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Anticancer Activity of Ethanol Extract of *Melastoma malabathricum* L. Leaf against Dalton Ascites Lymphoma

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Abstract
Aim: To study aims to evaluate the antitumor activity of ethanol extract of leaves of *Melastoma malabathricum* on DAL model in Swiss Albino mice. **Methods:** Evaluation of the antitumor effect of ethanol extract of leaves of *Melastoma malabathricum* on tumor growth and hosts survival time was made by the study of the following parameters: tumor volume, viable and non viable cell count and life span of host. **Results:** The results showed decrease in tumor volume and cell viability. Hematological studies revealed that, the Hb count decreased in DAL treated mice, whereas, it was induced by the drug treated animals and showed an increase in Hb near to normal levels. **Conclusion:** The results suggested that, the extracts of leaves of *Melastoma malabathricum* exhibited significant antitumor activity on DAL bearing mice.
***Keywords*:** *Melastoma malabathricum*, antitumor, lifespan, WBC.

INTRODUCTION

Cancer is considered one of the most common causes of mortality worldwide. Progress made in cancer therapy has not been sufficient to a significantly lower annual death rate from most tumor types, and there is an urgent need for new strategies in cancer control[1]. For centuries, people have been using plants for their therapeutic values. Today 85000 plants have been documented for therapeutic use globally [2] (Liu and Wang, 2008). The World Health Organization (WHO) estimates that almost 75% of World's population has therapeutic experience with herbal drugs. Cancer is one of the most dangerous diseases in humans and presently there is a considerable scientific discovery of new anticancer agents from natural products [3]. The potential of using the natural products as anticancer drugs was recognized in 1950's by U.S. Natural Cancer Institute (NCI) since 1950 major contributions have taken for the discovery of naturally occurring anticancer drugs [4].

Melastoma malabathricum belongs to the Melastomataceae family. It is also called the Singapore Rhododendron or Sendudok. It is a erect shrub or small tree 1.5 to 5m tall. It was traditionally used to treat diarrhoea, dysentery, leucorrhoea, hemorrhoids, wounds, and infection during confinement, toothache, flatulence, sore legs, and thrush and also it is used by the Jah hut people in Malaysia to cure diarrhea[5]. Biological activities such as anti-inflammatory and hepatoprotective activities were reported[6,7]. However, no work has been reported on the anticancer property of this plant. Keeping in view, the present study has been undertaken to investigate anticancer activity of the ethanol extract of *M.malabathricum* leaves against Dalton Ascites Lymphoma (DAL) tumor model.

MATERIALS AND METHODS

Collection

The leaves of *Melastoma malabathricum* L. were collected from Daudeli,Joide Taluk, Hubli District, North Karnataka. With the help of local flora, a voucher specimen (VOCB 1637) was retained in Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin for further reference.

Preparation of plant extract for anticancer activity

The leaves of *Melastoma malabathricum* DC were cut into small pieces, washed, dried at room temperature and the dried leaves were powdered in a Wiley mill. Hundred grams of powdered leaves were separately packed in a Soxhlet apparatus and extracted with ethanol. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extracts of leaves were used for preliminary phytochemical screening and anticancer activity.

Animals

Healthy male adult Swiss Albino mice (20-25gm) were used for the study. The animals were housed in microloan boxes in a controlled environment (temperature 25±20c) and 12 hr dark/eight cycle) with standard laboratory diet (Sai Durga feeds and foods, Bangalore) and water *ad libitum*. The mice well 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.

Tumor Cells

Dalton Ascites Lymphoma (DAL) cells were obtained from Division of Oncology Department of Biotechnology, Tamil Nadu, Veterinary and Animal Husbandary, Chennai, Tamil Nadu, India. The DAL cells were maintained *in vivo* in Swiss albino mice by weekly intra peritoneal (i. p) inoculation of 10^6cells / mouse after every ten days. DAL cells 9 days old were used for the screening of the anticancer activity.

