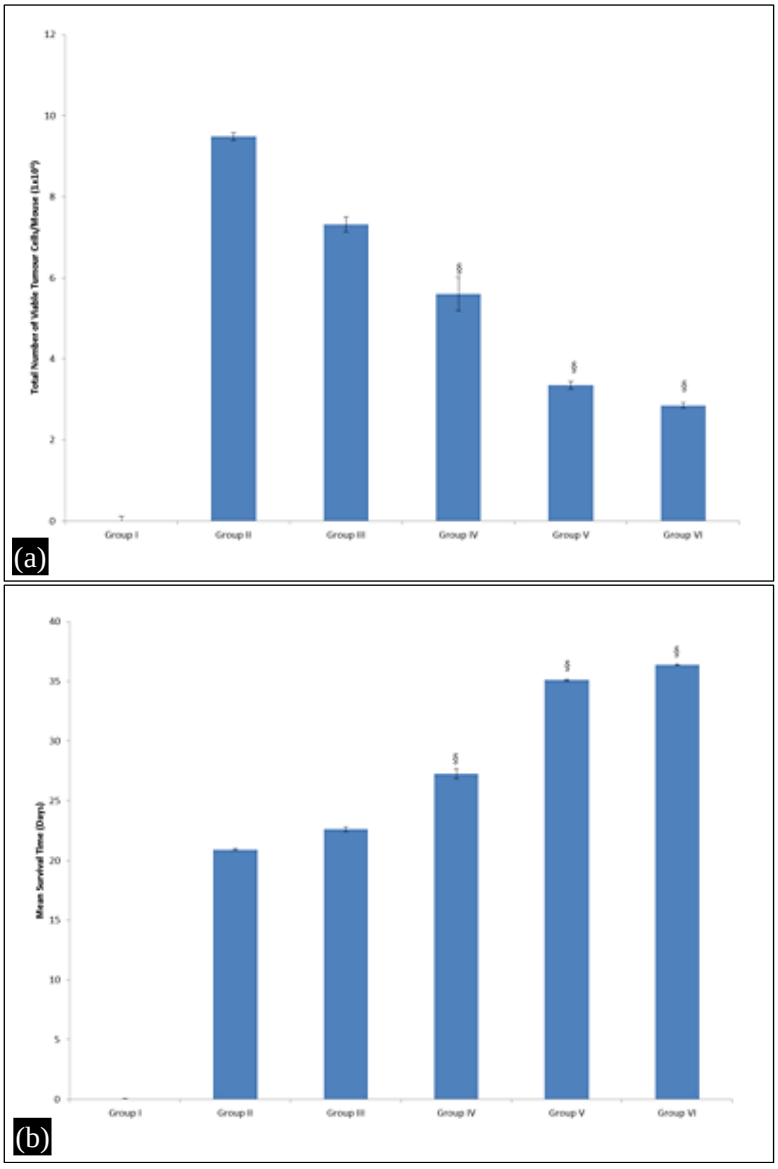
Studies on Survival Time and Hematological Parameters
Swiss albino mice were divided into six groups (n=6). All animals were injected with EAC cells (1×10^6 cells/mouse) intraperitoneally except for the normal group. This was taken on day 0. Group 1 served as the normal control and Group 2 served as the tumour control. These two groups received 2% DMSO. Group 3, 4 and 5 were treated with ELCV at 100, 200 and 300 mg/kg bw, respectively. Group 6 which served as the positive control was treated with 5-fluorouracil at 20 mg/kg bw. All these treatments were given 24 h after the tumour inoculation, daily for 13 days. On the 14th day after tumor inoculation, hematological parameters (hemoglobin, RBC and WBC) were measured from freely flowing tail vein blood of each mice of each group under sterilize condition [7]. Then mean survival time (MST) of each EAC cell inoculated group was noted.

Statistical Analysis
All values were expressed as Mean ± SEM (Standard Error of Mean). Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett's 't' test using SPSS statistical software of 14 version. $P<0.05$ was considered to be statistically significant when compared with control.

