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Research Article

In-vitro and *In-vivo* Antitumor Activity of *Catharanthus roseus*

Shabi Ruskin. R*, Aruna. S.R[1]
*Department of Biotechnology, Noorul Islam College of Arts and Science, Kumaracoil, TN, India, 629 180
[1]Department of Biotechnology, Noorul Islam College of Arts and Science, Kumaracoil, TN, India, 629 180

*Corresponding Author: R. Shabi Ruskin; Email: rshabiruskin@yahoo.com

Abstract: In the present study antitumor activity of *Catharanthus roseus* was evaluated using both *in vitro* and *in vivo* methods. *In vitro* antitumor activity of different extracts (methanol, ethanol, chloroform, ethyl acetate and acetone) of *Catharanthus roseus* was evaluated by the MTT assay method using MCF (breast cancer) cell lines. Then the Ethanolic Extract of *Catharanthus roseus* (EECR) is subjected to *in vivo* antitumor activity using Ehrlich ascites carcinoma (EAC) tumor model. In *in vitro* study ethanolic extract exhibited significant anti tumor activity. In *in vivo* study the potency of the extract was compared with standard drugs. Oral administration of EECR at the dose 50 and 100 mg/kg significantly increase the life span and decrease the tumour volume and cancer cell count of the tumor bearing mice. The result indicates that EECR possess significant anti tumor activity in both *in vitro* and *in vivo* studies.

Key words: *Catharanthus roseus*, Ehrlich ascites carcinoma, MTT assay, MCF cell line.

INTRODUCTION

Cancer is one of the most life-threatening diseases and serious public health problems in both developed and developing countries. It is a group of diseases characterized by the disregulate proliferation of abnormal cells that invade and disrupt surrounding tissues [1]. Medicinal plants are playing an important role as a source of effective anticancer agents and it is significant that 60% of currently used anticancer agents are derived from natural sources including plants[2]. Many plant-derived products have been reported to exhibit potent antitumor activity against several rodent and human cancer cell lines [3].One of the best approaches in the search of anticancer agents from plant sources is the selection of plants based on ethnomedical leads [4]. Thus, cancer patients who already got crippled with this disease, who are further burdened by drug-induced toxic side effects, have now turned to seek help from the complementary and alternative medicine hoping for a better cure [5].

The tropical plant Madagaskar Periwinkle (*Catharanthus roseus*) (L.) is an important medicinal plant of family Apocynaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities [6]. The leaves and stems are the sources of dimeric alkaloids, vinacristine and vinblastine that are indispensable cancer drugs, while roots have antihypertensive, ajmalicine and serpentine [7]. The extracts of Vinca have demonstrated significant anticancer activity against numerous cell types [8].

Pharmacological studies have revealed that *Catharanthus roseus* contains more than 70 different types of alkaloids and chemotherapeutic agents that are effective in treating various types of cancers – breast cancer, lung cancer, uterine cancer, melanomas, and Hodgkin's and non-Hodgkin's lymphoma [9].

MATERIALS AND METHODS

Plant material and Extraction

The aerial parts of *Catharanthus roseus* were collected from Neyyatinkara, Thiruvananthapuram district. The plant material was shade dried, powdered and extracted sequentially with methanol, ethanol, chloroform, ethyl acetate and acetone using soxhlet apparatus.

Tumor cell lines

MCF cell lines were obtained from NCCS Pune was maintained in 10% heat inactivated FBS in carbon dioxide incubator. The cells were trypsinised using 0.025% trypsin (cell culture grade HIMEDIA) upon reaching confluency. Ehrlich ascites carcinoma used in the present investigation is obtained from Chittaranjan National Cancer Institute (CNCI), Calcutta. This cell line is maintained by weekly intra-peritoneal injection of tumor cell suspension of 10^6 cells/mouse.

Experimental Animals

Healthy Swiss albino mice of either sex of 6-8 weeks age and weighing between 20-25 gm were used throughout the study. The animals were housed in plastic cages with saw dust bedding which was changed twice a week and maintained in standard lab conditions at 25±2ºC with a relative humidity of 55-65% under a 12 hr light/dark cycle. The animals were fed with standard pellet diet and tap water *ad libitum*. The study was approved by the Institutional Animal Ethical Committee, CPCSEA/CRC/IAEC/RPP-30.