Acute oral toxicity study

Acute oral toxicity was performed by following OECD guideline - 20 fixed dose procedure for ethanol extract of leaves of *Melastoma malabathricum* and it was found that, dose increasing up to 2000 mg / kg body weight, shown no toxicity or mortality in experimental mice. The LD50 of

ethanol extracts of leaves of *Melastoma malabathricum* as per OECD guidelines-420 is greater than 2000 mg/kg [8,9].

Antitumor activity

Healthy Swiss albino mice were divided in to six groups of six animals (n=6) each. The test samples were dissolved in isotonic saline (0.9% NaCl W/V) and used directly in the assay. DAL cells were collected from the donor mouse and were suspended in sterile isotonic saline. The viable DAL cells were counted (Trypan blue indicator) under the microscope and were adjusted at 1 X 10^6 cells/ ml. 0.1 ml of DAL cells per 10g body weight of the animals were injected (i. p) to each mouse of each group except normal saline group (Group I). This was taken as Day 0. Group I served as a normal saline control (1mL/kg, p.o) and group II served as DAL bearing control. On day 1, the ethanol extracts of *Melastoma malabathricum* at a dose of 150 and 300mg/kg each of the Group III, IV were administrated orally and continued for 14 consecutive days respectively. Group V served as tumor induced animal administrated with vincristine (80mg/kg body weight) for 14 consecutive days. On day 15, half of the animals (n=3) in each case were sacrificed and the remaining animals were kept to observe the life span study of the tumor hosts. The effect of ethanol extract of *Melastoma malabathricum* on tumor growth and host's survival time were monitored by studying parameters like tumor volume, tumor cell count, viable tumor cell count, nonviable tumor cell count, mean survival time and increase in life span[10,11].

Tumor growth response

The effect of ethanol extract of *Melastoma malabathricum* on tumor growth and hosts survival time were examined by studying the following parameters such as tumor volume, tumor cell count, viable tumor cell count, non viable tumor cell count, median survival time and increase in life span.

Determination of Tumor volume

The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube. Packed cell volume was determined by centrifuging the ascitic fluid at 1000 rpm for 5min.

Determination of Tumor cell count

The ascitic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension as placed on the Neubauer counting chamber and the number of cells in the 64 small squares was counted.

Estimation of viable and non viable tumor cell count

(Tryphan blue dye assay): The cells were then stained with tryphan blue (0.4% normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were non viable. These viable and non viable cells were counted.

Percentage increase of life span

(% ILS): Animals were inoculated (1 x 10^6 cells/ml) 0.1ml of DAL cells per 10g body weight of the animals was injected i.p) on day zero (day 0). A day of incubation was allowed for multiplication of the cells. Fourteen doses of the Test samples (150 mg/kg and 300 mg/kg, 0.1 ml/10g body weight) and control group was treated with same volume of Saline (0.9%sodium chloride solution) and compared with vincristine (80mg / kg body weight) were injected i.p from the first day up to the 9th day with 24 h intervals. The effect of ethanol extracts of leaves of *Polycarpae corymbosa* tumor growth was monitored by recording the mortality, daily for a period of 9 days and percentage increase in life span (% ILS) was calculated from the following equation.

$$\text{Increase in life span} = \frac{T-C}{C} \times 100$$

Body Weight

Body weights of the experimental mice were recorded both in the treated and control group at the beginning of the experiment (zero day) and sequentially on every 5th day during the treatment period.

Hematological studies

At the end of the experimental period, all mice were sacrificed by cervical dislocation. Blood was collected from freely flowing tail vein and used for the estimation of Haemoglobin content (Hb), Red blood cell count (RBC) and White blood cell count (WBC). WBC differential count was carried out from Leishman stained blood smears[12].

Statistical analysis

The data were analyzed using student's t test statistical methods. For the statistical tests, *p* values of less than 0.01 and 0.05 were taken as significant.

RESULTS

The present investigation indicates that the ethanol extract of *Melastoma malabathricum* leaves (EEMM) shows significant antitumor activities in DAL tumor bearing mice. The effects of ethanol extract of *Melastoma malabathricum* at the doses of 150 and 300mg/kg on survival time, %TLS, tumor volume, packed cell volume and tumor cell count (viable and nonviable cell) are shown in Table 1 and 2.

