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Antitumor Activity and Antioxidant Status of *Symplocos racemosa* Roxb Against Ehrlich Ascites Carcinoma in Swiss Albino Mice

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ABSTRACT

Antitumor and antioxidant status of ethanol extract (100 and 200mg/kg) of *Symplocos racemosa* (EESR) was evaluated against Ehrlich ascites carcinoma (EAC) bearing swiss albino mice. Acute and short term toxicity studies were performed initially in order to ascertain the safety of EESR. After 24 h of tumor inoculation, the extract was administered daily for 14 days intraperitoneally. After administration of last dose followed by 18 h fasting, the mice were sacrificed for observation of antitumor activity. The effect of EESR on the growth of transplantable murine tumor, life span of EAC bearing mice, hematological profile and liver biochemical parameters (lipid peroxidation, antioxidant enzymes) were estimated. Treatment with EESR decreased the tumor volume and viable cell count thereby increasing the lifespan of EAC bearing mice and brought back the hematological parameter more or less normal level. The effect of EESR also decreases the level of lipid peroxidation and increased the levels of catalase (CAT). The present work indicates that the ethanol extract of *Symplocos racemosa* exhibited antitumor effect by modulating lipid peroxidation and augmenting anti-oxidant defense system in EAC bearing mice.

Keywords: *Symplocos racemosa*, Ehrlich Ascites Carcinoma, hematological parameter, antioxidant, antitumor activity.

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INTRODUCTION

Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives each year. An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural agents to block the development of cancer in human beings. Plants, vegetables, herbs, and spices used in folk and traditional medicine have been accepted currently as one of the main sources of cancer chemo preventive drug discovery and development. A large and increasing number of patients in the world use medicinal plants and herbs for health purposes [1].

A simple but effective way of cancer may prevent oxidative damage. In the recent year; several antioxidant phenolic compounds have been recognized to have ability to induce apoptosis in various tumor cells of human origin. By virtue of antioxidant property they also prevent cell growth by inhibiting the protein responsible for cell growth. Ethanobotanical search reveals use of many traditional herbs in treatment of cancer, which are usually free from side effect, are economical and also easily assessable to humans [2]. The plant *Symplocos racemosa* (Family: Symplocaceae) is a low under shrub with wide distribution, mostly found in South India and Himalayas [3].The bark used traditionally for various diseases and cancer. The plant is popularly known as velli-lothi, in Tamil. The aim of present study was to evaluate the antitumor activity of the ethanol extract of *Symplocos racemosa* (EESR) against Ehrlich's Ascites Carcinoma (EAC) in mice.

MATERIALS AND METHODS

Preparation of extract

The bark portion of the plant was collected from Kolli Hills and authenticated by Dr. V. Sathyanathan, Epoch Pharma and Research Labs Pvt. Ltd. Chennai., TamilNadu, India. The coarse powder (250 g) was packed in a Soxhlet apparatus and subjected to hot continuous percolation for 72 h by using ethanol (95% *v/v*) as solvent. The extract was concentrated under vacuum and dried in a desiccator (Yield: 8.35% w/w) and used for pharmacological studies [4]. Phytochemical screening of the extract revealed the presence of carbohydrates, glycosides, alkaloids, terpenoids and phenolic compounds.

Animals

Adult Swiss male albino mice (18-25 g) were procured from Perundurai Medical College, Perundurai, Tamilnadu and used throughout the study. They were housed in polyacrylic cages in a controlled environment (Temperature 25 ± 28 °C and 12 h dark/light cycle) with standard laboratory diet and water *ad libitum.* All procedures described were reviewed and approved by the Institutional Animal Ethical Committee.

Tumor cells

EAC cells were obtained through the courtesy of Amala Cancer Research Center, Trissur, Kerala, India. The EAC cells were maintained by weekly intraperitoneal inoculation of 2×10^6 cells/mouse.

Toxicity study

An acute toxicity study relating to the determination of LD50 was performed [5-6].

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 $2x10^6$ cells/mouse) intraperitoneally except the normal group. This was taken as day zero. On the first day, 5 ml/kg body weight of normal saline (0.9% NaCl w/v) was administered in group 1 (Normal). Propylene glycol, 5 ml / kg per day was administered in group 2 (cancer control). EESR at different doses (100mg, 200mg) was administered into group 3 and 4 for 14 days intraperitoneally. To the group 5, standard drug Vincrystine (0.8mg/kg/day/mice) was administered, after the administration of last dose followed by 18 hours fasting 4 mice from each group were 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 [7-8].

