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Anti cancer Activity of Ethanolic Extract of *Crataeva magna* Lour (DC) against Ehrlich Ascitic Carcinoma Cell Lines in Mice

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Abstract

Cancer is the second leading cause of death worldwide next to cardiovascular diseases. Conventional therapies for treatment of cancer including chemotherapy and radiation therapy usually pose serious side effects. Use of herbal medicine for cancer treatment is gaining importance in this regard. India is a rich source of medicinal plants and a number of plant extracts are used against diseases in various medicinal systems. 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 Ehrlich ascites carcinoma (EAC) cell lines. EAC cells were injected intraperitoneally (1×10^6 cells/ml/mouse) to the mice. The activity was assessed using survival time, average increase in body weight, hematological parameters and solid tumor volume. Oral administration of EECM at the dose of 200 and 400 mg/Kg, significantly ($p < 0.001$) increased the survival time and decreased the average body weight of the tumor bearing mice. After 14 days of inoculation, EECM was able to reverse the changes in the hematological parameters and PCV consequent to tumor inoculation. Oral administration of EECM was effective in reducing solid tumor mass development induced by EAC cells. The results indicate that EECM possess significant antitumor activity on dose dependent manner. 5-Flurouracil (20 mg/kg) was used as a standard drug. All these findings enable to conclude that both doses of EECM possess a protective effect against EAC. From the result it was find out that the ethanolic extract of root bark of *Crataeva magna* Lour DC has potent dose dependent antitumor activity and that is comparable to that of 5-Fluorouracil.

Keywords: Ehrlich ascites carcinoma, *Crataeva magna* Lour DC, 5-Fluorouracil, Tumor volume, Lifespan, Hematological Parameters.

INTRODUCTION

The plant-derived compounds have always been an important source of medicines for various diseases and have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemo preventive effects [1]. Cancer is the second leading cause of death all over the world [2]. Cancer is a general term applied of series of malignant diseases that may affect different parts of the body. These diseases are characterized by a rapid and uncontrolled formation of abnormal cells; which may mass together to form a growth or tumor or proliferate throughout the body, initiating abnormal growth at other sites.[3,4] Cancer may affect people at all ages, even fetuses, but the risk for most varieties increases with age.[5] Cancer causes about 13% of all human deaths.[6] According to the American Cancer Society, around 7.6 million people die every year from cancer[7] A large number of medicinal plants and their constituents have been known to possess beneficial therapeutic potential[8]. India is a rich source of medicinal plants and a number of plant extracts are used against diseases in various systems of medicine such as Ayurveda, Unani and Siddha. Only a few of them have been scientifically explored [9].According to World Health Organization, more than 10 million new cases of cancer are diagnosed every year, and the statistical trends indicate that this number would double by 2020 [10]. It has recommended the evaluation of the plants effectiveness in conditions where we lack safe modern drugs [11]. This has lead to an increasing demand of research on anticancer natural products which produces minimal or no side effects[12].Most of the current anticancer drugs are derived from plant sources, which act through different pathways converging ultimately into activation of apoptosis in cancer cells leading to cell cytotoxicity [13]. There is a growing interest in the pharmacological evaluation of various plants used in the Indian traditional system of medicine.[14]

In developed countries at least one in five of the population can expect to die of cancer. Few categories of medications are commonly used with a narrow therapeutic index and have a greater potential of causing severe side effects. As the synthetic antineoplastic drugs posses comparatively more adverse effects, so herbal drugs are being evaluated as these are comparatively safe or non toxic to the host cell.[15]

Crataeva magna Lour DC (family Capparidaceae) is known as three leaved caper 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 [16-18]. 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[19]. The traditional plant used to treat various ailments in particular to Urolithiasis [20], Hepatoprotective [21], Cardio protective [22], anti arthritic and rubifacient [23-25]. Bark juice of this plant is given orally to prevent childhood diseases among the inhabitants of the Kanyakumari district [26]. The literature revealed that wide variety of

medicinally important compounds including friedelin, diosgenin, sitosterol, butulic acid, dodecanoic anhydride, methyl pentacosanoate, kaemferol-3-O-α-D-glucoside and quercitin-3-O-α-D-glucoside have been reported from *C. magna* [27]

In this present study was carried out to evaluate the anti-tumor activity of ethanolic extract of the root bark of *Crataeva magna* Lour DC belonging to family Capparidaceae against Ehrlich ascites carcinoma (EAC) in mice.

MATERIALS AND METHODS

Root bark of *Crataeva magna* Lour DC were collected in and around local forest area of Kanyakumari, Tamilnadu 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 whattman 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 ethanol yielded a number of fractions. The proportion of solvent systems used to obtain compound 1(10 mg) and compound 2 (15 mg) were hexane-EtOAc (80 : 20) and EtOAc –ethanol (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 [28].