Table 1: Antitumor activity of *Melastoma malabathricum* leaf extract in DAL Tumor bearing mice

Groups	*Dose (mg/kg)*	*Solid Tumor Volume*			
		15th day	*20th day*	*25th day*	*30th day*
Group I	Saline	3.93±0.054	5.013±0.034	6.21±0.089	7.11±0.034
Group II	150	3.24±0.031ns	3.84±0.064ns	4.19±0.034*	4.03±0.024*
Group III	300	3.54±0.017ns	3.21±0.035*	3.83±0.054**	2.91±0.039**
Group IV	20	2.89±0.039*	3.48±0.014*	3.21±0.011**	3.11±0.072**

Each Value is SEM of 5 animals Significance between tumor induced control vs drug treated group * p < 0.05 ; ** p < 0.01, DAL-Dalton Ascetic Lymphoma

Table 2: Antitumor activity of *Melastoma malabathricum* leaf extract on the survival time, life span, tumor volume and viable and non-viable cell count in tumor Induced mice

Treatment	*Mean Survival time (Days)*	*Increase of life span(%)*	*Packed cell volume*	*Viable cell count X 10^6 cells/ml*	*Non-viable tumor cells count X 10^6cells/ ml*
Group I	18.94±1.33	-	3.12±0.011	17.49±0.34	0.86±0.021
Group II	24.39±0.94*	28.77	1.93±0.013*	8.16±0.21**	1.24±0.034*
Group III	29.41±1.04**	55.49*	1.02±0.015**	4.22±0.62**	2.78±0.038*
Group IV	28.13±0.91**	48.52*	1.14±0.028**	4.78±0.41**	2.93±0.21**

Each Value is SEM of 5 animals * p < 0.05 ; ** p < 0.01 Significance between tumor induced control vs drug treated group.

Table 3: Antitumor activity of *Melastoma malabathricum* leaf extract on haematological parameters in DAL Tumor bearing mice

Parameter	*Hb (gm%)*	*RBC (million/mm^3)*	*WBC (10^3 cells/ mm^3)*	*Proteins (gm%)*	*Differential count*		
					Lymphocytes	*Neutrophils*	*Monocytes*
Group I	7.04±0.31	2.58±0.14	12.66±0.91	10.84±0.64	39.11±1.21	55.16±1.13	5.08±0.12
Group II	8.36±0.39ns	2.81±0.24	11.15±0.63	8.63±0.074	43.56±1.13	50.22±1.94	7.16±0.13
Group III	8.84±0.36*	3.49±0.14**	9.93±0.21*	7.34±0.52*	46.84±0.93	49.14±1.14	4.88±0.71*
Group VI	9.22±0.029*	3.04±0.036*	8.32±0.12**	7.11±0.24*	43.16±1.05ns	51.16±1.08	5.34±0.88

Each Value is SEM of 5 animals Significance between tumor induced control vs drug treated group * p < 0.05 ; ** p < 0.01 ; NS -Not significant ,DAL-Dolton Asetic Lymphoma

Preliminary phytochemical screening and acute toxicity

The phytochemical screening of ethanol extract of *M.malabathricum* leaves revealed the presence of alkaloid, catechin, coumarin, flavonoid, tannin, saponin, steroid, phenol, glycoside, terpenoids and xanthoproteins. Acute toxicity study revealed the non-toxic nature of the ethanol extract of *M.malabathricum.*

Effect on solid tumor volume

Treatment with ethanol extract of *M.malabathricum* leaves and 5 Fluorouracil at the doses of 150 and 300mg/kg significantly (p<0.01) reduces the solid tumor volume in a dose dependent manner as compared to that of the DAL control group.

Effect on mean survival time and increases life span

In the DAL control group, the mean survival time was 18.94±1.33 days, while it increased to 24.39±0.94 (150mg/kg) and 29.41±1.04 days (300mg/kg) days, respectively, in the ethanol extract of *M.malabathricum* treated groups, whereas the standard drug 5-Fluorouracil (20mg/kg) treated group had a mean survival time of 28.13±0.91 days. The percentage increase in survivals, it was found to be 28.77%, 55.49% and 48.52% respectively as compared to DAL control group.

