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Original Research Article

Assessment of Invivo Anticancer Activity of Justicia Adathoda Using Dal Cell Lines

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Abstract: Background: The medicinal plants have very important role in the health of human being as well as animals. The present study was thus designed to investigate the anticancer activity of ethanolic leaf extract of *Justicia adathoda*. **Method:** The antitumor activity of the ethanolic extract of leaves of Justicia adathoda (EEJA) has been evaluated against Dalton's ascitic lymphoma (DAL) in Swiss albino mice at the dose of 200 & 400 mg/kg, body weight. The experimental parameters used were tumour volume, tumour cell count, viable tumour cell count, mean survival time and increase in life span to assess antitumour activity. **Results:** The extract was administered orally for 14 consecutive days to tumor bearing group of animals. The extract increase the life span of DAL treated mice and restore the hematological parameters as compared with the DAL bearing mice in dose dependant manner. **Conclusion:** Administration of EEJA, showed significant reduction in the percent increase in body weight, tumor volume, tumor weight, viable cell count and increased non-viable cell count in tumor bearing mice when compared to the untreated mice of the DAL control group. The restoration of the haematological parameters towards the normal control was also observed. The results suggested that the *Justicia adathoda* exhibits significant anticancer activity.

Keywords: Dalton's ascitic lymphoma, Justicia adathoda (EEJA).

INTRODUCTION

Cancer or malignant neoplasm is a class of diseases in which a group of cells display uncontrolled growth, invasion and even sometimes metastasis (De Vita *et al.*, 2005; Thomas and Vinay, 2007). Currently, the treatment for cancer primarily includes surgery and chemotherapy, but the curative effects of the existing chemotherapeutic drugs are not good enough and they have plentiful side effects. The development of more effective drugs for treating patients with cancer has been a main attempt over the past 50 years. Hence, finding a reliable molecule for cancer treatment remained the prime aim of research scientists. Considering all these points, a number of natural and synthetic molecules/compounds have been studied for cancer drug discovery and some of them were found promising. Extensive studies seeking a new active plant extract have been carried out in the search for drug candidates that have high efficacy and safety (Gao *et al.*, 2005). This paper describes the screening of ethanolic extract of Justicia adathoda for its anti cancer activity against DAL cell lines.

Justicia adhatoda (L.) Nees (family Acanthaceae) is a shrub widespread throughout the tropical regions of Southeast Asia (Chakrabarty and Brantner, 2001). It is commonly known as Vasaka or Malabar nut. It is a perennial, evergreen and highly branched shrub (1.0 m to 2.5 mm height) with unpleasant smell and bitter taste (Patel and Venkata-Krishna- Bhatt 1984). It has opposite ascending branches with white, pink or purple flowers (Patel and Venkata-Krishna- Bhatt, 1984). It is a highly valuable Ayurvedic medicinal plant used to treat cold, cough, asthma and tuberculosis (Sharma *et al.*, 1992). Its main action is expectorant and antispasmodic (bronchodilator) (Karthikeyan *et al.*, 2009). The significance of Vasaka plant in the treatment of respiratory disorders can be understood from the ancient Indian saying, "No man suffering from phthisis need despair as long as the Vasaka plant exists" (Dymock *et al.*, 1893). Thus the frequent use of J. adhatoda has resulted in its inclusion in the WHO manual "The Use of Traditional Medicine in Primary Health Care" which is intended for health workers in

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south-east Asia to keep them informed of the restorative utility of their surrounding flora (WHO, 1990). The major alkaloids of the plant, vasicine and vasicinone, have been found to be biologically active and are the area under discussion of many chemical compounds and pharmacological studies.

METHODS

Plant extract used

Ethanolic extract of Justicia adathoda.

Experimental animals

Healthy Wistar Albino rats of either sex, weighing about 150-200 g were procured from animal house. The entire study was approved by the Institutional Animal Ethical Committee (IAEC) which is certified by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

The animals were kept in clean and dry polycarbonate cages and maintained in a well-ventilated animal house with 12 hours light – 12 hours dark cycle. The animals were fed with standard pellet diet and water was given *ad libitum.* For experimental purpose the animals were kept fasting overnight but allowed free access to water.

Acute toxicity class method (Ecobichon, 1997)

Procedure

Three healthy, Wistar Albino rats weighing 150-200 g were selected for the study. The rats were fasted over-night and provided with water *ad libitum*. Following the period of fasting, the animals were treated with the test extract at the dose of 2000 mg/kg body weight, orally. As most of the crude extracts possess LD50 value more than 2000 mg/kg body weight and this was used as starting dose.

After oral administration, the rats were observed on hourly basis for 24 hours to access mortality and to detect any changes in the autonomic or behavioral responses viz. alertness, spontaneous activity, salvation, respiration, urination, aggressiveness, irritability, convulsion and corneal reflex etc.