Acute Toxicity Studies (Ld_{50})

The oral acute toxicity study of the extract was carried out in Swiss albino mice using up and down procedure as per OECD, 2001 [29] Mice received ethanol extract at various doses (500-2,000 mg/Kg) orally by gavage. They were observed for toxic symptoms continuously for the first 4 h after dosing. Finally, the number of survivors was noticed after 24 h. In the toxicity study, no mortality occurred within 24 h under the tested doses of ethanolic extract of *cratavea magna* lour DC.

Technique for Inducing Tumor

Various technique for induction of cancer in animals, viz, chemically induced (using DMBA/croton oil, etc) [30] 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) [31-33] methods have been used in experimental studies of anticancer activity. In the present study, Ehrlich Ascites Carcinoma cell lines induced cancer in mice was used to evaluate the anticancer activity of ethanolic extract of *cratavea magna* lour DC.

Evaluation of Anticancer Activity

Induction of cancer using EAC cells

Ehrlich Ascites Carcinoma cell lines 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 EAC 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 x 10^6, this dilution was given intraperitoneally. Let the tumor grow in the mice for minimum seven days before starting treatments.

Effect of EECM on survival time [34]

Animals were inoculated with 1 × 10^6 cells/mouse on day '0' and the treatment with ethanolic extract of *cratavea magna* started 24 h after inoculation, at doses of 200 and 400 mg/Kg/day, *p.o.* The control group was treated with the same volume of 5% gum acacia solution. All the treatments were given for nine days. The median survival time (MST) and average body weight changes of each group, consisting of 6 mice, were noted. The antitumor efficacy of ethanolic extract of *cratavea magna* was compared with that of 5-fluorouracil (Dabur Pharmaceuticals, India; 5-FU, 20 mg/Kg/day, IP for 9 days). The MST of the treated groups was compared with that of the control group using the following calculation:

Increase in life span = (T – C) / C × 100

T = number of days the treated animals survived

C = number of days the control animals survived

Effect of EECM on hematological parameters

In order to detect the influence of EECM on hematological status of EAC bearing mice, a comparison was made among five groups (n = 5) of mice on the 14th day after inoculation. The groups were comprised of (I) Normal, (II) Tumor Control mice (III) Tumor bearing mice treated with EECM (200 mg/Kg/day, p.o. for 9 days), (IV) Tumor bearing mice treated with EECM (400 mg/Kg/day, p.o. for 9 days). Blood was drawn from each mouse by the retroorbital plexus method and the white blood cells (WBC), red blood cells (RBC), hemoglobin, protein and packed cell volume (PCV) were determined [35-37].

Effect of EECM on solid tumor

Mice were divided into four groups (n = 6). Tumor cells (1 × 10^6 cells/mouse) were injected into the right hind limb of all the animals intramuscularly. The mice of group I was served as control. Group II received EECM (200

mg/Kg/day, p.o.) and group III received EECM (400 mg/Kg/day, p.o.) for 5 alternative days. Tumor mass was measured from the 11th day of tumor induction. The measurement was carried out every 5 days for a period of 30 days. The volume of tumor mass was calculated using the formula V = 4/3 πr2, where 'r' is the mean of 'r1' and 'r2' which are the two independent radii of the tumor mass [38]

Statistical analysis

All values were expressed as mean ± SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett's t-test. p-values < 0.05 were considered to be statistically significant when compared to control.

RESULTS AND DISCUSSION

Effect of EECM on survival time

The effect of EECM on the survival of tumor bearing mice is shown in (Table 1). The MST of the control group was 16 ± 0.75 days, whereas it was 26 ± 0.92, 28 ± 0.76, 32 ± 0.21 and 31 ± 0.41 days for the groups treated with EECM (200 and 400 mg/Kg) and 5-FU (20 mg/Kg) respectively. The increase in the life span of tumor bearing mice treated with EECM (200 and 400 mg/Kg) and 5-FU was found to be 62.5%, 75%, 100% and 93.75% respectively. The effect of EECM on the inhibition of average increase in body weight is shown in Table 1. The average weight gain of tumor bearing mice was 13.3 ± 0.61 g, whereas it was 8.3 ± 0.84, 5.3 ± 0.66, 4.3 ± 0.36 and 4.0 ± 0.44 g for the groups treated with EECM (200 and 400 mg/Kg) and 5-FU (20 mg/Kg) respectively.

Effect of EECM on hematological parameters

Hematological parameters of tumor bearing mice on 14th day showed significant changes compared to the normal mice (Table 2). The total WBC count and PCV were found to increase with a reduction in the hemoglobin content of RBC. The differential count of WBC showed that the percentage of neutrophils increased while that of lymphocytes decreased. At the same time interval, EECM (200 and 400 mg/Kg) treatment could change these parameters near to normal. Maximum alteration occurred in the EGC treatment at the dose of 400 mg/Kg.

Effect of EECM on solid tumor

There was significant reduction in the tumor volume of mice treated with EECM (200 and 400 mg/Kg). Tumor volume of control animals was 6.62 ± 0.38 mL whereas it was 4.26 ± 0.18, 4.21 ± 0.1 and 4.17 ± 0.21 mL for the groups treated with EECM (200 and 400 mg/Kg) respectively (Table 3).

