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Anti-cancer activity of Ethanol extract of Stem bark of *Nyctanthes arbour tristis linn.*

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ABSTRACT

The aim of the present study is to evaluate the effect of methanolic extract of stem bark of *Nyctanthes arbortristis linn* (EENA) against Dalton's Ascitic Lymphoma (DAL) in Swiss Albino mice. DAL cells were injected intraperitonially (10^6 cells) to the mice. Two days after cells injection the animals were treated with 250 and 500 mg/kg of MEEV for 8 days. 5-fluorouracil (20 mg/kg) was used as reference drug. On day 11, cancer cell number, packed cell volume, decrease in tumour weight of the mice, increase in life span and haematological parameters were evaluated and compared with the same parameters in control. A significant increase in the life span and a decrease in the cancer cell number and tumour weight were noted in the tumour-induced mice after treatment with EENA. The haematological parameters were also normalized by EENA in tumour-induced mice. These observations are suggestive of the protective effect of EENA against Dalton's Ascitic Lymphoma (DAL).

Keywords: *Nyctanthes arbortristis linn, Dalton's Ascitic Lymphoma, Anticancer activity, 5-fluorouracil, Swiss Albino mice.*

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INTRODUCTION

Cancer is the second leading cause of death in the world [1]. Throughout history and across the world, the plant kingdom has provided a variety of medicines for cancer treatment. In modern times, plants have been a source of analgesics, anti-inflammatory, anti-asthmatics, anti-arrhythmic agents, anti-hypertensive, antimicrobial agents known to be numerous. Currently over 60% of the drugs are derived in one or other way from natural source including plant, marine organism and micro-organism [2]. There are worldwide efforts to discover anticancer agents from plants.

Nyctanthes arbour tristis L. [Oleaceae], a common wild shrub flourishing in the Sub-Himalayan tract in the States of Uttar Pradesh, Assam, Bengal, Madhya Pradesh and in the South upto Godavari [3, 4] has long been used in the Ayurvedic system of medicine for the cure of snake bite, bites of wild animals, cachexyia, cancer, sores, ulcers, dysentery menorrhagia [5] and obstinate sciatica [6]. The juice of the leaves is used in chronic and bilious fevers, rheumatism, as a laxative, diaphoretic and diuretic. The plant has been reported to be effective against leishmanial [7], viral [8] and amoebic [9] infections. The present study is to evaluate the anticancer activity in the root bark extract of the plant *Nyctanthes arbour tristis linn*.

MATERIALS AND METHODS

Plant Material

After proper identification of the Taxonomists in the Botanical Survey of India, Coimbatore, Tamil Nadu, the stem bark of the plant *Nyctanthes arbour tristis linn*. was collected from the surrounding areas of Namakkal, Tamil Nadu, India. The stem bark was dried in shade at room temperature and coarsely powdered using mechanical grinder. The powdered drug was then extracted successively with petroleum ether and ethanol for 24 hours. The extract was concentrated under reduced pressure. The dried extracts were stored under air tight containers.

Animals

The study was carried out after obtaining permission from Institutional Animal Ethics Committee (No. 1158/ac/07/CPCSEA) and CPCSEA regulations were adhered to during the study. Male Swiss Albino mice (20- 30 g) were selected for this study [10]. The animals were maintained under standard environmental conditions and fed with standard pellet feed and water *ad libitum*.

Determination of anti-tumour activity

The animals were acclimatized to our laboratory conditions. They were divided into five groups viz. Cancer induced animal (G1), Cancer induced animal treated with 20mg/kg of 5-Fluorouracil treated group (G2), 250mg/kg of root bark extract of *Nyctanthes arbour tristis linn* (G3) and 500mg/kg of root bark extract of *Nyctanthes arbour tristis linn* (G4), Normal control (G5) of six each and used for the study [12]. The DAL cells were procured from

Amala Cancer Institute, Thrissur, Kerala and injected intraperitonially (10^6 cells/mice) to all groups of animals. On the second day the animals of G3 and G4 were treated with 250 and 500mg/kg of MEEV while G2 with 5- fluorouracil (20 mg/kg) and the treatment was continued for next 10 days. G1 was not allocated any treatment after inoculation with DAL cells. The mice were observed for next 10 days for the development of ascitic tumour. On day 11, the following parameters were estimated. 1-Cancer cell number, 2-Packed cell volume (PCV), 3-Decrease in tumour weight of the mice, 4-Increase in life span (ILS).