The rats were observed regularly for 14 days to note the mortality or toxic symptoms. Since there was no death as per the guidelines, the study was repeated with the same dose to confirm the results.

INVIVO ANTI CANCER ACTIVITY

Experimental model (Jaslin Edward, 2015)

For the study of anticancer activity, an experimental model is selected in such way that it would satisfy the following condition;

- The animal should develop cancer rapidly and reproducibly.
- Pathological changes in the site of induction should result from cancer formation.
- The symptoms should be ameliorated or prevented by a drug treatment effective in human beings.
- The drug tested should be administered orally.
- Drug dosage should approximate the optimum therapeutic range for human, scaled the test animal weight.

Experimental Animals (Unnikrishnan, 1990)

Male Swiss albino mice (20-25 gm) were produced from animal experimental laboratory, and used throughout the study. They were housed in micro nylon boxes in a control environment (temp 25±2°C) and 12 hours dark /light cycle with standard laboratory diet and water *ad libitum.* The study was conducted after obtaining institutional animal ethical committee clearance. As per the standard practice, the mice were 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.

Technique for inducing tumor

Various technique for induction of cancer in animals, viz, chemically induced (using DMBA/croton oil, etc) (Agarwal, 2009) virus induced, cell line induced (sarcoma – 180, ULCA fibro sarcoma and Jensen sarcoma, mouse lung fibroblast cells L-929, Dalton's Lymphoma Ascites (DAL), Ehrlich Ascites Carcinoma (EAC) (Becerra, 2006, David, 1950 and Chitra, 2009) methods have been used in experimental studies of anticancer activity.

In the present study, DAL cell lines induced cancer in mice was used to evaluate the anticancer activity.

Induction of cancer using DAL cells

Dalton's Lymphoma ascites (DAL) cell was supplied by Amala Cancer Research Center, Thrissur, Kerala, India. The cells maintained in vivo in Swiss albino mice by intra peritoneal transplantation. While transforming the tumor cells to the grouped animal the DAL cells were aspirated from peritoneal cavity of the mice using saline. The cell counts were done and further dilutions were made so that total cell should be 1 x 10^6; this dilution was given intra peritonealy. Let the tumor grow in the mice for minimum seven days before starting treatments.

Treatment Protocol

Swiss Albino mice were divided into five group of six each. All the animals in four groups were injected with DAL cells (1×10^6 cells per mouse) intra peritonealy, and the remaining one group is normal control group (Sathiyanarayanan, 2006).

G1: Served as the normal control.
G2: Served as the tumor control. Group 1 and 2 receives normal diet and Water.
G3: Served as the positive control; was treated with injection fluorouracil at 20 mg/kg body weight, Intra peritonealy.
G4: Served as a low dose treatment control and was administered test extract EEJA (200mg/kg, n=6).
G5: Served as a high dose treatment control and was administered test extract EEJA (400mg/kg, n=6).

Treatment

In this study, drug treatment was given after the 24 hrs of inoculation, once daily for 14 days. On day 14, after 24 hrs the last dose, all mice from each group were sacrificed; blood was withdrawn from each mouse by retro orbital plexus method and the following parameters were checked.

Hematological parameters

- White blood cells (WBC)
- Red blood cells (RBC)
- Hemoglobin content (Hb)
- Platelet count
- Packed cell volume (PCV)

Serum enzyme and lipid profile

- Total cholesterol (TC)
- Triglycerides (TG)
- Aspartate amino Transferase (AST)
- Alanine amino Transferase (ALT)
- Alkaline Phosphatase (ALP)

Derived parameter

- Body weight
- Life span (%)
- Cancer Cell Count

RESULTS AND DISCUSSION

Effect on Tumor Growth

In the DAL tumor control group, the average life span of animals was found to be 48% whereas, 200 and 400 mg/kg of test extract EEJA showed increase life span to 73% and 83% respectively. These values were significant ($p < 0.001$) when compare with cancer control group mice. The average life span of 5- FU treated was found to be 90%, indicating its potent antitumor nature. The antitumor nature of test extract was evidenced by the significant ($p < 0.01$, $p < 0.001$) reduction of increase in body weight in animals treated with EEJA at the dose of 200 and 400 mg/kg test drug when compared to DAL tumor bearing mice. There was a significant ($p < 0.001$) reduction in packed cell volume and viable tumor cell count were found with 200, 400 mg/kg of EEJA when compared to the DAL tumor control.

Effect on Hematological Parameters

RBC count, Hb content, Platelets count were significantly ($p < 0.001$) decreased in cancer control group and were brought back to normal after treatment with 200, 400 mg/kg of EAJT and EEJT. WBC count was significantly ($p < 0.001$) increased in the DAL control group and was normalized by the treatment with test drug at the dose of 200 and 400 mg/kg of extracts. However, the standard 5-FU at the dose of 20 mg/kg body weight produced better result in all these parameters.

