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Anticancer activity of Ethanol extract of *Polygala javana* DC whole Plant against Dalton Ascites Lymphoma

Alagammal M.[1], Paulpriya K.[2] and Mohan V.R.[2*]
[1]Government Siddha Medical College, Palayamkottai, Tamil Nadu, INDIA
[2]Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin- 628008, TN, INDIA

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Abstract

The present study aims to evaluate the antitumor activity of ethanol extract of whole plant of Polygala javana on DAL model in Swiss Albino mice. Evaluation of the antitumor effect of ethanol extract of whole plant of Polygala javana 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. 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. The results suggeste that, the extracts of whole plant of Polygala javana exhibited significant antitumor activity on DAL bearing mice.

Keywords: *Polygala javana*, antitumor, lifespan, WBC.

Introduction

Cancer is still a major cause of mortality and morbidity in developing as well as in developed countries. Overall survival rate has only improved eligibility, despite advances in surgery, radiotherapy and chemotherapy. Molecular targeted agents are currently being studied in all treatment settings including that of chemoprevention, which is defined as the use of natural or synthetic non-essential dietary agents to interrupt the process of carcinogenesis and to prevent or delay tumor growth[1]. Due to the toxic and adverse side effects of synthetic drugs as well as conventional treatments are being failed to fulfill their objectives (tumor control), for these consequence herbal medicine has made a comeback to improve the fulfillment of our present and further health needs[2].

The use of natural products together with their therapeutic properties is as ancient as human civilization and, for a long time, mineral, plant and animal products were the main sources of drugs[3]. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants[4]. However, the potential use of plants as a source of new drugs is still poorly explored of the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and an even smaller percentage has been properly studied in terms of their pharmacological properties[5].

Polygala javana was traditionally used by Americans to treat snake bites[6] and as an expectorant to treat cough and bronchitis and it is considered as a powerful tonic[7] that can help to develop the mind and aid in creative thinking. *Polygala javana* is commonly known as 'Palpiranthai'. Paste prepared from fresh leaves is applied by Kanikkar tribal women on the breast twice a day to check lactation and to get relief from the pain developed while stopping mother feeding[8].

However, inspite of traditional use, pharmacology of its aerial whole plant has not yet been explored scientifically. So far no reports are available in anticancer activity of this plant. The present investigation was carried out to evaluate the anticancer activity of the ethanol extract of whole plant of *Polygala javana* DC against Dalton Ascites Lymphoma (DAL) tumor model.

Material and Methods

Collection: The whole plants of *Polygala javana* were collected in the month of February and March, 2010, from the Scott Christian College, Nagercoil, Kanyakumari district Tamil Nadu. The plant specimen were identified with the help of local flora and authenticated in Botanical survey of India, Southern Circle, Coimbatore, Tamil Nadu, and India. A voucher specimen was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of plant extract for anticancer activity: The whole plants of *Polygala javana* 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 anticancer activity.

Animals: Healthy male adult Swiss Albino mice (20-25gm) were used for the study. The animals were housed in microlon

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 - 420 fixed dose procedure for ethanol extract of whole plant of *Polygala javana* 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 *Polygala javana* as per OECD guidelines-420 is greater than 2000 mg/kg [9, 10].

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 106 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 *Polygala javana* at a dose of 100 and 200mg/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 *Polygala javana* 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[11,12].

Tumor growth response: The effect of ethanol extract of *Polygala javana* 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 106 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 (100 mg/kg and 200 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 *Polygala javana* 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.

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

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[13].

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 and Discussion

The acute toxicity study, ethanol extract of *Polygala javana* whole plant did not show any toxic effect upto the dose of 2000mg/kg body weight, accordingly 100mg/kg and 200mg/kg body weight were taken as low and high dose of whole plant of *Polygala javana* for the experiment. The present investigation indicates that, ethanol extract of whole plant of *Polygala javana* showed significant antitumor activity in DAL bearing mice.

The administration of ethanol extract of whole plant of *Polygala javana* to DAL bearing mice showed reduction in body weight, spleen, thymus, liver, kidney and lungs table 1. In the case of tumor growth response study, treatment with ethanol extract of whole plant of *Polygala javana* showed significant ($p < 0.01$) reduction in tumor volume. Table 2, table 3 depicts the effect of ethanol extract of whole plant of *Polygala javana* on life span, viable cell count and non viable cell count. It revealed that, there was an increase in mean survival time. Administration of ethanol extract of *Polygala javana* appreciably decreases the viable cell count compared to DAL bearing mice. Non viable cell count was significantly higher with increase in dosage of extracts. Table 4 showed that, haematological parameters of tumor bearing mice on day 15 were found to be significantly different as compared to the extracts of treated groups. In tumor bearing mice, it was found that, there was an increase in WBC count, and decrease in Hb content and RBC count. In differential count of WBC, presence of neutrophils and monocytes increased while the lymophocyte count decreased in the DAL control group. Treatment with *Polygala javana* whole plant at the dose 100mg/kg and 200mg/kg significantly ($p < 0.05$ and $p < 0.01$ respectively) increased the Hb and RBC count to normal levels. The total WBC count was found to be increased significantly in the DAL control group when compared to normal group. Administration of *Polygala javana* whole plant extracts (100mg/kg and 200mg/kg) in DAL bearing mice significantly ($p < 0.05$ and $p < 0.01$) reduced the WBC count as compared with DAL control.

