Research Article

EVALUATION OF ANTI-CANCER ACTIVITY OF THE EXTRACT OF *ALSTONIA VENENATA* (POISON DEVIL TREE) LEAVES

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ABSTRACT

The anticancer activity of the ethanolic extract of *Alstonia venenata* (Poison devil tree) leaves was evaluated in this study. In vitro cytotoxicity analysis of the leaf extract on DLA cells, EAC cells and the Normal splenocytes were studied. Tumour bearing animals treated with lower dose of 100 mg/kg, 250 mg/kgand higher dose of 500 mg/kg individually, shown a significant increase in survival. The average life spans of animals treated with plant leaf Extract was observed for 100mg dose 16.37 ± 4.60 days, 250 mg dose 20.62 ± 5.42& 500 mg dose 23.12 ± 5.56 days. Finally, the change in body weights of the animals suggests the tumor growth inhibiting property. The percentage increase in life span of the treated animals was found to be 30.79% for high dose (500 mg) when compared to untreated control.

Keywords: Anti-cancer, *Alstonia venenata*, cyclophosphamide, DLA cells, EAC Cells and splenocytes.

INTRODUCTION

Cancer is a group of disease due to genomic instability characterized by the uncontrolled growth and spread of abnormal cells coupled with malignant behavior, invasion, and metastasis. Now a days cancer is the most serious clinical problem worldwide and it affects both developing countries as well as developed countries. It is the second largest leading cause of death in developing countries. Majority of cancers are caused by physical in activity, aging population, environmental pollution, infections, poor nutrition, obesity, hormones and hereditary factors and also due to cancer predisposing life styles such as alcohol consumption, smoking etc.

According to 2013-2014 reports in India, more than 1300 people died every day, due to cancer, where the rate is 6% greater than the previous year. About 11 million people are diagnosed with cancer every year, out of which more than 7 million people are dying mainly due to the systemic metastatic nature of disease. Even, the rate of cancer incidence all around the world is faster than the increasing global population growth. It is predicted that, globally, there will be 20 million new cases and 12 million cancer deaths by 2020[1].

Drugs of natural origins are developed to overcome those draw backs caused by synthetic anti-cancer drugs and to make course of treatment more convenient because herbal drugs are not known for severe side effects. Natural products have afforded a rich source of compounds that have found many applications in the fields of medicine, pharmacy and biology. Within the sphere of cancer, a number of important new commercialized drugs have been obtained from natural sources, by structural modification of natural compounds, or by the synthesis of new compounds, designed following a natural compound as model. The search for improved cytotoxic agents continues to be an important line in the discovery of modern anticancer drugs.[2]

The plant known as *Alstonia scholaris* belongs to the same family and genus of *Alstonia venenata* reported to possess anti-cancer activity. Moreover, the presence of glycoside moieties like saponins, anthraquinones, terpenoids, flavonoids and alkaloids could inhibit tumour growth[3]. Both the plants *Alstonia scholaris* and *Alstonia venenata* are known to contain similar bioactive principles responsible for anti-tumour activities. Hence the present study has been designed to evaluate the anticancer activity of ethanolic extract of the leaves of the plant *Alstonia venenata* (Poison Devil Tree).

MATERIALS AND METHODS

Plant

The leaves of Alstonia Venenata was collected from the local region of Thrissur District in

Kerala and authenticated by Dr. N. Sasidharan, Taxonomist, KFRI, Peechi, Thrissur, Kerala, India. The collected leaves materials were cleaned to remove the adhered dust particles and were then dried in shade at room temperature (28±20C). The dried plant materials were coarsely powdered, weighed and stored in an air tight container till use.

Preparation of crude extract[4]

Leaves of *Alstonia Venenata* (Poison Devil Tree) were collected, dried and powdered. Leaf powder (30g) was exhaustively extracted in 300 ml Ethanol solvent for a day at room temperature (28±2^{0}C). Extraction of leaves residue was further repeated with Ethanol (300 ml each time) up to the filtrate become colourless. The filtrate from every extraction is pooled in to a beaker and the excess was evaporated under reduced pressure at 40^{0}C using a rotary evaporator to give concentrated crude ethanolic extracts, dried in oven at 50^{0}C. The weights of all the extracts was measured after solvent evaporation to get percentage yield and then kept into a glass container prior to use.

