

ANTITUMOR ACTIVITY OF AQUEOUS EXTRACT OF *COSTUS PICTUS* D. DON AGAINST DALTON'S ASCITES LYMPHOMA IN MICE

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Article Received on
05 Sept. 2017,

Revised on 27 Sept. 2017,
Accepted on 18 Oct. 2017

DOI: 10.20959/wjpr201714-9951

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ABSTRACT

The aim of the present investigation was to evaluate the antitumor activity of aqueous extract of *Costus pictus* leaves against Dalton's Ascites Lymphoma (DAL) induced tumor in Swiss albino mice. After 24 hours of tumor inoculation, aqueous extract at doses of 200mg/kg and 400 mg/kg body weight were administered daily for 14 days. After administration of last dose and 18hour fasting, the mice were sacrificed. Antitumor activity was assessed by monitoring tumor parameters, haematological parameters, serum biochemical parameters and histopathological evidences. Standard drug, 5- Furfural was used as positive control. Both the doses of aqueous extract treated group restored tumor parameters, hematological and biochemical parameters towards the normal levels when compared with the control group.

Histopathological studies also confirmed the protective influence. The findings of the present study indicate the protective effect of *C.pictus* leaf extracts.

KEYWORDS: Dalton's Ascites Lymphoma, *Costus pictus*, Antitumor, Serum biochemical parameters, Histopathology.

INTRODUCTION

Cancer is a group of disease involving abnormal growth of tissue in which the cells exhibit an uncontrolled division, relatively in an autonomous fashion, leading to a progressive increase in the number of dividing cell^[1] which spread throughout the body and may eventually cause death of the host. World Health Organization (WHO) reported that there are 7.6 million death in 2008 and it is estimated up to 13.1 million deaths in 2030.^[2] Conventional treatment of

cancer includes interventions such as psychosocial support, surgery, radiotherapy and chemotherapy. A combination of these methods is also used depending on the type and location of the cancer, the patient's age, general health and other factors. As these therapies are associated with adverse side effects, nontoxic chemopreventive agents derived from plants were proposed for treatment which in turn is less likely to cause side effects.

Plant-derived products can be exploited with sustainable comparative and competitive advantage. Screening of medicinal plants for anticancer activity has been a major interest since 1960s.^[3] *Costus pictus* D.Don is a perennial herb belonging to the family Costaceae. The plant has long narrow leaves which are spirally arranged around the stem. *C.pictus* is recognized by its yellow flower which grows intermittently throughout the year. Traditionally, the plant is valued for its tonic, antiseptic, stimulant and aphrodisiac properties.^[4] Leaves of this plant are used to cure diabetes, hence known as "Insulin plant". A decrease in blood glucose level with significant increase in plasma insulin in diabetic rats were observed with the administration of *C.pictus* aqueous extracts.^[5] Researches also have shown the plant possess a range of pharmacological properties like^[6], antispasmodic, antioxidant^[7,8], antibacterial, and antifungal^[9] effects apart from its anti-diabetic activity. However, no reports are available to prove the antitumor activity of *Costus pictus* leaves. Hence this study was planned to evaluate the *In vivo* antitumor activity of aqueous extract of *Costus pictus* leaves against Dalton's Ascites Lymphoma (DAL) tumor models.

MATERIALS AND METHODS

Collection of plant material: Fresh leaves of *Costus pictus* were collected and authenticated (BSI/SRC/5/23/Tech/2519) from Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore. The specimen is retained in the institute for future reference.

Preparation of plant extract: Leaves were washed, shade dried and powdered. 20g of powdered samples was dissolved in conical flasks with 150ml distilled water. The extraction was carried out for 48hour in a rotary shaker at 150-160 rpm. The extract was filtered using muslin cloth and residue is removed. The filtrate is then evaporated to dryness and the resulting pasty form extract were stored for future use.