RESULTS
The LD_{50} value of ELCV was assessed in Swiss albino mice and found to be 5000 mg/kg bw. Antitumour activity of ELCV against EAC cell bearing mice was evaluated by the parameters such as viable EAC cell (% inhibition in cell growth), mean survival time (MST), percentage (%) increase of life span (% ILS) and body weight gain. The average number of viable tumour cells per mouse of tumour control group

was found to be $(6.83\pm0.95)\times10^6$ cells/ml. Treatment with ELCV (100, 200 and 300 mg/kg bw) reduced the viable cells significantly ($P<0.05$) (Figure 1A).

The effect of ELCV on the survival of EAC bearing mice has been shown in Figure 1B. The MST of the control group was (19.71±0.41) days, whereas it was (25.37±1.13), (30.49±0.91), (35.70±1.19) and (41.98±0.21) for the groups treated with ELCV (100, 200 and 300 mg/kg bw) and 5-fluorouracil (20 mg/kg bw), respectively. The increase in the life span of EAC cell bearing mice treated with ELCV (100, 200 and 300 mg/kg bw) and 5-fluorouracil (20 mg/kg bw) was found to be 14.58, 37.88, 73.74 and 80.03%, respectively (Figure 1C). On the 14th day of tumour cell inoculation, the average weight gain of only EAC cell bearing mice was (34.68±0.11) g whereas it was (29.07±0.06), (24.78±0.07), (19.28±0.05) and (14.17±0.11) g for the groups treated with ELCV (100, 200 and 300 mg/kg bw) and 5-fluorouracil (20 mg/kg bw), respectively. ELCV at 300 mg/kg dose significantly reduced the weight gain ($P<0.05$) (Figure 1D).