Phytochemical screening

The ethanolic crude leaves extract of the plant was subjected to qualitative chemical screening for the identification of the tannins, alkaloids, flavonoids, saponin and glycosides using standard procedures.

Animal

Healthy Swiss albino mice (25-30g) of female sex were used for the experiments. They were maintained under standard conditions (temperature 22 ± 2^{o}C, relative humidity 60±5% and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. Experiments were conducted between 10:00 to 15:00h.

IN VITRO ANTICANCER ACTIVITY

Method: Trypan blue dye exclusion method[5]

Cytotoxicity of the *Alstonia Venenata* leaves extract towards cancer cells were assessed using Trypan blue exclusion method. Approximately 100 µl cell suspension from a stock of 1x10^7 DLA cells or EAC cells or spleen cells (normal cells) were diluted to 0.9 ml (By adding 0.8ml PBS,0.2M, pH 7.4) containing various concentrations of extracts in different test tubes. The tubes were then incubated at 37^{0}C for 3 hours. At the end of the experiments, 100µl trypan blue was added to each tube and waited for 3minute. The cell suspension (10µl) were applied on to a haemocytometer and observed under microscope. Live cells (non stained cells) and dead cells (blue stained cells) were separately counted and percentage cell death was determined. The percent cytotoxicity was calculated after comparing with the untreated control. The percentage values were plotted on a graph against concentrations and concentration needed to induce 50% cell death (IC_{50}) was determined.

Calculation

$$\%\text{Cell death} = \frac{\text{number of dead cells}}{\text{total number of cells}} \times 100$$

IN VIVO ANTICANCER ACTIVITY

Ehrlich Ascites Carcinoma (EAC) Induced Tumor Model[6]

Development of EAC

Ehrlich ascites carcinoma (EAC) cells collected from donor mice (Swiss albino) and suspended in sterile isotonic saline. A fixed number of viable cells (usually 2×10^6 cells/20 g body weight) were implanted into the peritoneal cavity of each recipient mouse[7]. The tumor cells multiplied relatively freely within the peritoneal cavity.

The ascetic tumor bearing mice (donor) was taken 12 days after tumor transplantation. The ascetic fluid was withdrawn using an 18 gauge needle into a sterile syringe. A small amount of tumor fluid was tested for microbial contamination. Tumor viability was determined by trypan blue exclusion test and cells counted using haemocytometer. The ascetic fluid was suitably diluted with saline to get a concentration of 10 million cells/ml of tumor cell suspension. 250 µl of this fluid was injected in each mouse by intra peritoneal route to obtain ascetic tumor. The drug treatment was started 24 hr after the tumor inoculation daily for 10 days[8].

Cyclophosphamide was given orally up to 10 days as standard. Tumor response was assessed on the basis of mean survival time (MST) and % increase in life span (% ILS).

Statistical Analysis

The data was expressed as Mean ± SEM and were analyzed by the one-way ANOVA.

RESULTS

In vitro cytotoxicity analysis of leaf extracts on dla, eac and noramal spleen cells

The results of *In vitro* cytotoxicity analysis of the leaf extracts of *Alstonia venenata* on DLA cells, EAC cells and the Normal splenocytes are depicted in table no:1.

A. Effect of leaf extracts on dla and eac cell lines

Table 1 shows the cytotoxic effect of plant extract on neoplastic cells in a short term assay. The toxicity observed was minimal however increased depending on dose in both these cell lines. IC50 value is expected above 100 µg/ml concentration.

B. Effect of crude leaf extracts on normal spleen cells

Fig.3 shows the cytoxicity of *Alstonia venenata* leaf extracts on primary splenocyte of rats. The cytotoxicity of the leaf extract was minimal in this study also. (Fig.1, Table 3) and the IC_{50} found to be more than 100 µg/ml.

Effect on ehrlich's ascites carcinoma (eac) induced tumor in mice

Effect on mean survival time, % increase in life span and average increase in body weight

The effect of crude leaf extract on the mean survival period was monitored. The untreated EAC cells induced tumour bearing animals had an average life span of 16 ± 4.4 Oral administration of extracts at the lower concentration of 100mg/kg body weight could not improve the life span however the higher dose 500 mg/kg body weight treated animals had an increase in life span of 23.12 ± 5.56 days accounting a 30% increase. Cyclophosphamide single dose used as standard drug at its therapeutic dose (15 mg/ml) increased the life span by 36% showing an average if span of 25.12 ± 4.2 days. Finally, the change in body weights of the animals suggests the tumour growth had been reduced in treated animals. The results are summarized in Table 2 and graphically depicted in Fig.4.