***In vivo* anticancer activity on Dalton's Ascites Lymphoma (DAL) induced cancer in mice**

Inbred Swiss mice of 2 months age, weighing 20 ± 5 g, were purchased from Government Veterinary College Mannuthi, Thrissur, India, for the study. The mice were obtained from the stock in breed colony, which was maintained by mating brothers and sisters. They were housed at room temperature of $24 \pm 2^\circ\text{C}$ under 12 hour light/12 hour dark cycle in the animal house. Mice were fed with commercial pellet diet and water *ad libitum* freely throughout the study. All animal procedures were performed after approval from the IAEC (Institution of Animal Ethical Committee IAEC NO: KMCRET/PhD/10/2016-17) and in accordance with the CPCSEA recommendations for the proper care and use of laboratory animals.

DAL - induced antitumor activity: Dalton's Ascites Lymphoma (DAL) cells were obtained from Amala Cancer Research Institute, Kerala, India. The cells were maintained *In vivo* in mice by injecting DAL cell suspension (1×10^6 cells per mouse) in to the peritoneal cavity of the animals. The freshly aspirated DAL cells from the peritoneal cavity of mice were washed with saline and given intraperitoneally to the experimental mice to develop ascitic tumor. Treatment was started after 24 hours of the tumor inoculation, continued once daily for 14 days and the antitumor efficacy of the extracts was compared with that of 5-Furfural (10mg/kg) and DAL control.

Experimental design: Swiss albino mice were divided into five groups of 12 animals each. All the animals in four groups except Group- I were injected with Dalton's Ascites Lymphoma (DAL) cells (1×10^6 cells per mouse) intraperitoneally. Group- I served as normal control and received normal saline. Group- II served as tumor control. Group-III was treated with 5FU at the dose of 10mg/kg, which served as positive control. Group- IV and Group- V received aqueous extract of *C.pictus* (AECF) leaves at dose 200mg/kg and 400mg/kg body weight respectively. Half of the animals in each group were sacrificed and the remaining animals were kept to observe the body weight, tumor volume, packed cell volume, mean survival time and increase in life span.

Determination of body weight

From the starting day to end of the study all the mice were weighed for every five days, after tumour inoculation. Average gain in body weight was determined and recorded. Percentage of increase in body weight was calculated by the formula:

% Increase in body weight = (Final body weight- initial body weight /initial body weight) ×100

Determination of Mean Survival Time (MST)

Each day after induction all the groups were checked for mortality & recorded the number of days survived by each mouse. Mean survival time (MST) and percentage increase in life span (ILS %) was calculated by using the formula:

Mean survival time = [1st Day of Death + Last Day of Death] / 2

ILS (%) = [(Mean survival of treated group/ Mean survival of control group)-1] x100

Determination of tumor volume: After 14 days treatment 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 and packed cell volume determined by centrifuging at 1000g for 5 minutes.

Estimation of hematological Parameters: Blood sample and intraperitoneal fluid was collected by mild anaesthization of mice with diethyl ether and then sacrificed by cervical dislocation. Blood was collected for estimation of RBC, WBC, differential cell count, hemoglobin percentage and platelet count by standard procedures.

Estimation of serum biochemical parameters: The separated serum sample was used for estimation of Alkaline Phosphatase (ALP), Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), totalbilirubin, totalprotein, urea, uric acid, creatinine, total cholesterol, triglycerides and HDL cholestrol by using semi auto analyzer (Photometer 5010 v₅₊) using standard enzymatic kits procured from Piramal Healthcare limited, Lab Diagnostic Division, Mumbai, India.

Histopathological techniques

Thin pieces of 3 to 5 mm thickness are collected from tissues, kept in fixative for 24-48 hours at room temperature. The section is then deparaffinised by xylol for 5 -10 minutes, removed xylol by absolute alcohol and then washed in water. It is then stained and mounted in DPX mountant or Canada balsam. The section were examined microscopically and photographed.

Statistical analysis

Values are expressed as mean± SEM. Statistical significance calculated by one way ANOVA followed by Dunnet's test.

RESULT AND DISCUSSION**Effect of *Costus pictus* on tumor parameters**

The administration of aqueous extract of *Costus pictus* (AECP) at doses of 200mg/kg and 400mg/kg to DAL bearing mice showed reduction in body weight (Table 1). A comparison of the result showed a dose dependent decrease in body weight when compared to that of control. The percentage increase in body weight was less in AECP treated mice and standard when compared to DAL control mice. The Mean Survival Time increased with administration of different doses, 200mg/kg and 400mg/kg of AECP and standard drug in DAL induced mice. The percentage increase life span in AECP treated mice increased by 18.23% (200mg/kg) and 27.27% (400mg/kg) whereas; standard drug 5-Furfural (10 mg/kg) treated mice showed 27.27% increase in life span (Table 2).