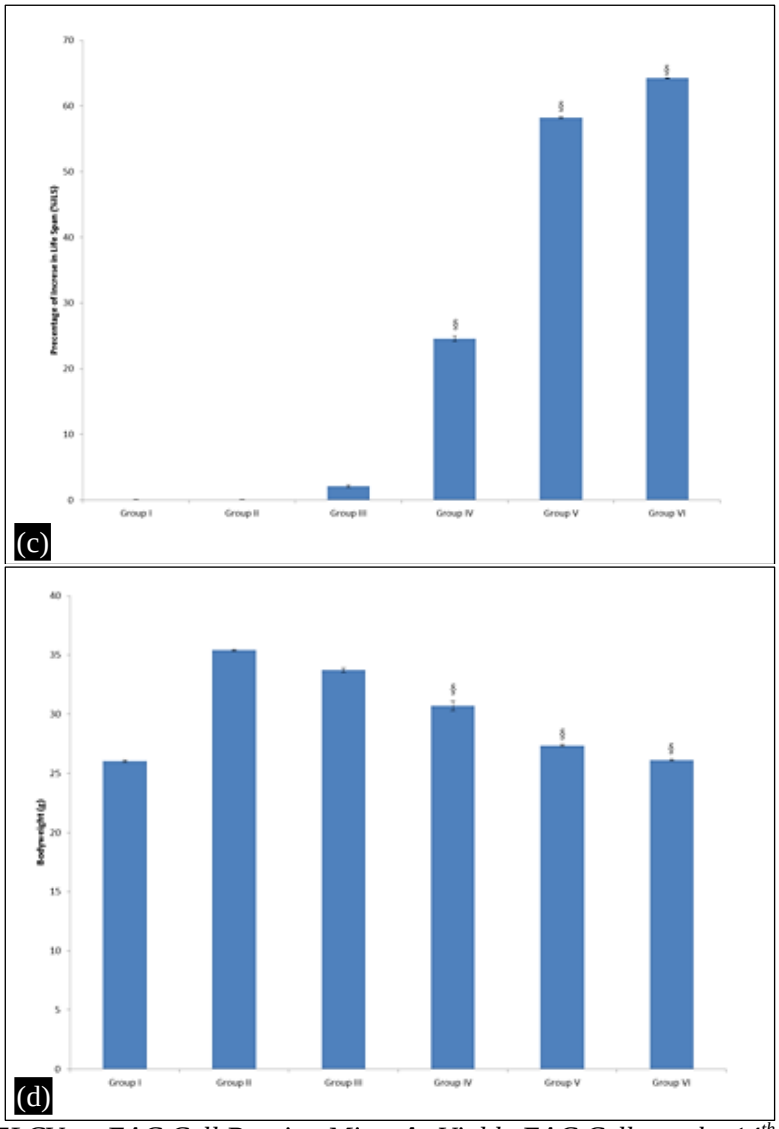


Fig. 1: *Effect of ELCV on EAC Cell Bearing Mice.* ***A:*** *Viable EAC Cells on the 14th Day after Tumor Cell Inoculation;* ***B:*** *Mean Survival Time;* ***C:*** *% Increase in Life Span;* ***D:*** *Body Weight Gain on the 14th day. Data are Expressed as Mean ± SEM (n=6). §P<0.05, between EAC Control and ELCV-Treated Group.*

Table 1: *Effect of ELCV on Hematological Parameters of EAC Cell Bearing Mice (Mean ± SEM) (mg/kg bw).*

Parameters	Treatment					
	Group I	Group II	Group III	Group IV	Group V	Group VI
Hb (%)	11.23±0.26	5.59±0.11	6.22±0.18	7.93±0.17	9.32±0.22§	9.76±0.13§
RBC (10^6 Cells/mm^3)	5.20±0.07	2.70±0.09	3.43±0.17	4.23±0.06	4.87±0.05§	5.23±0.18§
WBC (10^3 Cells/mm^3)	2.93±0.09	8.98±0.17	4.74±0.17	5.46±0.12	6.98±0.05§	7.98±0.15§

Data are Expressed as Mean ± SEM (n=6). §P<0.05, between EAC Control and ELCV-Treated Group.

Hematological parameters of EAC cell bearing mice on the 14th day showed significant changes when compared with normal mice (P<0.05) (Table 1). The total WBC count was found to increase with a reduction in the hemoglobin content and total RBC count. The WBC of EAC bearing was increased when compared with normal mice. At the same time interval, treatment of ELCV (100, 200 and 300 mg/kg bw) could change these parameters

near to normal. Maximum and significant alteration occurred in the ELCV treatment at the dose of 300 mg/kg ($P<0.05$). The differential counts were found to be similar to that of EAC cell bearing mice and treatment of ELCV could not normalize the differential count.

DISCUSSION

The results of the present study evidently reveal the tumour inhibitory activity of ELCV against EAC. The unswerving principles for appraising an anticancer drug are perpetuation of lifespan of the animal and decrease in WBC count of blood. Results of this study have exposed an increase in lifespan chaperoned by a lessening in WBC count in ELCV treated mice. It had noteworthy effect on increasing the life span of EAC bearing animals and also found to reduce the viable EAC cells in animal models. These results clearly validate the antitumour effect of ELCV against EAC. During the development of cancer chemotherapy, the main hitches are myelosuppression and anaemia [8].

The anaemia encountered in tumour bearing mice is mainly due to reduction in RBC and hemoglobin and this may occur either due to iron deficiency or hemolytic or myelopathic conditions [9]. Treatment with ELCV restored the hemoglobin content, RBC and WBC cell count to normal values. This indicates that ELCV possesses protective effect on the haematopoietic system.

A regular and rapid increase in ascetic fluid volume was observed in EAC bearing mice. Ascetic fluid is the direct nutritional source for tumour growth because it meets the nutritional requirements of tumor cells [10]. ELCV treatment reduced the number of viable cancer cell count and increased the lifespan. It may be suggested that ELCV can reduce the nutritional fluid volume and thereby arrests tumour growth and increases the life span.

Preliminary phytochemical screening indicated the presence of terpenoids, flavones, alkaloids, phenol, and glycosides in ELCV. These phytocompounds are identified to possess potent antitumor properties [11-15]. In addition, flavonoids could also induce mechanisms that may kill cancer cells and inhibit tumor invasion [16, 17]. The antitumour properties of ELCV may be due to these compounds. Further research work is needed to establish the exact antitumour mechanism action of ELCV as well as identify the main active phytochemicals responsible for the inhibition of EAC.

CONCLUSION

It can be concluded that etahnolic leaf extract of *C. verrucosa* exhibits strong antiproliferative activities in EAC tumor-transplanted mice.

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