Tumor growth response

Antitumor effect of EESR was assessed by observation of change with respect of body weight, ascitic tumor volume, packed cell volume, viable and non viable tumor cell count, mean survival time (MST) and percentage increase in life span (% ILS).

Tumor cell volume and packed cell volume

The mice were dissected for collecting ascitic fluid from peritoneal cavity. The transplantable murine tumor was carefully collected with the help of 5 ml sterile syringe and measured the tumor volume and the ascitic fluid was withdrawn, in graduated glass centrifuge tube and packed cell volume was determined by centrifuging at 1000 rpm for 5 min.

Viable and non viable cell count

For viable and non viable cell counting the ascitic cells were stained by the 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 non viable.

Mean survival time and percent increased in life span

The effect of EESR on tumor growth was observed by MST and % ILS. MST of each group containing 4 mice were monitored by recording the mortality daily for 6 weeks and % ILS was calculated by using following equation [9-10].

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

% ILS = [(MST of treated group/ MST of control group) – 1] x 100

Effect of EESR on hematological parameters

At the end of experimental period, half of the mice were killed the next day after an over night fasting by decapitation. Blood was collected from freely flowing tail vein and used for estimation of hemoglobin (Hb) content, red blood cell count (RBC), white blood cell count (WBC) and WBC differential count [5].

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 followed 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 remaining homogenate was centrifuged at 1500 rpm at 4 °C for 10 min. The supernatant thus obtained was used for the estimation of catalase and protein content.

Estimation of lipid peroxidation

Malondialdehyde (MDA), a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reactive substances (TBARS). To 0.2 ml of tissue homogenate, 0.2 ml of 8.1% sodium lauryl sulfate (SLS), 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% thiobarbituric acid (TBA) were added in succession. The volume of mixture was made up to 4 ml with distilled water. The mixture was incubated for 60 min at 95 ^{o}C in a temperature control water bath and cooled and added 5 ml of n-butanol: pyridine (15: 1) mixture was added and the contents were vertex thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min, the upper organic layer was separated and absorbance was read at 532 nm against an appropriate blank without the sample. The levels of lipid peroxides were expressed as n moles of thiobarbituric acid reactive substances (TBARS)/mg protein using an extinction coefficient of 1.56 x 10^5 $\mu^{-1}cm^{-1}$ [11].

Estimation of catalase

Catalase activity was measured by mixed 0.1 ml of supernatant to cuvette containing 1.9 ml of 50 mm phosphate buffer solution (pH 7.4). The reaction was started by the addition of 1.0 ml of freshly prepared 30 mm hydrogen peroxide. The role of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm was expressed as unit/mg protein. [12].

Estimation of protein content

The prepared 10% w/v liver homogenate in phosphate buffer solution (pH 7.4) was used for the estimation of protein content. The prepared homogenate was centrifuged at 1500 rpm for 15 min at 4 ^{o}C. The supernatant thus obtained was used for estimation. Test solution was prepared by using 0.2 ml serum, 5 ml biuret reagent and 3 ml distilled water

standard solution was prepared by using 3 ml solution of bovine albumin and 5 ml biuret reagent. The transmittance of the sample was read against the blank at 540 nm in UV visible double beam spectrophotometer. The amount of protein was expressed in g of protein in 100 ml [13].

Statistical analysis

The experimental results were expressed as mean ± S.E.M. data was assessed by ANOVA followed by the student t-test, P value < 0.05 was considered as statistically significant.

RESULTS

The present investigation indicates that the ethanol extract of *Symplocos racemosa* Roxb (EESR) showed significant antitumor and antioxidant activities in EAC bearing mice. The effect of EESR at the dose of 100 mg/kg and 200 mg/kg on various biochemical and biological parameters are discussed below.

Effect of EESR on mean survival time and tumor growth

In the EAC control group, the mean survival time was 24.00 ± 0.54 d, while it increased to 27.25 ± 0.71 (100 mg/kg) and 28.75 ± 0.94 (200 mg/kg) d, respectively in EESR treated mice. The mice treated with the standard drug Vincrystine (0.8 mg/kg) showed 33.25 ± 1.04 d for the same. Treatment with EESR at the doses of 100 and 200 mg/kg reduced the body weight, tumor volume, packed cell volume and viable tumor cell count in dose dependant manner as compared to that of EAC control mice. Further, non-viable tumor cell count at different doses of EESR was increased when compared with EAC control.