Table. 1: Effect of *Costus pictus* on body weight and percentage increase in body weight of DAL induced mice.

Groups	Body weight			Percentage increase in body weight (%)
	1 st Week	2 nd Week	3 rd Week	
Group-I	21±1.342	24.33±0.919	26.67±0.667	26.31
Group- II	54±33.613	14.67±6.561	21.17±9.509	66.66
Group- III	17.83±0.654	27.83±1.682	24.67±0.955	2.020
Group-IV	21.16±0.401	17.67±5.619	17.5±7.839	33.33
Group- V	20±0.447	20.67±6.581	16.5±5.23928	8.33

Values are expressed as ± SEM (n=6), *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table. 2: Effect of *Costus pictus* on Mean Survival Time, ILS, tumor volume and PCV.

Groups	Mean Survival Time (Days)	Increase in life span (ILS) (%)	Tumor volume	Packed Cell Volume (PCV)
Group-I	18.83±1.460	-	0±0	0±0
Group- II	12.83±0.786**	-	7.867±2.838	3.033±0.641***
Group- III	16.33±1.263	27.27	4.567±2.003	0.923±0.023
Group-IV	15.17±1.295	18.23	3.9167±1.855	1.886±0.363*
Group- V	16.33±1.263	27.27	5.333±1.994	2.27±0.319**

Values are expressed as ± SEM (n=6), *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

A significant reduction in tumor volume and Packed Cell Volume observed in AECP treated group at doses 200mg/kg and 400mg/kg when compared to the DAL tumor control mice. Group treated with standard drug also decreased tumor volume and PCV towards normal levels. The reliable criteria for judging the value of an anticancer drug is the prolongation of

the life span of animals.^[10] By decreasing the nutritional fluid volume and tumor proliferation *C.pictus* leaf extracts increased the life span of DAL bearing mice. Similar results have been obtained with the treatment of ethanol extract of *Polygala javana* on Dalton's Ascites Lymphoma increased the life span and mean survival time with a decrease in tumor volume.^[11]

Effect of *Costus pictus* on hematological parameters

The effect of AECP on hematological parameters is shown in Table 3. RBC count, total hemoglobin content and PCV decreased and WBC count increased in the DAL induced group as compared to the normal control group. Treatment with AECP increased the RBC count, total hemoglobin content and PCV and decreased WBC count towards the normal as compared to the DAL control group. Treatment with AECP at doses 200mg/kg and 400mg/kg brought back polymorphs, monocytes and eosinophils to more or less the normal levels with an increase in lymphocytes. The standard drug, 5- Furfural (10mg/kg) also produced better results. AECP treatment also reversed the MCH levels towards normal. The treatment of ethanol extract of *Cnidocolus chayamansa* against Dalton's Ascites Lymphoma brought back hemoglobin content, RBC and WBC count more or less to normal levels significantly.^[12] The improvement in the hematological profile of the tumor bearing mice followed by the treatment of aqueous extract of *C.pictus* leaves indicates that the plant possess the protective action on the hemopoietic system.

Table. 3: Effect of *Costus pictus* leaf extracts on hematological parameters.

Parameters	Group- I	Group- II	Group- III	Group- IV	Group- V
RBC	5.31±0.157	4.933±0.214	4.457±0.211	4.45±0.168	4.907±0.195
WBC	12.6±0.729	35.567±3.59***	22.5±1.528*	31.633±1.93**	27.83±2.0**
Total Hemoglobin	12.933±0.470	11.8±0.636	10.367±0.643	10.7±0.275	11.433±0.856
Packed Cell Volume	39.833±1.427	36.533±1.961	32.267±1.828	32.9±1.330	36.733±2.30
Polymorphs	4.333±0.760	13±1.095***	9±0.966	12.667±0.76***	11±0.730***
Lymphocytes	89.333±1.520	81±1.316**	83.667±1.173	83.667±1.646	86.667±0.918
Monocytes	2.667±0.210	4.333±0.210**	4±0.365*	3.666±0.557	3.333±0.210
Eosinophils	3.667±0.557	5±0.365	3.333±0.557	4±0.632	3±0.632
MCH	26.133±0.128	25.033±1.266	21.87±0.749*	23.633±1.023	25.267±0.641

