



**ANTITUMOR EFFECT OF *DECALEPIS HAMILTONII* ROOT EXTRACT
AGAINST EHRLICH ASCITES TUMOR IN MICE**

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ABSTRACT

The antitumor activity of *Decalepis hamiltonii* root extract (DHA) was investigated in Ehrlich Ascites Tumor (EAT) cell model in mice and the effect was compared with that of the anticancer drug, cyclophosphamide (CP). Treatment of EAT cell bearing mice with aqueous extract of *Decalepis hamiltonii* (50 mg/kg and 100 mg/kg b.w.) showed a significant reduction in ascites tumor volume, tumor cell counts and increased median survival time (MST). EAT cells induced alterations in hematological profile and the serum marker enzymes aspartate and alanine transaminases (AST, ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were restored by DHA treatment. The antitumor effect of DHA was comparable to that of the anticancer drug cyclophosphamide suggesting the anticancer potential of the root extract of *Decalepis hamiltonii*.

KEYWORDS: *Decalepis hamiltonii*, Antitumor, Cyclophosphamide, Ehrlich Ascites Tumor cells.



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INTRODUCTION

Cancer is a major ailment that affects several organs and chemotherapy is widely practiced to treat the disease¹. Chemotherapy, with or without radiation, although effective for many cancers, is accompanied by severe side effects due to the toxicity of the anticancer drugs². Several anticancer drugs are derived from plants such as taxol, cyclophosphamide, vincristine, vinorelbine and there is great interest in the search for newer and safer drugs from plants^{3,4}. Although several plants have shown promising anticancer potential in experimental animals, very few have led to developing a safer anticancer drug^{5,6}. There is a need to explore the anticancer potential of medicinal plants in order to discover new chemotherapeutic agents. *Decalepis hamiltonii* (Wight and Arn.) (family: Asclepiadaceae), a climbing shrub, grows in the forests of peninsular India. Its tuberous roots are consumed as pickles and juice for their health promoting properties in southern India. The roots are also used in folk medicine and ayurvedic (the ancient Indian traditional system of medicine) preparations as a general vitaliser⁷. Our earlier work has shown that the roots of *D. hamiltonii* possess potent antioxidant properties⁸. In our laboratory, several novel antioxidant compounds have been isolated and characterized from the roots of *D. hamiltonii*, which could be associated with their alleged health benefits⁹⁻¹¹. The root extract of *D. hamiltonii* also shows *in vivo* hepatoprotective and neuroprotective potential in the laboratory rat¹²⁻¹⁵. *D. hamiltonii* root extract has also been reported to have anti-inflammatory activity¹⁶. Since free radicals have been implicated in carcinogenesis, anticancer activity of plant extracts have also been associated with their antioxidant activity, we investigated if roots of *D. hamiltonii* could have antitumor potential in view of the presence of several bioactive molecules. In this study, we have shown the antitumor effect of *D. hamiltonii* root extract against Ehrlich Ascites Tumor cells in mice and compared its effect with that of the anticancer drug, cyclophosphamide.

MATERIALS AND METHODS

Trypan blue was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Cyclophosphamide (Ledoxan) was obtained from Dabur Pharma Limited, New Delhi, India. All other chemicals were purchased from Sisco Research Laboratories, Mumbai, India and were of highest purity grade available.

(i) Preparation of the root extract

Roots of *D. hamiltonii* were collected from B.R. Hills, Karnataka, India and the taxonomic identity of the plant has been previously reported⁸. The tuberous roots were washed with water followed by crushing with a roller to separate the inner woody core from the outer fleshy layer. The fleshy portion was collected, dried at 40°C and finely powdered. The aqueous extract was prepared by mixing the root powder in warm water (50°C) and allowed to stand for 24h, filtered with Whatman No. 1 paper and the filtrate was lyophilized and weighed. Aqueous extract of *D. hamiltonii* was chosen for this study as it has been shown to have high antioxidant activity and chemoprevention potential^{10,11}.

