EVALUATION OF ANTICANCER ACTIVITY OF *PLUMBAGO ZEYLANICA* LINN. LEAF EXTRACT

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ABSTRACT

Cancer is a malignant disease that is characterized by rapid and uncontrolled formation of abnormal cells which may mass together to form a growth or tumour, or proliferate throughout the body. Next to heart disease cancer is a major killer of mankind. Present study aims at a preliminary phytochemical screening and anticancer evaluation of *plumbago zeylanica* Linn. against Ehrlich Ascites Carcinoma in animal model. Results indicates that ethanolic extract of *plumbago zeylanica* Linn. possess significant anticancer activity and also reduce elevated level of lipid peroxidation due to higher content of terpenoids and flavonoids. Thus ethanolic extract of *plumbago zeylanica* Linn. could have vast therapeutic application against cancer.

KEY WORDS: Cancer, *plumbago zeylanica* Linn., lipid peroxidation, flavonoids, terpenoids.

INTRODUCTION

The chemotherapy of neoplastic disease has become increasingly important in recent years. An indication of this importance is establishment of a medical specialty in oncology in which the physician practices various protocol of adjuvant therapy. Most cancer patient now receives some form of chemotherapy, even though it is merely palliative in many cases. The relatively high toxicity of most anticancer drugs has fostered the development of supplementary drugs that may alleviate these toxic effects or stimulate the regrowth of depleted normal cells[1]. Plants have a long history of use in the treatment of cancer. Plants have played an important role as a source of effective anticancer agent, and it is significant that over 60% of currently used anticancer agents are derived in one way or another from natural sources, including plants, marine organism and microorganisms.

Plants have been prime source of highly effective conventional drugs for the treatment of many forms of cancer, and while the actual compounds isolated from the plant frequently may not serve as the drugs, they provides lead for the development of potential novel agents[2]. Therefore it was thought worth while to carry out preliminary phytochemical screening and screening of *Plumbago zeylanica* Linn. for anticancer activity against Ehrlich Ascites Carcinoma in animal model.

MATERIALS AND METHODS

Collection and Authentication of Plant

The plant leaves of *Plumbago zeylanica* Linn. was collected from Kolli hills, Salem District, Tamilnadu, India and was

authenticated. Reference number of the authentication report is BSI/SC/5/23/05.06/Tech/605.

Extraction Procedure

The leaves of *Plumbago zeylanica* Linn. were dried under shade and then made in to a coarse powder with a mechanical grinder. The powder was passed through sieve no. 40 and stored in an airtight container for further use. The dried powder material of leaves and stem (150gm) was first extracted with petroleum ether (60-80^{o}) in a soxhlet apparatus and after complete extraction (24 hrs) ,the solvent was removed by distillation under reduced pressure and resulting semisolid mass was vacuum dried using vacuum evaporator to yield a solid residue (petroleum ether extract). After the extraction with petroleum ether the same plant material was dried and again extracted with ethanol (95 % v/v) in soxhlet apparatus and after complete extraction (72 hr) the solvent was removed by distillation under reduced pressure and resulting semisolid mass was vacuum dried using vacuum evaporator to yield a solid residue (ethanolic extract) [3,4].

Phytochemical Tests

Various chemical tests were performed for the phytochemical identification of the ether and ethanolic extract of the plant leaves *Plumbago zeylanica* Linn. as per standard procedure[5]

Anticancer activity

Toxicity Evaluation (LD_{50})

(Karber's methods) Thirty mice including both male and female weighing 20–25 gm were selected for the study. LD_{50} was measured by Karber's methods[7].

Animals

Male Swiss albino mice weighing between 18-25 gm were used for present study. They were maintained under standard environmental conditions and were fed with standard pellet diet of water and *ad libitum*. The mice were acclimatized and laboratory condition for 10 days before commencement of experiment. All procedure described were reviewed and approved by the Institutional Animal Ethical Committee of J.K.K. Nataraja College of Pharmacy, Komarapalayam.

Cancer Cell line

EAC cells were obtained from Amala Cancer Research Center, Thrissur, and Kerala, India. They were maintained by weekly intraperitoneal inoculation of 10^6 cells / mouse.

Preparation of extract drug and mode of administration

In the present anticancer study, ethanolic extract of *plumbago zeylanica* (EEPZ) in the dose of 100 mg/kg and 200 mg/kg were prepared as suspension by dissolving the ethanolic extract in propylene glycol and sterile physiological saline containing Tween 20 to get the desired concentration[8, 9] .

Tumor Transplantation

Ehrlich's Ascites Carcinoma was maintained by serial transplantation from tumor bearing Swiss Albino mice. Ascetic fluid was drawn out from tumor bearing mice at the log phase (day 78 of tumor bearing) of the tumor cells. The tumor cell number was adjusted to $2X10^6$ tumor cells/ml. Sample showing more than 90 %

viability was used for transplantation. Each animal received 0.2 ml of tumor cell suspension containing 2 X 10^6 cells / ml intraperitoneally[10] .