Values are expressed as ± SEM (n=6), *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Effect of *Costus pictus* on serum biochemical parameters

The serum biochemical parameters of control, standard and treatment groups are shown in Table 4. The treatment with AECP significantly decreased the elevated SGOT, SGPT and

ALP as compared with the DAL control mice. Inoculation of DAL cells caused decrease in total protein level and an increase in total bilirubin in DAL induced mice, which on treatment with AECP at doses 200mg/kg and 400mg/kg body weight reversed towards normal levels. Levels of urea, creatinine and uric acid in DAL induced mice returned to normal after administration of AECP at doses 200mg/kg and 400mg/kg. Levels of total cholesterol and triglycerides increased in DAL induced mice, which on treatment with AECP reversed to normal levels. High Density Lipoprotein (HDL) levels also reversed towards normal in AECP treated group when compared to that of DAL control mice. Restoration of these marker enzymes level is an indication of stability of plasma membrane and also repairment of tissue damage caused by DAL cell induction.^[10]

Table. 4: Effect of *Costus pictus* on serum biochemical parameters.

Parameters	Group- I	Group- II	Group- III	Group- IV	Group- V
SGOT (U/L)	87.7±2.35	205.367±23.02***	173.4±10.86**	211.4±15.06***	194±14.69**
SGPT (U/L)	61.1±2.417	68.5±0.384	77.8±9.124	67.933±6.728	66±2.551
ALP (U/L)	16.3±0.096	19.333±2.292	27.8±2.942	11.78±2.726	22.467±1.570
Total Protein (mg/dl)	6.733±0.076	5.867±0.076***	5.033±0.05***	5.9±0.073***	6±0.193*8
Total bilirubin (mg/dl)	0.616±0.0462	0.953±0.037**	0.667±0.040	1.03±0.036***	0.99±0.080**
Urea (mg/dl)	37.667±2.853	55.467±3.007**	57.4333±3.8**	42.3±5.339	57.23±3.672**
Creatinine (mg/dl)	0.903±0.088	0.303±0.011*	0.783±0.090	0.686±0.117	0.903±0.296
Uric acid (mg/dl)	2.203±0.137	1.156±0.118*	2.233±0.0855	1.786±0.189	1.39±0.424
Total Cholesterol (mg/dl)	76.3±2.807	92.533±6.986	98.6±12.121	86.8±6.956	86.467±0.867
Triglycerides (mg/dl)	60.9±5.279	90.533±12.900	175.2±32.69***	67.733±5.566	64.667±7.637
HDL-Cholesterol (mg/dl)	46.667±1.734	43.767±1.460	34.633±4.341*	46.7±2.78	49.867±1.566

Values are expressed as \pm SEM (n=6), *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Effect of *Costus pictus* on histopathology

Histoarchitecture of the liver was done to observe the efficacy of aqueous extract of *Costus pictus* leaf on the morphological structure of the cells (Fig 1). Normal, untreated mice showed normal lobular architecture, normal portal triad, preserved hepatocytes with mild dilatation of central vein and sinusoid. There is no evidence of malignancy/ dysplasia in the section study. DAL induced mice liver tissues showed altered lobular architecture with dilatation of central vein and sinusoids. Hepatocytes showed cytoplasmic vacuolation and binucleation. Mice treated with AECP at dose 200mg/kg body weight showed lobular architecture with interface hepatitis and centrilobular necrosis. Portal triad shows no significant pathology. Central vein and sinusoids showed mild dilatation. However, mice treated with 400mg/kg body weight

and standard drug, 5-Furfural showed lobular architecture with reduced interface hepatitis, mild cytoplasmic vacuolation, mild dilatation of central vein and sinusoids with no evidence of malignancy/ dysplasia in the tissues. The normalization of histoarchitecture of liver tissues showed hepatoprotective nature of *C. pictus* leaf extracts.