(ii) Animals

Swiss albino mice were procured from the animal colony maintained at the Department of Zoology, University of Mysore, Mysore, India. The animals were kept at room temperature (25±2 °C), with 70±10% humidity and day light cycle of 12 hrs light and 12 hrs dark, and standard laboratory animal feed and water were provided *ad libitum*. Animals were acclimatized to the experimental conditions for a period of one week before the initiation of the experiment. Appropriate guidelines of the local animal ethics committee were followed for the animal experiments (UOM/1AEC/33/2011).

(iii) Experimental Protocol

Eight weeks old adult male mice (30-32 g) were divided into groups of 12 each. Dosage of DHA was selected based on our earlier studies¹⁵.

Group I	-	Control
Group II	-	EAT-bearing mice + Saline (1 ml/kg b.w.)
Group III	-	EAT- bearing mice + DHA (50 mg/kg b.w.)
Group IV	-	EAT- bearing mice + DHA (100 mg/kg b.w.)
Group V	-	EAT- bearing mice + CP (25 mg/kg b.w.) (diluted in saline)

The EAT cells were obtained from National Center for Cell Science, Pune, India and maintained by *in vivo* propagation in mice. EAT cells were grown in the peritoneal cavity of mice by peritoneal transplantation of 0.5 ml of cell suspension (10^6 cells/ml) in sterile citrated saline (0.9%). 24 hours after the inoculation of EAT cells, DHA (50 and 100mg/kg b.w) was administered orally and cyclophosphamide (25 mg/kg.b.w) was given daily by intraperitoneal injection for the next ten days. Six mice from each group were scarified after 24h of administration by ether anesthesia for the study of antitumor activity, hematological analysis, and biochemical assays. The remaining six animals in each of the group were maintained without any further treatment to determine the median survival time (MST) of the tumor bearing mice.

(iv) Antitumor activity

Body weights of the animals were recorded from the day of transplantation to sacrifice. The ascites tumor volume was determined by collecting the ascites fluid from the peritoneal cavity of mice by using syringe. Packed cell volume was determined by centrifuging the ascites fluid at 1000 rpm for 5 min. An aliquot of the ascites fluid (10 μ l) was used for counting the number of viable cells by staining with 0.4% trypan blue Mean survival time (MST) of animals was monitored by recording the mortality daily for six weeks and percent increase in life span (ILS) was calculated by the following formula:

$$\% \text{ ILS} = \{(\text{MST of treated group} / \text{MST of control group}) - 1\} \times 1000$$

$$\text{MST} = (\text{Day of 1}^{\text{st}} \text{ death} + \text{Day of last death}) / 2$$

(v) Hematological profile

On the 15th day post inoculation of EAT cells, blood was collected from the animal by retro-orbital puncture and the hematological parameters such as Red Blood Cells (RBC), White Blood Cells (WBC), differential count and hemoglobin content were determined by following the standard procedures¹⁷⁻¹⁹.

(vi) Biochemical assays

Blood was collected in polypropylene tubes, allowed to clot and the serum was separated by centrifugation at 2000g for 10 min and stored at 4 °C for biochemical analysis. Lactate dehydrogenase (LDH) activity was assayed by Kornberg's method²⁰. The reaction mixture consisted of NADH (0.02 M), sodium pyruvate (0.01 M) in sodium phosphate buffer (0.1 M, pH 7.4). The change in the absorbance was recorded at 340 nm at 30 s intervals for 3 min. The serum ALT, AST and ALP levels were measured spectrophotometrically by the standard enzymatic methods using commercial kits (Span Diagnostics Ltd, Surat, India).

(vii) Statistical analysis

The data are expressed as Mean \pm SEM. The data were evaluated statistically by using one way ANOVA followed by Dunnett's t-test. $p < 0.05$ were considered as statistically significant.

RESULTS

(i) Antitumor activity

Ehrlich ascites tumor bearing mice showed increased body weight gain due to ascites tumor volume whereas a significant reduction in the body weight was seen in the DHA administered groups. CP treated EAT bearing mice also showed decrease in body weight gain (Fig 1) (Table 1).