Drug Treatment Schedule

Male swiss albino mice were divided into 5 groups (n = 8). All the groups were injected with EAC cells (0.2 ml of 2 X 10^6 cells/mouse) intraperitoneal except the normal group. This was taken as day zero. From the first day normal saline 5 ml/kg/mouse/day and propylene glycol 5 ml/kg /mouse/day was administered to normal and EAC control groups respectively for 14 days intraperitoneal. Similarly EEPZ at different doses (100 mg and 200 mg/kg/mouse/day) were administered in groups 3, 4, 5 respectively after the administration of last dose followed by 18 hrs. Fasting 4 mice form each group was sacrificed for the study of antitumor activity, hematological and liver biochemical parameters. The remaining animals in each of the groups were kept to check the mean survival lime (MST) and percent increase in life span of the tumor bearing hosts.[12,13,14] Various parameters like Body weight of animals, Life span of animals, Cytological studies on cell lines, Hematological parameter, RBC, WBC, Hemoglobin, differential count, Biochemical parameters evaluated in the present study.

Anticancer effect of EEPZ was assayed by observation of change with respect of body weight, ascitic tumor volume, packed cell volume, viable and non viable tumor cell count, mean survived time (MST) and percentage increase in life span (%ILS) [10].

Tumor Cell Volume and Packed Cell Volume

The mice were dissected to collect ascitic fluid from peritoneal cavity and centrifuged to determine packed cell volume at 1000 rpm for 5 min[11]. The transplantable murrane tumor was carefully collected to measured the tumor volume.

Viable and non viable cell count

Viable and non viable cell counting of the ascetic cell was done by staining with tryphan blue (0.4 % in normal saline), dye exclusion test and count was determined in a neubauer counting chamber. The cells that did not take up the dye were viable and those that took the stain were not viable[10] .

Mean survival time and percent increased in life span

The effect of EEPZ on tumor growth was observed by MST and % ILS. MST of each group continuing 4 mice were monitored by recording the mortality daily for 6 weeks and % ILS was calculated by using following equation[10,11].

MST = (Day of first death + Day of last death)/2

$$\%\ ILS = \frac{MST\ of\ treated\ group}{MST\ of\ control\ group} (-1) \times 100$$

Effect of EEPZ on hematological parameters

Blood was collected from each mice by intracardial puncture with blood anticoagulant (Heparin) and while blood cells (WBC), red blood cells (RBC); hemoglobin and differential count were determined in group comprise of I) Tumor bearing mice (control), II) Tumor bearing mice treated with\EEPZ (100 mg/kg/mice/day), III) Tumor bearing

mice treated with EEPZ (200 mg/kg/mice/day), VI) Normal group[12].

Biochemical Assay

After the collection of blood samples the mice were sacrificed and their liver was excised. The isolated liver was rinsed in ice cold normal saline fallowed by cold phosphate buffer having pH 7.4, and blotted dry and weighed. A 10% w/v homogenate of liver was prepared in ice cold phosphate buffer (pH 7.4) and a portion were utilized for estimation of lipid peroxidation and other portion of the same after precipitation of proteins with TCA was used for estimation of glutathione remaining homogenate were centrifuged at 1500 rpm at 4^{o}C for 15 min. The supernatant thus obtained was used for the estimation of superoxide dismutase, catalase and protein content[13] .

Statistical Analysis

The experimental result were expressed as mean $\pm$ SEM. Data were assessed by the student t-test $P<0.05$ was considered as statistically significant.

RESULTS

Phytochemical screening

It suggests that ethanolic extract of plant contain terpenoid, phytosterols, flavanoid and saponin. (Table 1.)

Toxicity Evaluation (LD_{50})

In acute toxicity study, the given extract of *Plumbago zeylanica* did not show any mortality up to the dose of 2000 mg / kg. The extract shows sedation, hypnosis, mild muscle relaxant property.

Anticancer Activity

Administration of EEPZ reduces the tumour volume, packed cell volume and viable tumour cell count in a dose dependant manner when compared to EAC control mice. In EAC control mice the median survival time was 22±0.25 days. Whereas, it was significant increased median survival time (24±0.33, 29±0.49) with different doses (100 and 200 mg/kg) of EEPZ and standard drug respectively. The mean survival time and effect of EEPZ (100mg/kg, 200mg/kg) at different doses on tumour volume, viable and non viable cell count, are shown in table 2 and 3.

EEPZ at the dose of 100 and 200 mg/kg the haemoglobin content in EAC bearing mice were increased to 10.6±0.057and 11.45±0.057.The haemoglobin contents in the EAC control mice (9.8± 0.02) was significantly decreased as compare to normal mice (12.85± 0.25). (Table-7)The total WBC count was significantly higher in the EAC treated mice when compared with normal mice. Whereas EEPZ treated mice significantly reduced the WBC count as compared to that of control mice. Significant changes observed on differential count when extract treated mice compared with EAC control mice. (Table 4).