Effect on tumor growth

Treatment with ethanol extract of *M.malabathricum* at the doses of 300mg/kg significantly (p<0.01) reduced the packed cell volume and viable tumor cell count ina dose dependent manner as compared to that of the DAL control group. Furthermore, nonviable tumor cell counts at different doses of ethanol extract of *M.malabathricum* were increased in a dose dependent manner.

Effect on hematological parameters

As shown in Table 3, hemoglobin content and RBC count in the DAL control group was decreased. Treatment with ethanol extract of *M.malabathricum* at the doses of 150 and 300mg/kg increased the hemoglobin content and RBC count to more or less normal levels. The total WBC counts and protein was found to be increased in the DAL control group. Administration of ethanol extract of *M.malabathricum* at the doses of 150 and 300mg/kg in DAL bearing mice reduced the WBC count and protein as compared with the DAL control. In a different count of WBC, the presence of neutrophils increased, while the lymphocyte count decreased in the DAL control group. Treatment with ethanol extract of *M.malabathricum* at different doses changed these altered parameters more or less to the normal levels.

DISCUSSION

Cancer chemoprevention has been defined as a process facilitated by blocking induction of neoplastic process or preventing transformed cells from progression to malignant phenotypes by administration of one or more chemical entities, either as synthetic drugs or naturally occurring phytoconstitutents. Recent studies on tumor inhibitory compounds of plant origin have yielded an impressive array of research on medicinal plants. The efficacy of *M.malabathricum* against Dalton Ascites Lymphoma (DAL) described in the present investigation offer the potential for reaching on understanding of anticancer potency. The reliable criteria for judging the value of any anticancer drug are prolongation of lifespan and decrease of WBC from blood[13,14]. The EEMM treated animals at the doses 300mg/kg significantly decrease the tumor volume, tumor cell count and brought back the hematological parameters to more or less normal levels. There was a regular and rapid increase in ascetic fluid volume of DAL bearing mice. Ascetic fluid is direct nutritional requirements of tumor cells [15]. It may be concluded that EEMM by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of DAL bearing mice. Thus, EEMM has antitumor activity against DAL bearing mice.

Usually in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia [16]. 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 order to haemolytic or myelopathic conditions [17]. In DAL control group, a differential count the presence of neutrophils increased, while the lymphocyte count decreased, the observed leucocytopenia indicates a common symptom of immunosuppression in many types of cancer[18,19] and one of the causes of neutrophilia is myeloid growth factors which are produced in malignant process as part of a paraneoplastic syndrome. In addition to this another factor granulocyte colony stimulating factor produced by the malignant cells has also been attributed to be the cause of neutrophilia because of its action on bone marrow granulocytic cells in cancer. After the repeated, EEMM able to reverse the changes in altered neutrophils and lymphocytes count [20,21]. Treatment with EEMM also brought back the haemoglobin content RBC and WBC count more or less to normal levels and this indicates that EEMM possess protective action on the haematopoietic system.

The results of the present study demonstrates that the ethanol extracts of *M.malabathricum* increased the life span of DAL tumor bearing mice, reduce tumor volume and improve the haematological parameters. The association between flavonoids and reduced cancer risk has been reported in previous studies that showed a decrease in cancer risk with consumption of vegetables and fruits rich with flavonoids [22,23]. The results of this study are accordance with this finding since the phytochemical screening showed the presence of flavonoids in ethanol extracts of *M.malabathricum*. While the presence of alkaloids with flavonoids in *M.malabathricum* extracts may explain its superior activity compared with other plants studied [24].The anticancer activity of total flavonoids and alkaloids isolated from different plants were reported earlier [25, 23]. Plants derived compounds have played an important role in the development of several clinical useful anticancer agents [26]. Since the phytochemical screening, *M.malabathricum* showed the presence of alkaloids, flavonoids, terpenoids, steroids, saponins, glycosides and phenols which could make the plants useful for treating anticancer drug. Further, the isolation of the compounds responsible for the activity has to be taken up which may result in a modern drug from these plants.

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