Effect of Decalepis hamiltonii root extracts treatment on Ehrlich Ascites Tumor bearing mice

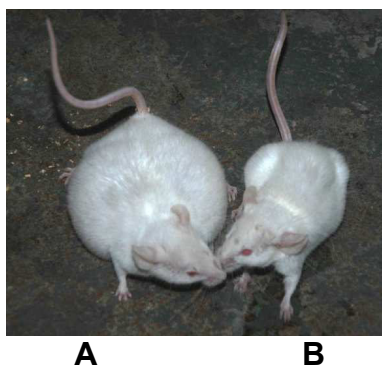


Figure 1

A) EAT bearing mice without treatment; B) EAT bearing mice with DHA treatment.

There was a marked increase in the ascites fluid volume and packed cell volume in EAT bearing animals, whereas in DHA treated groups the ascites fluid volume and packed cell volume was significantly reduced. Furthermore, DHA treatment reduced the number of cancer cells as compared to the untreated EAT bearing mice. Reduction in cancer cell count was also seen in CP treated EAT bearing mice (Fig 2).

Table 1
Effect of DHA treatment on EAT bearing mice: body weight

Group	Body weight		Weight Gain %
	Initial	Final	
I	30.4 ± 2.42	31.7 ± 2.1	1.15±0.10
II	29.8 ± 2.17	44.8 ± 1.7	7.60±0.31**
III	31.2 ± 1.3	36.0 ± 1.2	5.20±0.24*
IV	30.3 ± 2.1	34.6 ± 2.3	3.45±0.18*
V	29.7 ± 1.7	32.4 ± 1.0	2.30±0.16*

Group I – control, Group II– EAT bearing mice, Group III– EAT+DHA (50mg / kg. b.w.), Group IV– EAT + DHA (100mg / kg.b.w.), Group V– EAT + CP (25mg / kg.b.w.). Values are mean of 6 mice ± SEM. ** p < 0.01, and * p < 0.01. ** Values are significantly different from control, * Values are significantly different from EAT bearing mice.

Antitumor activity of DHA treatment on EAT bearing mice

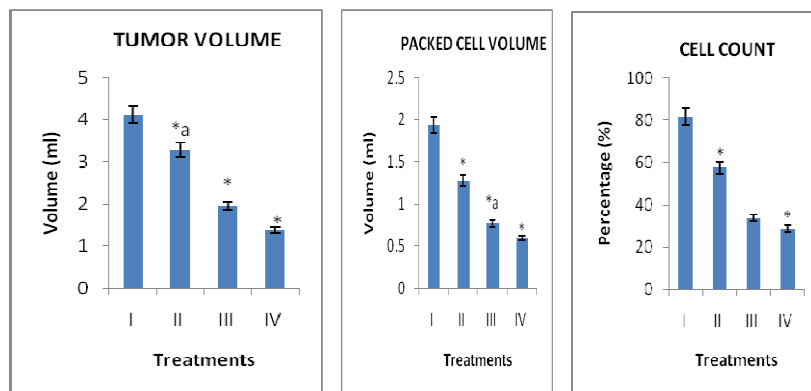


Figure 2

Treatments: I– EAT bearing mice, II– EAT+DHA (50mg / kg. b.w.), Group III– EAT + DHA (100mg / kg.b.w.), Group IV– EAT + CP (25mg / kg.b.w.). Values are mean of 6 mice ± SEM. *a p < 0.05 and * p < 0.01. * Values are significantly different from EAT bearing mice.

DHA treatment significantly increased the survival time of tumor bearing mice (Fig 3). The Mean survival time of EAT bearing mice was 18.86 ± 0.24 days, whereas, administration of the DHA significantly prolonged the MST to 28.69 ± 0.61 days and 32.24 ± 0.54 days, for 50 and 100 mg/kg.b.w, respectively. Cyclophosphamide

(25 mg/kg) treated groups improved MST to of 38.24 ± 1.16 days. The increase in the life span of tumor bearing mice treated with DHA (50 and 100 mg/kg) and cyclophosphamide were 56.41, 77.43 and 91.18 respectively as compared to the EAT bearing groups.