Determination of Haematological Parameters

Apart from the above mentioned parameters, the effects of EENA on haematological parameters were also studied in the mice of all the groups. Blood was collected from all groups of animals by retro-orbital puncture and counted for RBC and WBC. For comparison a normal control group (G5) was used which was neither inoculated with cancer cells nor treated *Statistical analysis*. The results are expressed as mean ± S.E.M. The evaluation of the data was done using one way ANOVA followed by Newman-Keul's multiple comparison test; $p< 0.05$ implied significance.

RESULTS

The extract at the dose of 500mg/kg reduced the cancer cell number to 0.72 ± 0.16 x 10^6 cells in the treated mice (Table 1). Following inoculation with DAL cells, there was profound proliferation of tumour cells in the peritoneal cavity of the mice. As a result the PCV in the tumour control mice was found to be high (52.22%). Administration of the extract had reduced the PCV to 34.72%. Also a decrease in tumour weight was noted in the EENA treated mice (Table 1). The percentage increase in lifespan (ILS) of the EENA treated mice increased by 32.14% (Table 1). Regarding the effect of EENA on the haematological parameters, it was found that the tumour bearing mice showed reduced number of RBC but an increase in WBC compared to normal control mice. Following treatment with EENA, RBC count was elevated to 4.02 ± 0.35 x $10^5 \mu l^{-1}$ whereas WBC count was reduced to 5.25 ± 0.64 x 10^3 μl^{-1} (Table 2).

DISCUSSION

Cancer is a group of more than 100 different diseases characterized by uncontrolled cellular growth, local tissue invasion and distant metastases [13] and the free radicals have been implicated in carcinogenesis [14]. Supportive to this, many plant extracts containing antioxidant principles have been reported to possess anti tumour activity. Based on this, it was contemplated to carry out this study. In the present study, intraperitoneal inoculation of DAL cells in the mice produced an enormous increase in the cancer cell count, which indicated that there is progression of cancer in the animals. The reliable criterion for judging the value of any anticancer drug is the prolongation of life span of the animal and disappearance of leukemic cells from blood [15]. The acquired results illustrate the anti tumour effect of EENA against DAL in Swiss albino mice. A significant enhancement of MST and peritoneal cells counts were observed (Tables 1 and 2).

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Analysis of the haematological parameters showed a minimum toxic effect in mice which was considered as cured [16] by EENA treatment. Eleven days after transplantation, EENA treated group was able to reverse the changes in the haematological parameters consequent to tumour inoculation.

Table-1 Effect of EENA on DAL induced mice.

Groups	Cancer cell Number (x 10^6)	Packed Cell Volume (%)	Increase in Tumour weigh	Number of Days survived	Increase in Life Span
G1	1.52 ± 0.12	42.22 ±0.14	9.56 ± 0.48	19 ± 0.2	-
G2	0.54 ± 0.24*	24.31 ± 0.18**	4.16 ± 0.40**	27 ± 3.2*	39.12%
G3	0.80 ± 0.13*	34.25 ± 0.34*	6.73±0.43*	23 ± 1.2*	25.32%
G4	0.62 ± 0.16*	29.70 ± 0.58**	5.35 ± 0.54**	25 ± 2.2*	32.14%

Values are represented as mean ± SEM of six animals.
One-way ANOVA followed by Newman-Keul's multiple comparison test
* $p < 0.05$, ** $p<0.001$ compared to G1.
G1 – Control (DAL induced, non-treated)
G2 – 20 mg/kg of 5-flourouracil treated group
G3 – 250 mg/kg of EENA treated group
G4 – 500 mg/kg of EENA treated group

Table-2. Effect of MEEV on haematological parameters

Groups	Total WBC (x 10^3) μl^{-1}	Total RBC (x 10^5) μl^{-1}
G1	7.35 ± 0.10	2.64 ± 0.40
G2	5.32 ± 0.58**	4.54 ± 0.64*
G3	6.06 ± 0.52**	3.42 ± 0.42*
G4	5.25 ± 0.43**	4.02 ± 0.35*
G5	4.34 ± 0.64**	5.32 ± 0.42**

Values are represented as mean ± SEM of six animals.
One-way ANOVA followed by Newman-Keul's multiple comparison test.
*$p<0.05$, ** $p<0.01$ compared to G1.
G1 – Control (DAL induced, non-treated)
G2 – 20 mg/kg of 5-flourouracil treated group
G3 – 250 mg/kg of EENA treated group
G4 – 500 mg/kg of EENA treated group
G5 – Normal control

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