Biochemical assay

Biochemical assay indicated that EEPZ significantly reduced the elevated levels of lipid peroxidation and thereby it may act as an antitumor agent. The level of lipid peroxidation, catalase and protein content were summarized in table 8 and graphical representation shown. (Table 5)

DISCUSSION

The plant leaves and stem of *Plumbago zeylanica* Linn. were found to contain

higher amount of Triterpenoids. In present study anticancer potential of the plant was estimated in EAC bearing carcinoma Cell. Ethanolic extract of *Plumbago zeylanica* Linn. had considerably reduced tumour volume and increased the life span of the test animals. It is also found that EEPZ significantly reduced the elevated levels of lipid peroxidation and thereby it may act as an antitumour agent.

CONCLUSION

The ethanolic extract of *Plumbago zeylanica* Linn. possessed significant anticancer and antioxidant activity due to its higher terpenoids and flavonoids content. Further investigation on different biological activities of this plant with different modes will not only validate the types of activities claimed by ayurvedic, siddha and traditional practitioners, but also will bring out innovation in the field of therapeutics.

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Table 1: Result of phytoconstituent identification tests of ethanol extract of *Plumbago Zeylanica* Linn..

Phytoconstituent	Phytosterol	Flavonoids	Triterpenoids	Saponin
Ethanol Extract	+	+	+	+

Table 2: Effect of EEPZ on survival time on EAC bearing mice

Sr. No.	Experimental groups	Mean survival time (MST) days	% increase in life span
1	Normal control (normal saline 5 ml/kg b.w.)	-	-
2	EAC control	22±0.25	-
3	EAC + EEAV (100 mg /kg)	24±0.33	9.09
4	EAC + EEAV (200 mg / kg)	29±0.49	31.81
5	EAC + Vincrystine (0.8 mg / kg) std	31±0.55	40.90

Values are mean ± SEM (Standard error of mean), Number of mice in each group (n=4), $P < 0.001$, Experimental group was compared with EAC control.

Table 3: Effect of EEPZ on tumor volume, packed cell volume, viable and non viable tumor cell count of EAC bearing mice.

Parameters	EAC control	EEAV 100 mg / kg	EEAV 200 mg/kg	Standard vincristine 0.8 mg/kg
Body weight	26.11±0.12	24.34±0.16	23.28±0.13	23.9±0.02
Tumor volume (ml)	5.82±0.042	4.22±0.051	3.42±0.082	2.42±0.13
Packed cell volume (ml)	2.12±0.104	1.75±0.043	1.05±0.092	1.15±0.03
Viable tumor cell count % 10^7 cells /ml	11.25±0.098	7.78±0.18	4.85±0.23	4.90±0.015
Non viable tumor cell count X 10^7 cells / ml	0.5±0.017	0.92±0.023	1.47±0.021	1.23±0.81

Values are mean ± SEM. No. of mice in each group (n = 4), $P < 0.01$, experimental groups was compared with EAC control Weight of normal mice=20±0.15

Table 4: Effect of EEPZ on hematological parameters of EAC treated mice.

Parameter	Normal saline 0.5 ml/kg	EAC control 2 X 10^6 cells / mice	EAC + EEAV 100 mg/kg	EAC + EEAV 200 mg / kg	EAC Cell + Vincristine 0.8 mg/kg
Hemoglobin (gm)	12.85±0.25	9.8±0.02	10.6±0.057	11.45±0.18	11.7±0.045
Total RBC million/mmcu	6.65±0.18	3.8±0.035	4.75±0.032	5.42±0.22	5.8±0.054
Total WBC Million/mmcu	7.8±0.045	20.07±0.068	11.92±0.042	8.85±0.059	9.12±0.055
Lymphocyte	77.75±0.19	33.37±0.56	52.7±0.50	60.72±0.36	59.12±0.30
Monocyte	1.7±0.035	0.82±0.024	1.15±0.014	1.2±0.045	1.32±0.024
Granulocyte	29.97±0.46	52.6±0.37	40.87±0.2	31.72±0.63	41.65±0.29

Values are mean ±SEM, (n =4), EAC control group compared with normal group, Experimental group compared with EAC control. $P < 0.01$, $^*P < 0.05$

Table 5: Effect of different doses of EEPZ on different biochemical parameter in EAC bearing mice.

Parameter	Normal saline 0.5 ml/kg	EAC control 2 X 10^6 cells / mice	EAC + EEAV 100 mg/kg	EAC + EEAV 200 mg / kg
Lipid peroxidation n mole MDA/gm of tissue	0.92±0.02	1.36±0.09	1.27±0.04	1.13±0.02
Catalase (units /mg tissues)	2.51±0.72	1.71±0.15	1.75±0.13	2.34±0.23
Protein content (gm / 100 ml)	12.66±0.69	17.25±0.76	16.50±0.70	16.10±0.55
Superoxide dismutase	4.37±0.41	3.20±0.71	2.30±0.48	2.65±0.02

Values are mean ±SEM, (n =4). EAC control group compared with normal group, Experimental group compared with EAC control. $P < 0.05$, *$P< 0.05$

Fig 1. *Plumbago zeylanica* Linn.