Effect of DHA on mean of survival time in EAT bearing mice

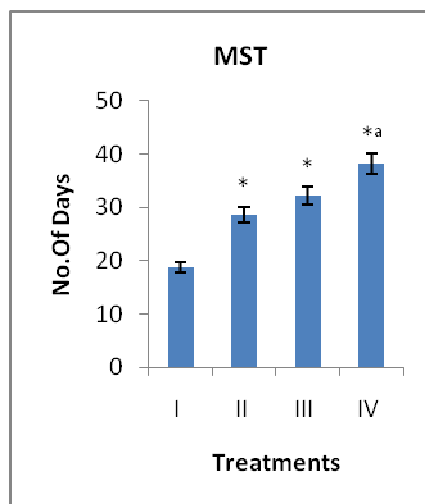


Figure 3

Treatments: I- EAT bearing mice, II- EAT+DHA (50mg / kg. b.w.), Group III- EAT + DHA (100mg / kg.b.w.), Group IV- EAT + CP (25mg / kg.b.w.). Values are mean of 6 mice ± SEM. *a p < 0.05 and *p < 0.01. * Values are significantly different from EAT bearing mice.

(ii) Hematological profile

Hematological profile of EAT bearing mice was significantly altered compared to normal mice (Table 3). There was a significant decrease in Hb content and RBC count whereas, WBC count, were markedly increased in EAT bearing mice. Administration of DHA to EAT bearing mice

significantly restored the RBC count, Hb content and WBC count as compared to that of untreated EAT mice. In a differential count, neutrophils were increased while lymphocytes were decreased in DHA treated mice. DHA treatment restored hematological parameters comparable to that of CP treated mice.

Table 3

Effect of DHA treatment on hematological profile of mice with Ehrlich Ascites tumor

Groups	Hb (g %)	RBC (10^6 million/ mm^3)	WBC (10^3 million cells / mm^3)	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)
I	12.76±0.17	5.23±0.07	8.051±0.17	75.1±0.46	21.8±0.51	1.72±0.16
II	8.18±0.4**	3.55±0.12**	21.92±0.78**	34.2±1.7**	62.3±1.63**	1.7±0.1**
III	9.74±0.17*	4.11±0.05*	16.18±0.82	56.3±1.44*	37.5±1.86	1.52±0.24*
IV	11.22±0.08*	4.64±0.04*	12.87±0.16*	67.7±2.80*	25.9±1.74*	1.31±0.21
V	12.63 ± 0.45*	4.92 ± 0.14 *	10.08 ± 0.23 *	70.26± 3.04 *	21.00 ± 1.24 *	1.12± 0.32

Group I – control, Group II– EAT bearing mice, Group III– EAT+DHA (50mg / kg. b.w.), Group IV– EAT + DHA (100mg / kg.b.w.), Group V– EAT + CP (25mg / kg.b.w.). Values are mean of 6 mice ± SEM. ** p < 0.01, and * p < 0.01. ** Values are significantly different from control, * Values are significantly different from EAT bearing mice.

(iii) Biochemical assays

Activity of the serum marker enzymes, AST, ALT, LDH, and ALP, were elevated in the EAT bearing mice as compared to that of normal control group. Treatment of EAT bearing mice with DHA and cyclophosphamide showed a significant restoration of the enzyme activities (Table 4).

Table 4
Effect of DHA treatment on serum enzymes profile in mice with Ehrlich Ascites tumor

Groups	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	LDH (U/L)
I	32.58±1.08	26.28±2.07	78.64±2.23	873.10 ± 26.24
II	62.27±3.19**	56.02±3.18**	121.03±4.12**	1420.60 ± 102.10**
III	45.07±1.07*	44.04±1.54*	99.60±2.50*	1051.00 ± 71.19*
IV	36.03±2.32*	35.75±0.62*	84.73±3.16*	960.00 ± 41.34*
V	39.16±1.12*	32.15±1.12	81.34±1.42*	907.00 ± 36.12

Group I – control, Group II – EAT bearing mice, Group III – EAT+DHA (50mg / kg. b.w.), Group IV – EAT + DHA (100mg / kg.b.w.), Group V – EAT + CP (25mg / kg.b.w.). Values are mean of 6 mice ± SEM, ** p < 0.01, * p < 0.01. ** Values are significantly different from control, * Values are significantly different from EAT bearing mice.