Table No.1 Effect of EECM on the life span, body weight and Median survival time

Treatment	Number of animals	% ILS Life span	Increase in Body weight grams	Median survival time (days)
Tumour control	6	-	3.3 ± 0.61	16 ± 0.75
5 Fluoro uracil	6	93.71%	4.0 ± 0.44*	31 ± 0.41*
EECM (200mg/Kg)	6	75%	5.3 ± 0.66*	28 ± 0.76*
EECM (400mg/Kg)	6	100%	4.3 ± 0.36*	32 ± 0.21*

All values are expressed as mean ± SEM for 6 animals in each group.
*p < 0.001; **p < 0.01 when compared with control. Data were analyzed by one-way ANOVA followed by Dunnett's test.

Table No. 2 Effect of EECM on Haematological Parameters

N = 5 animals in each group. Values are expressed as mean ± SEM.

Treatment	Total WBC Cells /mlx10^3	RBC Count Mill/cumm	HbGm/dl	PCV %	Differential count (%)		
					Lymphocytes	Neutrophils	Monocytes
Normal	13.85 ±1.80	5.60±0.86	13.65 ±1.30	16.40±2.45	70.7 ± 1.1	30.3 ± 0.21	1 ± 0
Cancer Control	15.65±2.60^{a**}	4.48±0.20^{a**}	8.36 ±0.92^{a**}	32.40±3.25^{a**}	60 ± 3.92	38 ± 3.2	1 ± 0
200mg/kg EECM	12.60±1.75^{b**}	5.20±0.78^{b**}	12.30±1.45^{b**}	18.40±1.50^{b**}	83 ± 4.74*	15 ± 1.82	2 ± 0
400mg/kg EECM	11.42±1.90^{b**}	5.45±0.58^{b**}	11.40±1.32^{b**}	22.40±1.70^{b**}	65.5 ± 0.21*	27.8 ± 0.25*	1 ± 0

* p < 0.001; **p < 0.01; ***p < 0.05 when compared with control. Data were analyzed by one-way ANOVA followed by Dunnett's test.

Table No.3 Effect of EECM on the solid tumor volume

Treatment	Solid tumor volume (ml)			
	15th day	20th day	25th day	30th day
Tumor control	3.99 ± 0.23	4.63 ± 0.26	5.13 ± 0.41	6.62 ± 0.38
EECM (200mg/Kg)	2.13 ± 0.21*	3.58 ± 0.16*	3.86 ± 0.21*	4.21 ± 0.1*
EECM (400mg/Kg)	2.27 ± 0.37*	3.27 ± 0.37*	3.66 ± 0.19*	4.17 ± 0.21*

N = 6 animals in each group. Values are expressed as mean ± SEM.
*p < 0.001when compared with control. Data were analyzed by one-way ANOVA followed by Dunnett's test.

DISCUSSION

Cytotoxicity is one of the chemotherapeutic targets of antitumor activity[39].Ehrlich ascites tumor is a rapidly growing carcinoma with very aggressive behavior . Most of the clinically used antitumor agents possess significant cytotoxic activity in cell culture systems. It is able to grow in almost all strains of mice. The Ehrlich ascitic tumor implantation induces a local inflammatory reaction, with increasing vascular permeability, which results in an intense edema formation, cellular migration, and a progressive ascitic fluid formation. The ascitic fluid is essential for tumor growth, since it constitutes a direct nutritional source for tumor cells [40].Preliminary Phytochemical study indicated the presence of flavonoid, alkaloids and tannins in ethanolic extract of *Cratavea magna*. Flavonoids have been shown to possess antimutagenic and antimalignant effect [41].Furthermore flavonoid have a chemo preventive role in cancer through their effect on signal transduction in cell proliferation and angiogenesis [42] Also, quercetin has been proved to inhibit human breast cancer cells [43] and prostate cancer cells[44] The present observation suggests that flavonoid and several other compounds present in the ethanolic extract might be responsible for its cytotoxic and anticancer properties. Identification and characterization of the active principles from ethanolic extract needs to be done to support this hypothesis. Thus, from this study, it is likely that ethanolic extract has high cytotoxic and antitumor properties, suggesting a potential role of ethanolic extract as a powerful chemotherapeutic agent for cancer. However, further research work is required to establish the exact mechanism of action of EECM at molecular level. This study should help to confirm the effectiveness of *Cratavea magna* in the treatment of cancer.

CONCLUSION

In conclusion, ethanolic extract of *Cratavea magna* significantly inhibited tumor in induced cancer in swiss albino mice. This activity involves restoration of hematopoietic parameters, median survival time and increased lifespan of the animals. These results suggest that *Cratavea magna* might be a good choice for the treatment of cancer. No toxic symptoms were observed for all two doses during the period of study. This may be used to development of effective therapeutic approaches towards the prevention or treatments of various immune conditions and different types of cancer.

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