Table-1
Effect of ethanol extract of *Polygala javana* on relative organ weights of tumor induced and drug treated mice

Treatment	Relative Organ Weight (g/100g body wt.)					
	Body weight (g)	Spleen	Thymus	Liver	Kidney	Lungs
Group I	20.14±1.75	0.39±0.031	0.10±0.037	2.78±0.71	0.93±0.20	0.41±0.021
Group II	34.54±1.22[a]	0.48±0.019	0.19±0.051[a]	3.41±0.58[a]	1.86±0.11[a]	0.68±0.032[a]
Group III	29.31±0.89*	0.44± 0.12	0.14± 0.061	3.01± 0.16	1.44±0.030	0.64± 0.011
Group IV	26.33±1.43*	0.39±0.021	0.17±0.032	2.84±0.31	1.13±0.041	0.59±0.054
Group V	20.98±1.86**	0.36±0.023	0.14±0.015	2.81±0.64	1.09±0.029	0.45±0.034

Each Value is SEM ± 6 individual observations: * $p < 0.05$; ** $p < 0.01$; Compared to DAL control vs. drug treated groups [a]: $p < 0.05$; Compared to DAL control vs Normal Control

Table-2
Antitumor activity ethanol extract of *Polygala javana* on solid tumor volume in tumors induced mice

Treatment	Solid Tumor Volume			
	15th day	20th day	25th day	30th day
Group I	-	-	-	-
Group II	3.65±0.16	3.98±0.27	4.36±0.53	4.98±0.53
Group III	3.21 ± 0.38*	3.42 ± 0.24*	3.56 ± 0.13*	3.68 ± 0.31*
Group IV	3.12±0.36[NS]	3.23±0.30*	3.12±0.63*	2.67±0.34**
Group V	3.18±0.21[NS]	3.04±0.14**	2.63±0.53**	2.31±0.49**

Each Value is SEM of 6 animals Significance between tumor induced control vs drug treated group * $p < 0.05$; ** $p < 0.01$; NS :Not significant

Table-3
Antitumor activity of ethanol extract of *Polygala javana* 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 II	17.21±0.56	-	3.20±0.031	14.68±2.01	0.83±0.031
Group III	21.86± 0.32 *	27.02	1.56 ±0.032*	9.34 ±0.32*	0.94± 0.11*
Group IV	27.58±0.44*	60.02	1.34±0.054*	8.67±0.21*	1.23±0.033[NS]
Group V	30.68±0.45*	78.26	0.98±0.074**	6.54±0.66**	2.89±0.038*

Each Value is SEM of 6 animals Significance between tumor induced control vs drug treated group * $p < 0.05$; ** $p < 0.01$; NS :Not significant

Table–4
Anticancer activity of ethanol extract of *Polygala javana* on hematological parameters in DAL Tumor bearing mice

Parameter	Hb (gm%)	RBC (million/mm^3)	WBC (10^3 cells/ mm^3)	Differential count		
				Lymphocytes	Neutrophils	Eusinophill
Group I	**14.34±0.46**	**3.96±0.24**	**9.67±0.68**	**52.18±1.44**	**43.81±0.97**	**4.35±0.74**
Group II	7.94±0.76**	2.97±0.17*	16.56±0.95	30.29±1.34	62.32±1.51	7.36±0.87
Group III	9.28± 0.28*	3.21±0. 33*	14.36±0.34*	34.21±1.11*	58.36±0.89*	6.96±0.51*
Group IV	11.67±0.87*	3.97±0.12*	10.45±0.38*	45.58±1.52	50.32±1.23	4.01±0.22
Group V	13.04±0.55**	4.13±0.65**	8.91±0.33*	50.48±1.32	41.23±1.37	7.84±0.47

Each Value is SEM of 6 animals Significance between tumor induced control vs drug treated group * $p < 0.05$; ** $p < 0.01$;

The present study was carried out to investigate the antitumor potential of whole plant of *Polygala javana* against DAL bearing mice. The ethanol extract treated animals at the doses of 100 and 200 mg/kg significantly inhibited the tumor volume, packed cell volume, tumor (viable) cell count and brought back the haematological parameters to more or less normal levels.

In DAL tumor bearing mice, a regular rapid increase in ascitic tumor volume was observed. Ascitic fluid is the direct nutritional source for tumor cells and rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells[14]. Treatment with ethanol extract of *Polygala javana* inhibited the tumor volume, viable tumor cell count and increased the life span of the tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the life span of animals[15]. It may be concluded that, ethanol extract of *Polygala javana* by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of DAL bearing mice. Thus, ethanol extract of *Polygala javana* has antitumor activity against DAL bearing mice.

Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anaemia[16]. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or haemoglobin percentage, and this may occur either due to iron deficiency or due to haemolytic or myelopathic conditions[17,18]. Treatment with ethanol extract of *Polygala javana* whole plant brought back the haemoglobin (Hb) content, RBC and WBC count more or less to normal levels. This clearly indicates that, *Polygala javana* whole plant possesses protective action on the hemopoietic system.

Plant derived compounds have played an important role in the development of several clinical useful anticancer agents[19]. Phytol, Ledene oxide (I) and Squalene were reported in the ethanol extract of *Polygala javana* whole plant by GC-MS analysis. These compounds may play a role in anticancer activity[20]. Further study is required for isolating specific compound.

Conclusion

The ethanol extract of *Polygala javana* whole plant showed significant antitumor activity. The results for its DAL bearing antitumor activity showed that, this plant has highly potent antitumor agents. Further isolation and purification of bioactive compounds from *Polygala javana* may reveal the presence of potent novel anticancer agent from *Polygala javana.*

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