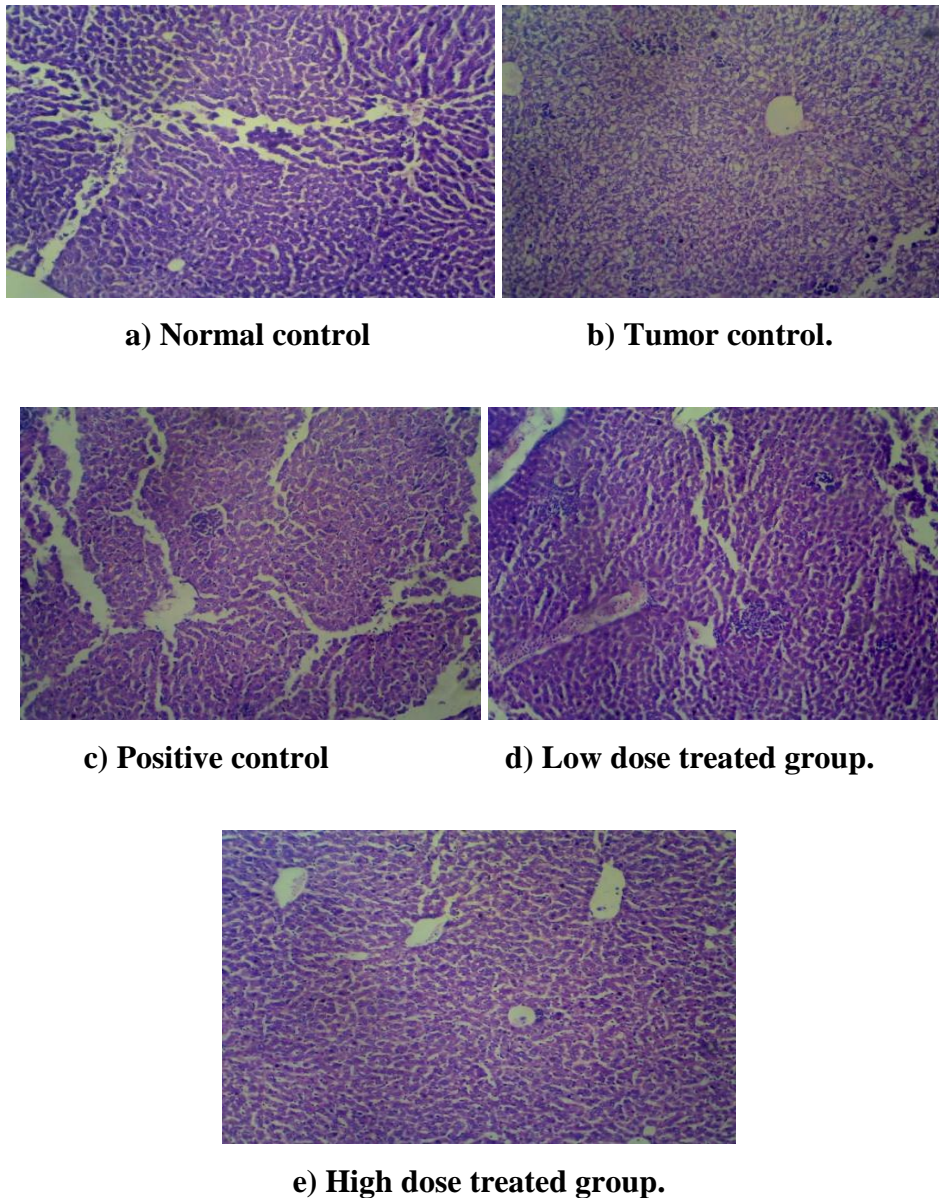


Fig. 1: Histopathological observations in liver of experimental animals.

CONCLUSION

In the present study antitumor activity of aqueous extract of *Costus pictus* leaves were evaluated against Dalton's Ascites Lymphoma induced cancer in mice. The extract treatment at the doses, 200mg/kg and 400mg/kg body weight inhibited the tumor activity by restoring the tumor parameters, haematological parameters, serum biochemical parameters and

histoarchitecture of liver tissues. The normalization of these parameters clearly indicates the antitumor and hepatoprotective effect *Costus pictus* leaf extracts.

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