Effect on Biochemical Parameters

In DAL inoculated mice, there was significant ($p < 0.001$) increase of the level of Total Cholesterol, Triglycerides Aspartate amino Transferase, Alanine amino Transferase and Alkaline Phosphatase when compared to the normal group. The treatment with EEJA at the dose of 200 and 400 mg/kg body weight reversed these changes towards the normal level. The treatment with standard 5- FU also gave the similar results.

Table.1 Effect of test drug on Hematological parameters

Treatment	Total WBC Cells/mlx10^3	RBC Count Mill/cumm	Hb gm/dl	Platelets Lakhs/cumm
G1	9.38 ±0.60	3.51 ± 1.55	12.55 ±2.65	3.54±0.40
G2	13.79 ±1.29^{a**}	1.58±1.10^{a**}	7.26 ±1.3*^{a**}	1.70±0.55^{a**}
G3	10.15 ±1.34^{b**}	2.25±1.35^{b**}	11.7 ±1.26^{b**}	2.66±0.18^{b**}
G4	12.16±1.87^{b*}	2.80±0.86^{b*}	9.55±1.45^{b*}	1.40±0.50^{b*}
G5	11.41 ±2.10^{b**}	2.47±0.38^{b**}	10.10±1.74^{b**}	2.56 ±0.94^{b**}

All values are expressed as mean ± SEM for 6 animals in each group.
**a – Values are significantly different from control (G_1) at $p < 0.001$
*b – Values are significantly different from cancer control (G_2) at $p < 0.01$
**b – Values are significantly different from cancer control (G_2) at $p < 0.001$

Table.2 Effect of test drug on serum Enzymes and lipid proteins

Treatment	Cholesterol (mg/dl)	TG (mg /dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
G1	103.20 ± 1. 94	124.76 ± 3.65	40.25 ± 1.38	38.72 ±1.25	130.22 ± 2.08
G2	141.15 ± 2.90^{a**}	210.32 ± 4.92^{a**}	90.60 ± 2.05^{a**}	60.86 ± 2.65^{a**}	245.56 ± 3.27^{a**}
G3	112. 30 ± 3. 47^{b**}	157.35 ± 3.16^{b**}	57.75 ± 1.70^{b**}	42.89 ± 1.62^{b**}	165.64±2.17^{b**}
G4	128. 80 ± 2. 35^{b*}	190. 50 ± 2.38^{b*}	80.45 ± 2.70^{b*}	57.40 ± 2.70^{b*}	201.05±3.36^{b*}
G5	121. .32 ±3.16^{b**}	170.53 ± 2.27^{b**}	71 .48 ± 1.32^{b**}	43.52 ±1.43^{b**}	190.56±2.40^{b**}

All values are expressed as mean ± SEM for 6 animals in each group.
**a – Values are significantly different from control (G_1) at $p < 0.001$
*b – Values are significantly different from cancer control (G_2) at $p < 0.01$
**b – Values are significantly different from cancer control (G_2) at $p < 0.001$

Table. 3 Effect of test drug on the life span, body weight and cancer cell count of tumor induced mice.

Treatment	% ILS Life span	Increase in Body weight grams	Cancer cell count ml × 10^6 Cells/ml	PCV %
G1	>30 days	1.35 ± 0.42	-	13.72 ± 1. 85
G2	48%	7.83 ± 1.72^{a**}	2.32 ± 0.27^{a**}	31.52 ± 3. 84^{a**}
G3	90%	2.75 ± 0.65^{b**}	1.85 ± 0.52^{b**}	18.76 ± 2.82^{b**}
G4	71%	5.55 ± 1.78^{b*}	1.65 ± 0.48^{b*}	26.28 ± 2.85^{b*}
G5	78%	6.58 ± 0.95^{b**}	1.24 ± 0.12^{b**}	20.37 ± 2.20^{b**}

All values are expressed as mean ± SEM for 6 animals in each group.
**a – Values are significantly different from control (G_1) at $p < 0.001$
*b – Values are significantly different from cancer control (G_2) at $p < 0.01$
**b – Values are significantly different from cancer control (G_2) at $p < 0.001$

Where,

G_1 - Normal control,
G_2 - Tumor control, Group 1 and 2 receives normal diet and Water.
G_3 - Positive control (fluorouracil at 20 mg/kg),
G_4 - Treatment control (test drug 200mg/kg),
G_5 -Treatment control (test drug 400mg/kg).

CONCLUSION

The results have clearly shown that EEJA has brought hemoglobin content and also the RBC count to normal which is a big crisis encountered in cancer treatment due to myelosuppression and anaemia,. Analysis of the other hematological parameters showed minimum toxic effect in the mice which were treated with EEJA. After treatment, EEJA treated groups were able to reverse the changes in the haemotological parameters consequent due to tumour induction. All these data point to the chances of developing an ethanolic extract of leaves of *Justicia adathoda* as a novel, potential agent in the area of cancer management. Further studies to characterize the active principle and elucidate the mechanism of action of EEJA should be carried out.

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