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ANTICANCER ACTIVITY OF ETHANOL EXTRACT OF *POLYCARPAEA CORYMBOSA* (L.) LAM WHOLE PLANT AGAINST DALTON ASCITES LYMPHOMA

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ABSTRACT

The present study aims to evaluate the antitumor activity of ethanol extract of whole plant of *Polycarpaea corymbosa* on DAL model in Swiss Albino miceThe following parameters: tumor volume, viable and non viable cell count and life span of host were evaluated DAL bearing in swiss albino mice. The above said parameter were also evaluation by administrating ethanol extract of whole plant of *Polycarpaea corymbosa.* The results showed a 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. The results suggested that, the extracts of whole plant of *Polycarpaea corymbosa* exhibited significant antitumor activity on DAL bearing mice.

KEYWORDS: ***Polycarpaea corymbosa*, antitumor, lifespan, WBC.**

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INTRODUCTION

Over the past few decades, cancer has remained as the largest cause of mortality worldwide and the number of individuals living with cancer is steadily expanding. Hence, a major portion of the current pharmacological research is involved with the anticancer drug design customized to fit new molecular targets[1]. Due to the enormous propensity of plants, which synthesize a variety of structurally diverse bioactive compounds, the plant kingdom is a potential source of chemical constituents with antitumor and cytotoxic activities. Traditionally various plants have long been used in the treatment of cancer[2-7].

Polycarpaea corymbosa(L.) Lam. belongs to "Caryophyllaceae" is commonly known as "Palllipoondu" in *Palliyar* tribals of Sirumalai hills,Western Ghats Tamil Nadu. Paste prepared from the whole plant is taken once in a day for a period of 2-3 weeks to treat jaundice by the palliyars[8]. Biological activities such as anti-inflammatory and hepatoprotective activities were reported[9,10]. 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 *P. corymbosa* whole plants against Dalton Ascites Lymphoma (DAL) tumor model.

MATERIALS AND METHODS

i. Collection

The whole plant of *Polycarpaea corymbosa* (L.) Lam were collected from Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. With the help of local flora, a voucher specimen was retained in Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin for further reference.

ii. Preparation of plant extract for anticancer activity

The whole plants of *Polycarpaea corymbosa* DC were cut into small pieces, washed, dried at room temperature and the dried whole plants were powdered in a Wiley mill. Hundred grams of powdered whole plant 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 whole plant were used for preliminary phytochemical screening and anticancer activity.

iii.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.

iv.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.

v. Acute oral toxicity study

Acute oral toxicity was performed by following OECD guideline - 20 fixed dose procedure for ethanol extract of whole plant of *Polycarpae corymbosa* 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 whole plant of *Polycarpae corymbosa* as per OECD guidelines-420 is greater than 2000 mg/kg [11,12].

vi. 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 *Polycarpae corymbosa* 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 *Polycarpae corymbosa* 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[13,14].

vii. Tumor growth response

The effect of ethanol extract of *Polycarpae corymbosa* 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.

viii. 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.

ix. 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.

x. 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.

xi. 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 whole plant of *Polycarpaea 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.

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

xii. 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.

xiii. 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[15].

xiv. 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 *P.corymbosa* whole plant (EEPC) shows significant antitumor activities in DAL tmor bearing mice. The effects of ethanol extract of *P.corymbosa* 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.

Preliminary phytochemical screening and acute toxicity

The phytochemical screening of ethanol extract of *P.corymbosa* whole plant 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 *P.corymbosa.*

Effect on solid tumor volume

Treatment with ethanol extract of *P.corymbosa* whole plant 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 21.54±1.62 (150mg/kg) and27.25±0.84 days (300mg/kg) days, respectively, in the ethanol extract of *P.corymbosa* 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 13.72%, 43.87% and 48.52% respectively as compared to DAL control group.

Effect on tumor growth

Treatment with ethanol extract of *P.corymbosa* at the doses of 300mg/kg significantly ($p<0.01$) reduced the packed cell volume and viable tumor cell count in a dose dependent manner as compared to that of the DAL control group. Furthermore, nonviable tumor cell counts at different doses of ethanol extract of P.corymbosa 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 *P.corymbosa* 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 *P.corymbosa* 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 *P.corymbosa* at different doses changed these altered parameters more or less to the normal levels.

Table 1

Antitumor activity of Polycarpae corymbosa whole plant 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	3.19±0.057ns	3.27±0.063*	3.24±0.031**	3.11±0.057**
Group III	300	2.94±0.063*	3.34±0.058*	3.08±0.026**	2.82±0.072**
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 ;NS -Not significant ,DAL-Dalton Asetic Lymphoma***

Table 2

Antitumor activity of Polycarpae corymbosa whole plant 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 10^5 cells/ml	Non-viable tumor cells count X 10^5cells/ ml
Group I	18.94±1.33	-	3.12±0.011	17.49±0.34	0.86±0.021
Group II	21.54±1.62*	13.72	3.04±0.022	11.93±0.12	0.93±0.64
Group III	27.25±0.84**	43.87*	1.23±0.017**	4.93±0.32**	3.12±0.27**
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 Polycarpae corymbosa whole plant 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	8.14±0.11ns	2.61±0.41	10.16±0.35ns	7.39±0.14*	53.55±1.89*	50.18±1.23	6.19±0.93
Group III	9.56±0.024*	3.16±0.48*	9.84±0.16*	7.81±0.19*	50.11±1.34*	44.19±1.17	3.81±0.62*
Group IV	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 Ascetic Lymphoma***

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 *Polycarpae corymbosa* 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[16,17]. The EEPC 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 increase in ascetic fluid volume of DAL bearing mice. Ascetic fluid is direct nutritional requirements of tumor cells[18]. It may be concluded that EEPC by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of DAL bearing mice. Thus, EECP has antitumor activity against DAL bearing mice.

Usually in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia[19]. 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[20]. 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[21,22] 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, EEPC able to reverse the changes in altered neutrophils and lymphocytes count[23,24]. Treatment with EEPC also brought back the haemoglobin content RBC and WBC count more or less to normal levels and this indicates that EEPC possess protective action on the haematopoietic system. The results of the present study demonstrates that the ethanol extracts of *Polycarpae corymbosa* 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 [25,26]. The results of this study are accordance with this finding since the phytochemical screening showed the presence of flavonoids in ethanol extracts of *Polycarpae corymbosa*. While the presence of alkaloids with flavonoids in *Polycarpae corymbosa* extracts may explain its superior activity compared with other plants studied [27].The anticancer activity of total flavonoids and alkaloids isolated from different plants were reported earlier [28, 26]. Plants derived compounds have played an important role in the development of several clinical useful anticancer agents [29]. Since the phytochemical screening, *Polycarpae corymbosa* 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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