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Antitumor activity of Coleus spicatus Benth. against Dalton's Ascitic Lymphoma

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Abstract: The tumor inhibitory effect of an ethanolic extract of *Coleus spicatus* Benth (EECS) synonym of *Plectranthus canninus* Roth aerial parts on the growth of Dalton's ascitic lymphoma (DAL) and on the life span of tumor bearing mice were studied. EECS treatment showed significant increase in the life span and a decrease in the cancer cell number and tumor weight were noted in the tumor induced mice after treatment with EECS. Hematological parameters like red blood cell count, hemoglobin content and platelet were also corrected by EECS in tumor induced mice. The result of the present work suggest that EECS of aerial parts has an anti tumor effect against Dalton's ascitic lymphoma (DAL).
Keywords: *Coleus spicatus*; Dalton's Ascitic Lymphoma; Anticancer agents.

1. Introduction

Coleus spicatus Benth (labiatae) is an important plant in Indian system of medicine. It is a perennial fleshy herb, in arid places on rocky ground among bushes found in Salem and Coimbatore district of Tamil Nadu, India[1]. It grows upto 50cm in height with branchlets hispid in nature[2]. Traditionally this plant is used as a stimulant treatment of cough[3], diuretic, cytotoxic, and various phytoconstituents viz coleon S and T, α – amyrin tormentic acid, flavones, kumata kinin, 3,7-dimethyl quercetin and sitosterol were isolated from aerial parts of the plant[4].

Many Indian spices[5] and plants[6] are quoted to be useful in different types of cancer. In spite of numerous medicinal uses attributed to this plant, there are no pharmacological evidences that support the use of this medicinal species yet. Therefore, it is necessary to investigate the anti tumor activity of *Coleus spicatus* in order to determine whether the medicinal uses are supported by pharmacological effect.

Here we report the result of invivo experimental study with the ethanolic extract from the aerial parts of *Coleus spicatus* (EECS) against (DAL).

2. Materials and methods

2.1 plant material

The aerial parts of *Coleus spicatus* were collected from various places of Salem district, Tamil Nadu, India and identified by Dr.V.Chelladurai, Ex. Professor Medicinal plant survey for Siddha, Government of India. Tirunelveli, Tamil Nadu, India. A voucher specimen (HS 034) was kept in the herbarium of the Department of Pharmacognosy, Ezhuthachan College of pharmaceutical sciences, Marayamuttom, Thiruvananthapuram, Kerala, India.

2.2 Animals

Male swiss albino mice (20-25gm) were studied for this study[7]. They were housed in micro nylon boxes in a control environment (temp 25±2ºC) and 12 hours dark light cycle and relative humidity with standard laboratory diet and water adlibitum.

2.3 Preparation of extract

The shade dried aerial parts of *Coleus spicatus* were powdered coarsely and about 1kg of this powder was macerated with ethanol 70%(v/v) during 72h. The obtained extract was filtered and concentrated in a rotary evaporator under reduced pressure. The yield was 26.8 gm w/w.

2.4 Preparation of drug

The extract was dried in vacuum and resuspended in water before use. The phytochemical screening gave positive results for carbohydrate, polysterols, saponins, tannins and flavanoids.[8,9]

2.5 Determination of anti tumor activity.

2.51 Grouping of animals

The animals were divided into five groups viz G_1, G_2, G_3, G_4, and G_5 of six each and used for the study.

2.52 Induction of cancer using DLA cells

DLA cells were obtained through the courtesy of Amala cancer institute, Thrissur, Kerala, India. The cells maintained in swiss albino mice by intraperitoneal transplantation (10^6cells/mouse) for four groups, remaining one group (G_1) is serve as normal control. On the second day animals of G_3 were treated with 5-fluorouracil (20mg/kg). While the mice G_4 were treated with EECS 100mg/kg and G_5 were treated with EECS 200mg/kg. The treatment was continued for the next 14 days G_2 was not allocated any treatment after inoculation with DAL cells. The mice were observed for the next 15 days for the development of ascitic tumor. On day 15, the mice were sacrificed for cancer studies.