The increase in the life span of tumor bearing mice treated with EESR and Vincrystine ($P<0.01$) as compared to the control group.

Effect on hematological parameters

Hemoglobin content and RBC count in the EAC control mice was decreased when compared to normal group. Treatment with EESR at the dose of 100 and 200 mg/kg in EAC treated mice were increased the hemoglobin content (10.42 and 11.1 g% respectively). Moderate change in the RBC count was observed in the extract treated mice. The total WBC counts were found to be increased in EAC control mice when compared with normal mice. Administration of EESR at the dose of 100 and 200 mg/kg in EAC bearing mice reduced WBC count when compared with EAC control. In differential count, the percentage of lymphocyte decreased and percentage of granulocyte increased in EAC control, while EESR treated mice lymphocyte was increased and granulocyte was decreased as compared with EAC control mice [14-15].

Effect on biochemical parameters

Table no. 4 showed that the level of lipid peroxidation in liver tissue was increased in EAC control mice 1.66 n moles as compared to the normal mice 0.80 n moles. Treatment with EESR (100 and 200 mg/kg) were significantly decreased the lipid peroxidation level (1.46 and 1.28 n moles) in a dose dependant manner. The catalase level was decreased in EAC control mice (1.72 unit/mg of protein tissue) when compared with normal mice (2.40 unit/mg of protein in tissue) treatment with EESR at the doses of 100 and 200 mg/kg it back to normal level (1.43 and 2.22 unit/mg of protein in tissue) [16].

DISCUSSION

Natural products have been regarded as important sources that could produce potential chemotherapeutic agents. Plant derived compounds, in particular have a special case in anticancer therapy and some of the new chemotherapeutic agents currently available for use in chemical setting include pacilitaxel, vincristine, podophyllotoxin and camptothecin, a natural product precursor for water soluble derivatives. Obviously natural products are extremely important as sources of medicinal agents. Although there are some new approaches to drug discovery, such as combinatorial chemistry and computer based molecular modeling design, none of them can replace the importance of natural products in drug discovery and development [16].

The present investigation was carried out to evaluate the antitumor activity and antioxidant status of EESR in EAC bearing tumor mice. The EESR treated animals at the doses of 100 and 200 mg/kg significantly inhibited the tumor volume, packed cell volume, tumor viable cell count, and brought back the hematological parameter to more or less normal levels. The extract also restored the hepatic lipid peroxidation and free radical scavenging enzyme catalase in tumor-bearing mice to near normal levels.

In EAC tumor bearing mice, a regular rapid increase in ascitic tumor volume was observed. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in ascetic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells. Treatment with EESR inhibited the tumor volume, viable tumor cell count and increased the life span of the tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of life span of animals. It may be concluded that EESR by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of EAC bearing mice.

In cancer chemotherapy the major problem are of myelosuppression and anemia. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency or due to hemolytic or myelopathic condition. Treatment with EESR brought back the hemoglobin content, RBC and WBC cell count near to normal values. This indicates that EESR posses protective action on the heamoto-pioetic system.

Excessive production of free radicals resulted in oxidative stress, which leads to damage of macromolecules such as lipids can induce lipid peroxidation *in vivo.* Increased

lipid peroxidation would cause degeneration of tissues. Lipid peroxide formed in the primary site would be transferred through the circulation and provoked damaged by propagating the process of lipid peroxidation. MDA, the end product of lipid peroxidation was reported to be higher in carcinomatous tissue than in non diseased organs. EESR reduced the elevated level of lipid peroxidation [17-25].

CONCLUSION

The present study demonstrated that the ethanol extract of *symplocos racemosa* (EESR) increased the life span of EAC tumor bearing mice and decreased lipid peroxidation and thereby augmented the endogenous antioxidant enzymes in the liver. All these parameters suggest that the EESR exhibit potential antitumor and antioxidant activities.

Further investigation is in progress to identify the active principles involved in this antitumor activity and to investigate their mechanism.