Anti tumor activity

In vitro anti tumor activity of *Catharanthus roseus* on MCF cell line

MTT cell viability assay

The cell culture suspension was washed with 1 X PBS (Phosphate Buffered Saline) and then added 200 µl MTT [3-(4, 5-Dimethyl thiazole-2yl)-2, 5-diphyhyl tetra-zolium Bromide] solution to the culture flask (MTT 5 mg/volume dissolved in PBS). Filtered through a 0.2 µm filter before use. Then incubated at 37°C for 3 hours, removed all MTT solution, washed with 1 X PBS and added 300 µl DMSO to each culture flask and incubated at room temperature for 30 minutes until all cells get lysed and homogenous color was obtained. The solution was then transferred to centrifuge tube and centrifuged at top speed for 2 minutes to precipitate cell debris. Debris was dissolved using DMSO. OD was measured at 540 nm using DMSO blank. Then the percentage viability was calculated.

Calculation

$$\% \text{ of viability} = \frac{\text{Mean absorbance of sample}}{\text{Mean absorbance of control}} \times 100$$

***In vivo* anti tumor activity of EECR on Ehrlich Ascites Carcinoma**

Group I containing 6 animals, served as a normal control, for which inoculation cell was not done. The remaining animals were inoculated with Ehrlich ascites induced carcinoma (1×10^6 cells/mouse) and divided into 9 groups containing 6 animals in each group. Group II served as tumour control and Group III served as positive control, was treated with 5-FU (20 mg/kg) body weight. Group IV was treated with Tetra hydro amento flavone (50 mg/kg), Group V was treated With Cirsimaritin (50 mg/kg). Group VI was treated with Rhinacanthone (20 mg/kg). Group VII was treated with Pterostilbene (50 mg/kg). Group VIII was treated with EECR at a dose (50 mg/kg). Group IX was treated with EECR at a dose (100 mg/kg) body weight. All the treatments were given orally after 24 hrs of inoculation and continued once daily for 14 days. After the last dose, all mice from each group were sacrificed and blood was collected by retro-orbital plexus method and was centrifuged and serum was used for the estimation of biochemical parameters like cholesterol, triglycerides (TGL), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alanine phosphatase (ALP). The ascitic fluid was collected from the peritoneal cavity and measured for tumour volume and cancer cell count.

Evaluation of clinical parameter

The ascitic fluid (0.1) from the peritoneal cavity of each mouse was withdrawn from tumor bearing mouse at the log phase (day 7-8 of tumor bearing) of the tumor cells by sterile syringe. The freshly drawn fluid was diluted with (0.9%) of ice cold normal saline or sterile phosphate buffer solution (PBS) and the tumor cell number were adjusted to 2×10^7 tumor cells/ml by using haemocytometer. The viability of cells was checked by tryphan blue dye (0.4%) exclusion assay. The cells were stained with by tryphan blue (0.4% in normal saline) dye. The cells that did not take the stain were nonviable. The viable and nonviable cells were counted using haemocytometer.

$$\text{Cell count} = \frac{\text{No. of cells} \times \text{Dilution}}{\text{Area} \times \text{Thickness of liquid film}}$$

Derived Parameters

A) Body weight

All the mice were weighed, from the day 1 to day 15 of the study. Average body weight on the 15th day was determined.

B) Percentage increase in life span (ILS)

Survival time of treated group was compared with those of control using the following calculation.

Increase in life span = T-C/C×100.

Where,

T = Number of days the treated animal survived

C = Number of days the control animal survived

Statistical Analysis

Results are expressed as ±SEM and the test of significance of the results were evaluated by Students T test. Significance of the data was evaluated by ONE WAY ANOVA followed by Dennett's test as a positive test of Significance. For all groups except vehicle control $P < 0.05$

RESULT AND DISCUSSION

***In vitro* antitumor activity of *Catharanthus roseus* against MCF cell line**

The *in vitro* antitumor activity of *Catharanthus roseus* extracts (acetone, chloroform, ethanol, ethyl acetate and methanol) was evaluated on MCF (breast cancer) cell lines by using MTT assay method. MTT assay is the reliable assay to assess the *in vitro* cytotoxicity of the anticancer compounds [10]. The result indicates that the ethanolic extract has lowest percentage (11.01%) of viability and shows significant antitumor activity. All the other solvent extracts show cytotoxic effect. The MTT cell viability assay was shown in **Table 1**