2.53 Tumor growth response

The effect of EECS on tumor growth and host survival were estimated by evaluating packed cell volume, cancer cell count, percentage increase in life span (ILS) of the tumor hosts, increase in body weight respectively[10]. Median life span of each group (containing six mice) was noted and anti-tumor activity of the test compounds compared with that of control group by measuring %ILS.

$$\%ILS = \frac{Life\ span\ of\ treated\ group}{Life\ span\ of\ control\ group} - 1 x 100$$

2.54 Determination of hematological parameters

Apart from the above mentioned parameters, the effects of EECS on hematological parameters were also studied in the mice of all groups. Blood was collected from the all mice on the groups by puncturing retro orbital plexus and counted for RBC, WBC, Hb platelets and packed cell volume.[11,12]

2.55 Determination of biochemical parameters

The effect EECS on lipids like total cholesterol, triglycerides and liver enzyme[13] parameters like AST, ALT, ALP was studied in all groups[14].

2.56 Statistical analysis

The results expressed as mean ±SEM. The evaluation of the data was done using oneway ANOVA followed by newman-keul's multiple range test. Difference below $P<0.05$ implied significance.

Table No 1: Effect of EECS on the life span, body weight and cancer cell count of tumor induced mice.

Treatment	Number of animals	% ILS Life span	Increase in Body weight grams	Cancer cell count ml X 10^6
G_1	6	>30 days	2.30±0.52	-
G_2	6	48%	$7.78±1.24^{a**}$	$2.45±0.30^{a**}$
G_3	6	90%	$3.44±0.65^{b**}$	$1.40±0.42^{b**}$
G_4	6	70%	$6.50±1.15^{b*}$	$1.85±0.55^{b*}$
G_5	6	78%	$5.60±0.95^{b**}$	$1.64±0.42^{b**}$

All values are expressed as mean ± SEM for 6 animals in each group.

G_1 – Normal Control, G2 –Cancer Control, G3 –Positive control, G_4 –Treatment control (low dose EECS),G_5– Treatment control (High dose EECS).

All values are expressed as mean ± SEM for 6 animals in each group.

**a Significantly different from normalcontrol (G_1) at $P < 0.001$

*b Significantly different from cancer control (G_2) at $P < 0.01$

**b Significantly different from cancer control (G_2) at $P < 0.001$

Table 2: Effect of EECS on Hematological parameters

Treatment	Total WBC Cells /ml×10^3	Rbc Count Mill/cumm	Hb Gm/dl	PCV %	Platelets Lakhs/cumm
G1	10.05 ±1.45	4.05±0.95	12.30 ±2.25	14.45±2.60	3.05±0.80
G2	14.65 ±2.60^{a**}	2.65±0.55^{a**}	7.30 ±1.04^{a**}	31.45±4.55^{a**}	1.70±0.48^{a**}
G3	11.78 ±1.92^{b**}	3.85±0.90^{b**}	11.6 ±1.75^{b**}	19.25±2.40^{b**}	2.68±0.65^{b**}
G4	13.40±2.15^{b*}	3.05±0.68^{b*}	9.45±1.40^{b*}	25.22±3.85^{b*}	1.98±0.28^{b*}
G5	12.50 ±2.25^{b**}	3.35±0.80^{b**}	10.80±1.85^{b**}	21.30±2.25^{b**}	2.22 ±0.48^{b**}

All values are expressed as mean ± SEM for 6 animals in each group.
**a Significantly different from Normal control (G_1) at P < 0.001
*b Significantly different from cancer control (G_2) at P < 0.01
**b Significantly different from cancer control (G_2) at P < 0.001