Table 1 Effect of ethanol extract of *Symplocos racemosa on* survival time of EAC bearing mice

Experimental groups	Mean survival time (MST) days	% increase in life span
Normal control (normal saline 5 ml/kg b.w.)	-	-
EAC control	24.00 ± 0.54	-
EAC + EESR (100 mg/kg)	27.25 ± 0.71	10.63%
EAC + EESR (200 mg/ kg)	28.75 ± 0.94	21.27%
EAC + Vincrystine (0.8 mg/kg)	33.25 ± 1.04	40.42%

Values are mean ± SEM (Standard error of mean). Number of mice in each group (n=8). P < 0.01, Experimental group was compared with EAC control.

Table 2 Effect of ethanol extract of *Symplocos racemosa* on tumor volume, packed cell volume, viable and non viable tumor cell count of EAC bearing mice

Parameters	EAC control	EESR (100 mg/kg)	EESR (200 mg/kg)	Vincrystine (0.8 mg/kg)
Body weight (g)	27.11 ± 0.30	24.48 ± 0.17	23.40 ± 0.14	24.00 ± 0.03
Tumor volume (ml)	5.70 ± 0.05	4.57 ± 0.04	4.07 ± 0.06	2.48 ± 0.13
Packed cell volume (ml)	3.07 ± 0.06	2.17 ± 0.04	1.82 ± 0.04	1.17 ± 0.03
Viable tumor cell count (x 10^7 cells/ml)	11.64 ± 0.09	8.11 ± 0.02	5.37 ± 0.05	4.92 ± 0.01
Nonviable tumor cell count (x 10^7 cells/ml)	0.64 ± 0.01	1.0 ± 0.03	1.35 ± 0.02	1.32 ± 0.08

Values are mean ± SEM (Standard error of mean). Number of mice in each group (n=8). P < 0.01, Experimental group was compared with EAC control.

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Table 3 Effect of ethanol extract of *Symplocos racemosa* on hematological parameters of EAC bearing mice

Parameters	Normal saline (0.5 ml/kg)	EAC control ($2x10^6$ cells/mice)	EAC + EESR (100 mg/kg)	EAC + EESR (200 mg / kg)	EAC Cell + Vincrystine (0.8 mg/kg)
Hemoglobin (gm)	12.15 ± 0.18	9.25 ± 0.12	10.42 ± 0.07	11.10 ± 0.09	11.52 ± 0.06
Total RBC (million/mmcu)	7.37 ± 0.13	3.57 ± 0.07	4.72 ± 0.08	5.5 ± 0.04	6.02 ± 0.07
Total WBC (million/mmcu)	7.87 ± 0.05	20.02 ± 0.08	11.72 ± 0.14	8.9 ± 0.09	9.05 ± 0.16
Diff. count Lymphocyte	77.90 ± 0.47	33.40 ± 0.36	52.02 ± 0.38	60.3 ± 0.27	59.05 ± 0.29
Monocyte	1.82 ± 0.04	0.82 ± 0.02	1.17 ± 0.02	1.30 ± 0.02	1.35 ± 0.03
Granulocyte	30.51 ± 0.27	52.20 ± 0.38	40.72 ± 0.26	31.50 ± 0.45	41.80 ± 0.29

Values are mean ± SEM (Standard error of mean). Number of mice in each group (n=8). $P < 0.05$, $P < 0.01$ Experimental group was compared with EAC control.

Table 4 Effect of ethanol extract of *Symplocos racemosa* on different biochemical parameters of EAC bearing mice

Parameters	Normal saline (0.5 ml/kg)	EAC control ($2x10^6$ cells/mice)	EAC + EESR (100 mg/kg)	EAC + EESR (200 mg/kg)
Lipid peroxidation (n mole MDA/g of tissue)	0.80 ± 0.13	1.66 ± 0.01	1.46 ± 0.02	1.28 ± 0.01
Catalase (units/mg tissues)	2.40 ± 0.63	1.72 ± 0.10	1.43 ± 0.12	2.22 ± 0.28
Protein content (g/100 ml)	13.00 ± 0.38	17.06 ± 0.62	16.41 ± 0.13	15.95 ± 0.58

Values are mean ± SEM (Standard error of mean). Number of mice in each group (n=8). $P < 0.01$, Experimental group was compared with EAC control. $P < 0.05$, Experimental group was compared with EAC control.

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