DISSCUSSION

The objective of the present study was to evaluate the anti-cancer property of the plant Alstonia venenata (Poison devil tree) leaves. In this study, the extract of *A. venenata* leaves showed considerable cytotoxicity towards neoplastic cells (DLA cells and EAC cells). However towards the primary spleen cells, cytotoxicity was minimal, The Phytochemical evaluation of ethanolic extract of A. venenata leaves showed positive result for various bioactive secondary metabolites such as saponins, alkaloids, flavonoids, terpenoids, tannins, carbohydrate, glycosides, coumarin, proteins, anthraquinones, Quinones and phenols. Terpenoids and alkaloid class of compounds are good anti-cancer agents. It is expected that these compounds may be accountable for its cytotoxic property. Moreover the extracts exposed good radical scavenging efficacy.

On DLA cells the leaf extracts showed an increase in cytotoxic effect with increase in dose. Maximum cytotoxicity and IC50 value of the leaf extract observed above 100 µg/ml concentration. Similarly in the EAC cell population, the leaf extracts showed minimal cytotoxicity and the cell death increases with increase in dose. The leaf extract had an IC50 more than 100 µg/ml on EAC cells. The cytoxicity of leafextracts on primary splenocytes of rats also showed the minimal cytotoxic activity in this study also. The IC50 value found to be more than 100µg/ml. Tumour bearing animals treated with lower dose of 100 mg/kg, 250 mg/kg and higher dose of 500 mg/kg individually, shown a significant increase in survival period and Cyclophosphamide single dose used as standard at its therapeutic dose (15mg/ml). The average life spans of animals treated with plant leaf Extract was observed for 100mg dose 16.37 ± 4.60days, 250 mg dose 20.62 ± 5.42& 500 mg dose 23.12 ± 5.56 days. Finally, the change in body weights of the animals suggests the tumor growth inhibiting property. The percentage increase in life span of the treated animals was found to be 30.79% for high dose (500 mg) when compared to untreated control.

CONCLUSION

The present study concluded that the ethanolic extract of *Alstonia venenata* (Poison devil tree) has significant potential anti-cancer activity. All these results clearly indicate that the crude leaf extract has remarkable capacity to inhibit the growth of ascites tumour induced by EAC cell line in the experimental animals. Perhaps the presence of cytotoxic and anti-oxidant components of the leaf extracts might be imparting the anti-cancer efficacy.

The exact mechanism for the anti-cancer activity of the *A. venenata* leaves is still unknown. Further studies are needed to isolate the bioactive principles responsible for anti-cancer activity and to determine the exact mechanism of action.