DISCUSSION

Cancer chemoprevention by natural products in order to inhibit malignant cell transformation, prevent invasion and metastasis would be desirable²¹. The use of herbal medicine or dietary supplements are promising approaches in the management of cancer treatment²²⁻²⁴. Many of the plant derived anticancer drugs such as vinblastine, vincristine, taxol and camptothecin have greatly contributed to the efficacy of cancer chemotherapy²⁵. However, these plant derived anticancer drugs have side effects and toxicity. Hence, there is a great potential for the discovery of newer anticancer drugs from the untapped reservoir of the plant kingdom. The edible roots *Decalepis hamiltonii* contains many bioactive compounds showing chemopreventive properties^{10, 12, 14}. In this study, we have demonstrated the antitumor effect of *D. hamiltonii* aqueous extract in EAT tumor bearing mice. EAT cell transplanted mice showed weight gain and increase in tumor volume due to growth of tumor cells²⁶. Administrations of DHA suppressed EAT tumor growth as shown by the decreased cell number and enhanced survival time. Our results indicate that DHA shows both preventive and curative effect in EAT bearing mice. As expected, administration of the antitumor drug, CP to EAT bearing mice significantly prolonged the MST. Prolongation of lifespan of the tumor bearing animal is

considered as a significant measure for evaluating an anticancer drug²⁷. DHA treatment prolonged the lifespan of tumor bearing mice and the effect was comparable but slightly lower than that of CP, which could be attributed to the lower concentrations of the bioactive constituents in the extract.

A rapid increase in ascetic fluid volume is seen in EAT bearing mice and the tumor cells use ascetic fluid as a nutritional source²⁸⁻³⁰. Treatment with DHA showed a significant decrease in the tumor volume and cell count which suggests that DHA was effective in arresting the tumor growth. However, it needs to be determined whether tumor suppressive effect of DHA is due to the inhibitory influence on cell multiplication or promoting cancer cell death by apoptosis. In cancer therapy, major problems encountered are myelosuppression and anemia^{31, 32}. The anemic changes seen in tumor bearing mice as evident from reduction in RBC or Hb content which could be due to hemolytic or myelopathic conditions³³. Treatment with *D. hamiltonii* extract to EAT bearing mice restored the Hb content, RBC and WBC count and was comparable to that of normal mice which indicates the protective action of DHA against tumor-induced changes in the hemopoietic system³⁴. During malignancy the tumor marker enzymatic changes reflect overall changes in

metabolism and they are increasingly appreciated as critical determinants of tumor cell behavior with sensitivity and specificity³⁵. Elevation in the activities of serum enzymes AST and ALT is associated with hepatic dysfunction in tumor bearing mice³⁶. Treatment with the aqueous extract of *D. hamiltonii* prevented the rise in the enzyme activities in the EAT tumor bearing mice which is consistent with reduced tumor growth. Altered serum enzyme levels in EAT bearing mice are indicative of changed metabolism due to cancerous growth which was restored to normal levels by DHA treatment indicating the physiological consequences towards normalcy.

CONCLUSION

In conclusion, Results from this study shows *in vivo* antitumor effect of the aqueous extract of *D. hamiltonii* in EAT bearing mice. This could be

attributed to the presence of bioactive constituents including natural antioxidants present in the root extract of *D. hamiltonii*, which exert a marked effect in the prevention of tumor development and possibly induction of apoptosis in tumor cells. However, this needs to be substantiated by further studies. The edible roots of *D. hamiltonii* have a history of human use and, therefore, could be a good source of a natural therapeutics that suppress tumor growth besides its general chemopreventive potential. Recently, we have shown that aqueous extract of *D. hamiltonii* ameliorates the toxicity of the anticancer drug, CP, which clearly demonstrates that the beneficial effects of DHA without comprising the efficacy of CP and rises the possibly of using DHA as a adjunct in cancer chemotherapy³⁷. This study further adds to the medicinal value of the roots of *D. hamiltonii*.

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