***In vivo* antitumor activity of EECR against Erlich ascitic carcinoma**

The *in vivo* antitumor activity of EECR was carried out by using Ehrlich ascites carcinoma. In this study an increase in life span, decrease in tumour volume and cancer cell count was observed with groups treated with EECR (Group VIII and Group IX) when compared to tumour control animals (Group II). Transplantable tumor cells such as EAC is a rapidly growing cancer cells with aggressive behavior [11]. The tumor implantation includes a local inflammatory reaction, with increasing vascular permeability, which results in an intense ascetic fluid accumulation [12]. The ascitic fluid is essential for tumor growth, since it constitutes a direct nutritional source for tumor cells [13]. The drug which reduces ascitic fluid volume might be a good anticancer agent. The reliable criterion for judging the value of anticancer drug is the prolongation of

life span of tumor inoculated mice [14]. The effect of EECR on total body weight, lifespan, survival time, tumor volume and Cancer cell count was shown in the **Table 2**

Effect of EECR on biochemical parameters

The inoculation of Ehrlich ascites tumor cells to tumour control animals (Group II) caused significant increase in the level of biochemical parameters when compared to normal animals (Group I). The treatment with EECR (Group VIII and Group IX) at the dose of 50 mg/kg and 100 mg/kg decreased the level of biochemical parameters when compared to tumour control animals (Group II). Almost similar results were observed for other groups. The result was shown in **Table 3**.

Table: 1 *In vitro* studies of anticancer activity against MCF (Breast cancer) cell line

Extracts	OD	% of Viability
Control	0.772	100
Acetone	0.381	49.35
Chloroform	0.382	50.38
Methanol	0.484	62.69
Ethyl acetate	0.445	57.64
Ethanol	0.085	11.01

Table: 2 Effect of EECR (Ethanolic Extract of *Catharanthus roseus*) on survival time and viable cell count

Treatment	Total Body Weight (gm)	Mean survival time	Life Span (%)	Tumor volume (ml)	Cancer cell count ml × 10^6
Group I	22.2±0.5	-	>>30 days	-	-
Group II	28.3±0.6	22.2±2.0	-	4.2±0.02	4.21±0.10
Group III	22.7±0.9	45.1±1.2	95.9	1.3±0.02	2.04±0.01
Group IV	23.9±1.1	35.8±0.4	61.26	1.1±0.03	1.41±0.02
Group V	23.2±0.8	36.5±0.3	64.4	2.2±0.01	2.20±0.06
Group VI	23.9±0.4	34.8±0.1	56.75	1.0±0.05	2.10±0.03
Group VII	23.2±0.5	36.7±0.3	65.31	0.5±0.06	1.05±0.08
Group VIII	25.5±0.6	26.5±0.6	19.36	1.5±0.03	1.90±0.05
Group IX	24.8±0.6	32.5±0.6	46.3	1.2±0.01	1.10±0.12

All values are expressed as Mean ± SEM for six animals in each group. All values are found out by using One way ANNOVA followed by Dunnetts post hoc test of significance.

Table 3 Effect of EECR (Ethanolic Extract of *Catharanthus roseus*) on biochemical parameters

Treatment	Cholesterol mg/ml	TGL mg/ml	AST	ALT	ALP
Group I	113.30±0.42	133.30±0.23	38.50±0.52	31.60±0.11	124.70±019
Group II	141.34±0.10	220.4±0.35	87.35±0.20	56.75±0.40	241.5±0.69
Group III	122.60±0.20	141.94±0.50	54.60±0.13	41.38±0.50	161.5±0.68
Group IV	120.50±0.19	140.96±0.30	52.10±0.10	39.15±0.30	160.41±0.65
Group V	130.50±0.12	152.04±0.56	58.15±0.16	40.32±0.15	130.45±0.50
Group VI	132.40±0.15	142.56±0.16	56.20±0.20	43.50±0.30	165.40±0.30
Group VII	135.10±0.23	143.16±0.21	56.40±0.10	44.10±0.26	166.52±0.51
Group VIII	136.35±0.40	191.32±0.31	82.12±0.32	52.10±0.22	235.03±3.12
Group IX	139.40±0.32	161.31±0.31	85.36±1.10	55.20±0.13	232.10±3.10

All values are expressed as Mean ± SEM for six animals in each group. All values are found out by using One way ANNOVA followed by Dunnetts post hoc test of significance.

CONCLUSION

On the basis of the above results it is concluded that the ethanolic extract of *Catharanthus roseus* possess significant anticancer activity. The activity may be due to the presence of one or more phytochemical constituents present in the extract. Further studies warranted, for isolation of the constituents responsible for the activity and also to explore the exact mechanism of action of the activity. Hence there is a hope in the pharmaceutical industry, that even more powerful commercial drugs can be developed sooner, using plant derivaties, to effectively treat cancer and save mankind.

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