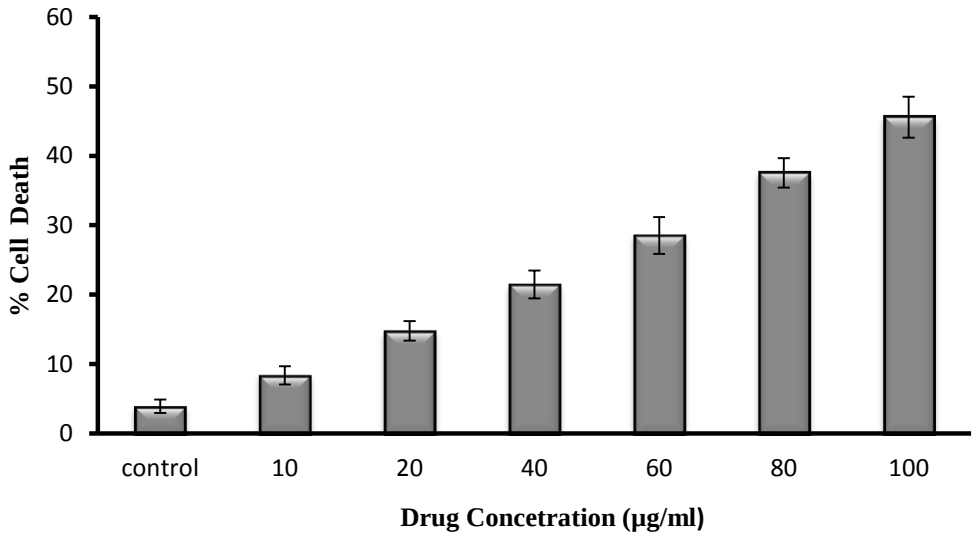
ACKNOWLEDGEMENT

Authors are sincerely thankful to Dr. KARUNAKAR HEGDE Professor and Head. Department of pharmacology, Srinivas College of pharmacy, Karnataka for their contribution and facilities provided regarding our work.

Table 1: IC_{50} values of *Alstonia venenata* leaf extracts

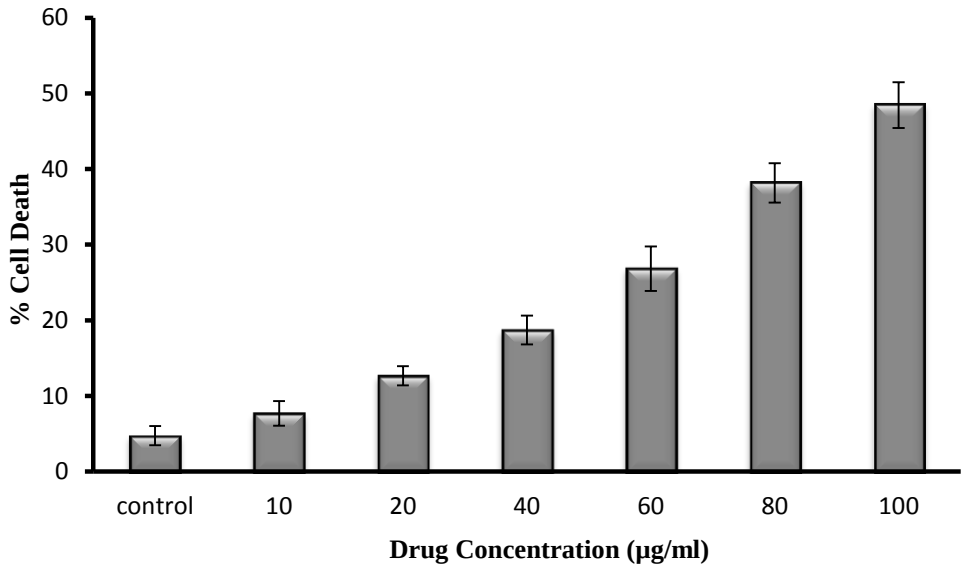
S. No.	Test compound	IC_{50}values (µg/ml)		
		DLA	EAC	Spleen
1	**Extract**	Above 100	Above 100	Above 100

(All data expressed as mean ±SD of three individual experiments)



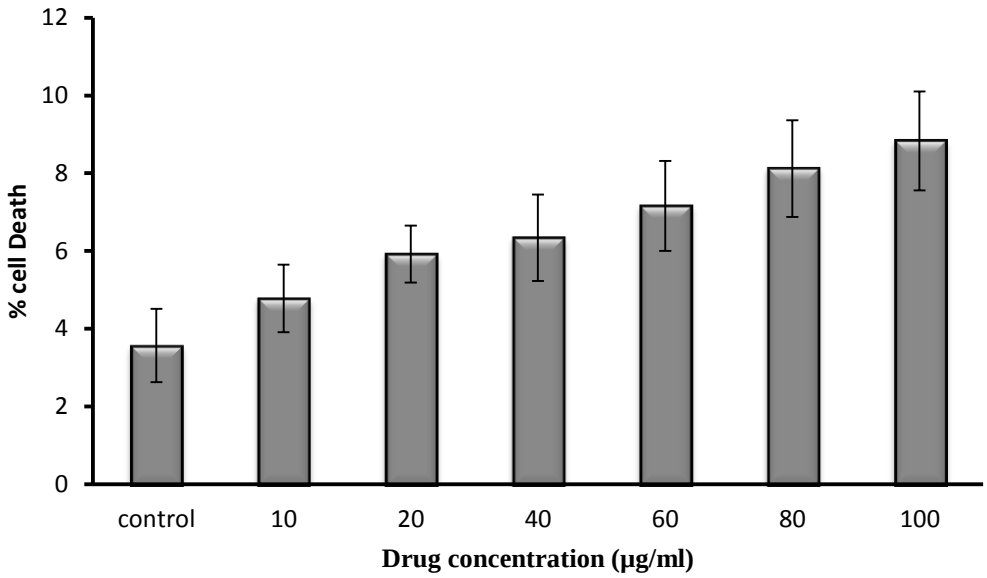
(Values are ±SD of at least three consecutive experiments)

