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Research Article

Anti-Cancer Activity of Methanol Extract of Root Bark of *Erythrina variegata linn.*

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ABSTRACT

The aim of the present study is to evaluate the effect of methanolic extract of root bark of *Erythrina variegata* (MEEV) 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 MEEV. The haematological parameters were also normalized by MEEV in tumour-induced mice. These observations are suggestive of the protective effect of MEEV against Dalton's Ascitic Lymphoma (DAL).

Keywords: *Erythrina variegata, Dalton's Ascitic Lymphoma, Anticancer activity, 5-fluorouracil, Swiss Albino mice.*

INTRODUCTION

The genus *Erythrina* (Leguminoceae) is distributed in the tropical and subtropical regions of the world and encompasses over 100 species. The antibacterial and anti inflammatory properties of *Erythrina variegata linn* are utilized in Chinese herbal medicine for the treatment of pyrexia, scabies and septicaemia. *Erythrina variegata* is a tall ornamental tree distributed throughout upper Gangatic plains of India and Nepal. The bark of the plant is astringent, febrifuge, anti-bilious and anthelmintic. It is also useful in opthalmia and skin diseases. The leaves are used in fever, inflammation and joint pain. The juice of the leaves is used to relieve ear ache and toothache [1]. The roots are used as bronchitis, febrifuge and as an insecticide. The roots are also used in the treatment of cancer, convulsions and used to treat pimples [2]. It has the reputation to stimulate lactation and menstruation and is used as laxative, diuretic and expectorant [3]. Although many compounds have been reported from the genus, *Erythrina*, previous phytochemical investigations with *E. variegata* revealed the occurrences of orientanol B, erycristagallin, cristacarpin, sigmoidin K, 2-(γ,γ-dimethylallyl)- 6a-hydroxyphaseollidin, erystagallin A [4], eryvarins A and B [5], bidwillon B [6], eryvarins F and G [7], alpinum isoflavone, isococculinine, decarbomethoxyerymelanthine, erysodienone, erythritol, erysodine [8], erysovine, stachydrine, sterols, fixed oils and fatty acids [9]. The present study is to evaluate the anticancer activity in the root bark extract of the plant *Erythrina variegata linn*.

MATERIALS AND METHODS

Plant Material:

After proper identification of the Taxonomists in the Botanical Survey of India, Coimbatore, Tamil Nadu, the root bark of the plant *Erythrina variegata linn* was collected from the surrounding areas of Trichy, Tamil Nadu, India. The root 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 methanol 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 (20-30g) were selected for this study [10]. The animals were maintained under standard environmental conditions and fed with standard pellet feed and water *ad libitum*.

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Table-1 Effect of MEEV on DAL induced mice.

Groups	Cancer cell Number (x 10^6)	Packed Cell Volume	Increase in Tumour weight	Number of Days survived	Increase in Life Span
G1	1.62 ± 0.12	56.32 ±0.14	10.86 ± 0.48	20 ± 0.2	-
G2	0.64 ± 0.24*	27.36 ± 0.18**	3.86 ± 0.40**	29 ± 3.2*	39.12%
G3	0.90 ± 0.13*	40.35 ± 0.34*	8.03±0.43*	25 ± 1.2*	25.32%
G4	0.72 ± 0.16*	35.80 ± 0.58**	5.64 ± 0.54**	27 ± 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 MEEV treated group
G4 – 500 mg/kg of MEEV treated group

Acute toxicity studies:

Acute toxicity study was carried out on root bark extract of *Erythrina variegata linn (*MEEV) following OECD guidelines [11]. The extract was found to be safe up to 2000 mg/kg of body weight

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 *Erythrina variegata*(G3) and 500mg/kg of root bark extract of *Erythrina variegata*(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 intraperitoneally (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)

Table-2. Effect of MEEV on haematological parameters

Groups	Total WBC (x 10^3) μl^{-1}	Total RBC (x 10^5) μl^{-1}
G1	7.65 ± 0.10	2.84 ± 0.40
G2	5.12 ± 0.58**	4.74 ± 0.64*
G3	6.16 ± 0.52**	3.52 ± 0.42*
G4	5.45 ± 0.43**	4.12 ± 0.35*
G5	4.64 ± 0.64**	5.12 ± 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 LELR treated group
G4 – 500 mg/kg of LELR treated group
G5 – Normal control

Determination of Haematological Parameters

Apart from the above mentioned parameters, the effects of MEEV 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 (56.32%). Intraperitoneal administration of the extract had reduced the PCV to 35.80%. Also a decrease in tumour weight was noted in the MEEV treated mice (Table 1). The percentage increase in lifespan (ILS) of the MEEV treated mice increased by 32.14% (Table 1). Regarding the effect of MEEV 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 MEEV, RBC count was elevated to 4.12 ± 0.35 x $10^5 \mu l^{-1}$ whereas WBC count was reduced to 5.45 ± 0.43 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 MEEV against DAL in Swiss albino mice. A significant enhancement of MST and peritoneal cells counts were observed (Tables 1 and 2).

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

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