Table 3: Effect of EECS on serum Enzymes and lipid proteins

Treatment	Cholesterol (mg/dl)	TGL (mg /dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
G_1	100.20±3.62	125.8±4.45	38.50 ±1.28	38.40 ±1.40	128.45 ±2.25
G_2	142.90±4.64^{a**}	208.20±6.78^{a**}	88.6±2.75^{a**}	61.33±2.40^{a**}	240.65±4.30^{a**}
G_3	110.40±3.92^{b**}	157.35±3.80^{b**}	56.30 ±1.95^{b**}	42.40±1.75^{b**}	165.25±2.32^{b**}
G_4	127.65±4.44^{b*}	188.94±3.75^{b*}	78.45 ±2.33^{b*}	56.40±2.25^{b*}	202.35±3.40^{b*}
G_5	121.30±3.85^{b**}	170.65±3.28^{b**}	71.52±1.60^{b**}	44.40 ±1.55^{b**}	190.45±2.55^{b**}

All values are expressed as mean ± SEM for 6 animals in each group.
G_1 – Normal Control, G2 –Cancer Control, G3 –Positive control, G_4 –Treatment control (low dose EECS), G_5– Treatment control (High dose EECS).
All values are expressed as mean ± SEM for 6 animals in each group.
**a Significantly different from control (G_1) at P < 0.001
*b Significantly different from cancer control (G_2) at P < 0.01
**b Significantly different from cancer control (G_2) at P < 0.001

Results and discussions

The intraperitoneal inoculation of DAL cells in the mice produces increased proliferation of cells. EECS reduced the cancer cell count to 1.85±0.55×10^6, 1.64±0.42×10^6 cells in the treated mice (Table 1). The EECS treated mice survived upto 35 days where as tumor control mice survived upto 20 days only. As a PCV in tumor control mice was found to be high (31.45±4.55%). Oral administration of the low and high dose of EECS extract had reduced the PCV 25.22±3.85% and 21.30±2.25% respectively. The percentage increase in life span of EECS treated mice increased by 70% and 78% respectively. Extract treatment reduces the tumor weight and hence increased the life span of cancer induced mice. Regarding the hematological parameters, cancer control mice showed reduced RBC count but increase in WBC count than normal group. The treatment with low and high dose of EECS also raised the RBC count significantly to 3.05±0.68 millon/cumm, 3.35±0.80 million/cumm respectively. Similarly both dose restored the WBC value to 13.40±2.15 cells/ml × 10^3, 12.50±2.25 cells/ml × 10^3 respectively. Hb content in cancer control mice reduced significantly when compared with normal group. But, the EECS doses increased Hb content to 9.45±1.40 gm/dl, 10.80±1.85 gm/dl (Table 2). The EECS extract restored the normal platelet count in treated mice. The inoculation of DLA cells caused significantly increase in the level of Total Cholesterol, Aspartate amino Transferase, Alanine amino Transferase, Alkaline Phosphatase in the tumor control animals(G_2), when compared to the normal group. The treatment with EECS at the dose of 100 and 200 mg/kg body weight reversed these changes towards the normal level (Table No. 3).

Lymphoma is defined as malignant tumors of lymphoreticular orgin i.e.from lymphocytes and histiocytes and their precursor cells[15]. Many studies have reported the useful effects of plant products against DAL. The free radicals have been implicated in carcinogenesis[16]. Many plant extract containing anti oxidant principles have been reported to possess anti-tumor activity[17]. Hence plants containing

glycosides, flavanoids, etc.. are constantly being screened for anti tumor activity. Some of the phytoconstituents like triterpenes and flavones were isolated from this plant. Hence this plant was selected to study the anti tumor activity against DAL.

Intraperitoneal inoculation of DAL cells in mice produced an enormous increase in the cancer cell count which indicated that there is progression of cancer in mice. The decrease in tumor cell number observed in the EECS treated mice of G_4 and G_5 indicates that the test drug is having significant inhibitory effect on the tumor cell proliferation. The increase in tumor weight of G_4 and G_5 may be due to accumulation of peritoneal fluid as an abnormal enlargement of peritoneal cavity was observed in tumor weight and hence increased the life span. From the hematological studies it is understood that the significant rise in WBC in $G_{2,}$ might be defensive mechanism against cancer cells. As the progression of cancer was brought under control by EECS, the WBC count got reduced in $G_{3.}$ These observations on the effect of EECS on various parameters studied to evaluate the antitumor activity enabled us to conclude that it possesses antitumor activity. However further investigations are essential to study the mechanism of action of EECS.

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