Fig. 1: Cytotoxic study on DLA cells



(Values are ±SD of at least three consecutive experiments)

Fig. 2: Cytotoxic study on EAC cells



(Values are ±SD of at least three consecutive experiments)

Fig. 3: Cytotoxic study on Normal spleen cells

Table 2: Effect of A. venenata leaf extracts on mean survival time, % increase in life span of EAC tumour bearing mice

Treatment	MST (days)	Percentage Increase in Life span
Tumor control(EAC cells)	16 ± 4.40	……
100mg/kg dose	16.37 ± 4.60	2.26
250 mg/kg dose	20.62 ± 5.42	22.40
500 mg/kg dose	23.12 ± 5.56	30.79
Std (Cyclophosphamide)	25.12 ± 4.232	36.30

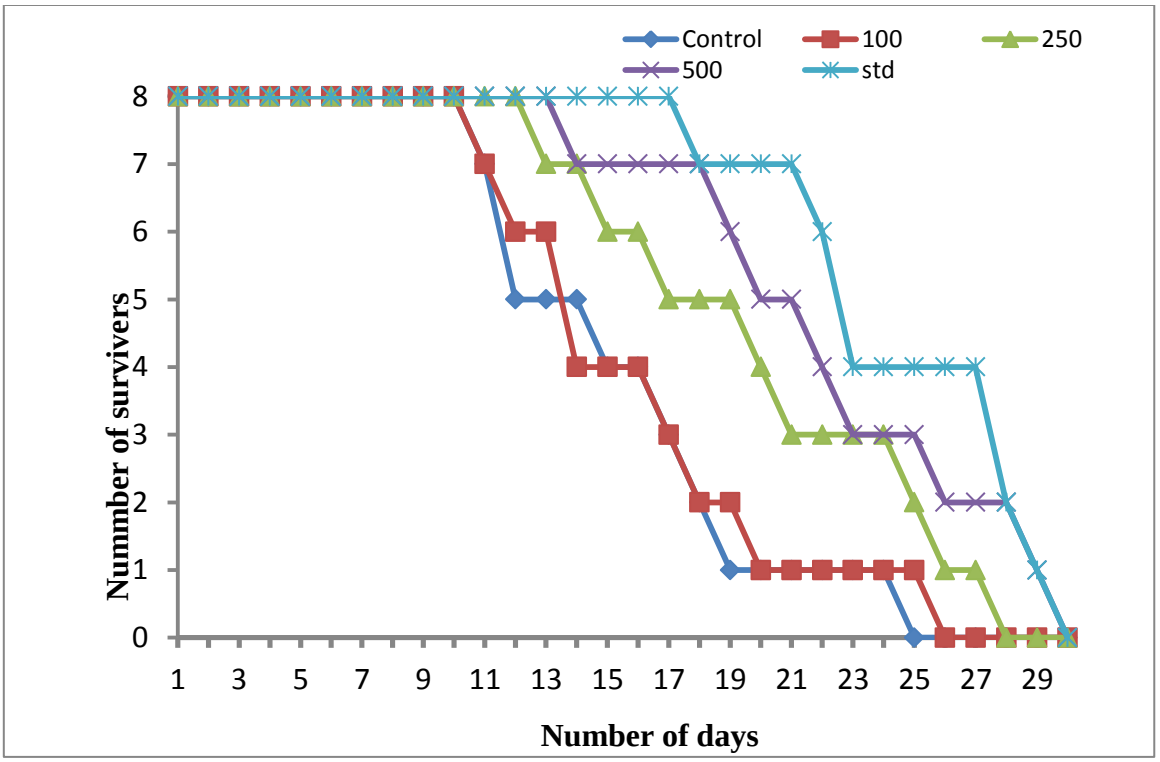


Fig. 4: Effect on mean survival time, %increase in life span

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