

Antitumor Activity of *Diospyros peregrina* on Ehrlich Ascites Carcinoma in Mice

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Abstract

The methanol extract of *Diospyros peregrina* (Ebenaceae) bark (MEDP) were evaluated for antitumor activity against Ehrlich ascites carcinoma (EAC)-bearing swiss albino mice. The extract was administered at the doses of 200 and 400 mg/kg body weight per day for 14 days after 24 h of tumor inoculation. After the last dose and 18 h fasting, the mice were sacrificed. The present study deals with the effect of MEDP on the growth of transplantable murine tumor, life span of EAC-bearing hosts and hematological profile MEDP caused significant ($P < 0.01$) decrease in tumor volume, packed cell volume, and viable cell count; and it prolonged the life span of EAC-tumor bearing mice. Hematological profile converted to more or less normal levels in extract-treated mice. The results indicate that MEDP exhibited significant antitumor activity in EAC-bearing mice.

Keywords: *Diospyros peregrina*; Ehrlich ascites carcinoma; Antitumor.

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1. Introduction

Cancer is a class of diseases in which a cell or a group of cells display uncontrolled growth, invasion and sometimes metastasis. According to WHO estimates, globally 10 millions new cancer cases are diagnosed each year. It caused about 13% of all human deaths in 2007. It is estimated that by the year 2020, there will be 20 million new cancer cases with 12 million deaths. India is a rich source of medicinal plants and a number of plant extracts are used against diseases in various systems of medicine such as ayurveda, unani, and siddha. Only a few of them have been scientifically explored. Plant derived natural products such as flavonoids, terpenes; alkaloids [1-3] and soon have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemopreventive effects [4].

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The genus *Diospyros* L. (Ebenaceae), which is distributed throughout the tropics, is characterized by its ability to produce triterpenes of the lupine series. The genus *Diospyros* consists of 240 species, 59 of which are distributed in India, Thailand, Japan, Nigeria, South Africa and Philippines.

Diospyros peregrina can be seen mostly as trees and rarely shrubs. Various bioactive compounds were isolated from this plant. Roots contain dihydroflavonol glycoside 5, 7, 3, 5' - tetra hydroxyl - 3' - methoxy flavones [5], 4'-O- α -L-rhamnopyranoside (5), leaves contain triterpenes, anthocyanin [6], fruits contain lup-20 (29)-3n-3 α , 27-diol-29, lup-20 (29)-3n-3 β -diol-29, taraxerone, sitosterol, gallic acid, peregrinol, fruit pulp contain hexacosane, hexacosanol, β -sitosterol, monohydroxy triterpene ketone, betulin, β -D-glycoside of β -sitosterol, betulinic acid, methyl ester acetate, methylester B-D-glycoside of β -sitosterol [7].

Many naturally occurring substances have been tested for anticancer activity on experimental animals resulting in the presence availability of some 30 effective anticancer drugs [8]. *D. peregrina* is a plant indigenous to India. Besides its traditional use for the treatment of dysentery and menstrual problems different parts of the plant are of different therapeutic values. To mention a few the plants is used in snake bite, intermittent fever, wound and ulcer healing [9, 10]. It is also reported to possess antifertility [11], hepatoprotective [12], hypoglycemic, antiviral and antiprotozoal activity [13]. The aim of the study to evaluate the antitumor activity of the methanolic extract of bark of *D.peregrina*.

2. Materials and methods

2.1. Plant material

The plant *D. peregrina* (Family: Ebenaceae) was collected in the month of august 2009 from the Talakona forest, Chittor district. The plant material was taxonomically identified by the taxonomist, S.V University, Tirupathi.

2.2. Preparation of methanolic extract

The dried powder material of the bark of the *D. peregrina* was extracted with methanol (yield 8.78%) in a soxhlet apparatus. The methanol extract was then distilled, evaporated, and dried in vacuum. Preliminary qualitative analysis of the methanol extract showed the presence of alkaloid, tannin, C-glycoside, saponin, reducing sugar and triterpenes. The methanol extract of *D. peregrina* (MEDP) was used for the present study.

2.3. Animals

The study was carried out after obtaining permission from Institutional animal ethics committee (No. 160/SPIPS/Wgl/IAEC/2010) and CPCSEA regulations were adhered to during the study. Male swiss albino mice (20- 25 g) were selected for this study. The

animals were maintained under standard environmental conditions and fed with standard pellet feed and water *ad libitum*.

2.4. Tumor cells

EAC cells were obtained from Centre for Cellular and Molecular Biology (CCMB) (Hyderabad, India). The EAC cells were maintained by intraperitoneal inoculation of 2×10^6 cells /mouse.

2.5. Antitumor activity

Male swiss albino mice weighing 20 ± 2 g. were than divided into 5 groups (n =12). All the groups were injected with EAC cells (0.2 ml of 2×10^6 cells/mouse) intraperitoneally except the normal group. This was taken as day zero. On the first day, 5 ml /kg of normal saline was administered in group 1 (Normal). Normal Saline, 5 ml/kg per day, was administered in group 2 (EAC control). MEDP at different doses (200 and 400 mg/kg per day) and the standard drug 5-fluorouracil [14] (20 mg/kg) were administered in groups 3, 4 and 5 respectively for 14 days orally. After the last dose and 18-h fasting, six mice from each group were sacrificed for the study of antitumor activity, hematological parameters. The rest of the animal groups were kept to check the survival time of EAC-tumor bearing hosts.

2.6. Effect of MEDP on tumor growth response

The antitumor effect of MEDP was assessed by change in the body weight, ascites tumor volume, packed cell volume, viable and nonviable tumor cell count, mean survival time (MST), and percentage increased life span (% ILS). MST of each group containing six mice was monitored by recording the mortality daily for 6 weeks and % ILS was calculated using following equation [15, 16]:

$$\text{MST} = (\text{Day of first death} + \text{Day of last death}) / 2$$

$$\text{ILS (\%)} = [(\text{Mean survival time of treated group}) / (\text{Mean survival time of control group}) - 1] \times 100$$

2.7. Effect of MEDP on hematological studies

Blood was withdrawn from each mouse by retro orbital plexus method and the hemoglobin content, red blood cell (RBC), and white blood cell (WBC) counts were measured [17,18]. Differential leukocyte count of WBC was carried out from leishman stained blood smears [19] of normal, EAC control, and MEDP treated groups, respectively.

2.8. Effect of MEDP on *in vitro* cytotoxicity

Short-term cytotoxicity was assessed by incubating 1×10^6 EAC cells in 1 ml phosphate buffer saline with varying concentrations of the MEDP at 37°C for 3 h in CO_2 atmosphere ensured using a McIntosh field jar. The viability of the cells was determined by the trypan blue exclusion method. [20]

2.9. Statistical analysis

The experimental results were expressed as the mean \pm S.E.M. Data were assessed by ANOVA followed by Student's t-test; *P* value of < 0.05 was considered as statistically significant.

3. Results

The present investigation indicates that the MEDP showed significant antitumor activity in EAC-bearing mice. The effects of MEDP at the doses of 200 and 400 mg/kg on survival time, % ILS, tumor volume, packed cell volume, and tumor cell count (viable and nonviable cell) are shown in Table 1.

Table 1. Effect of the methanol extract of MEDP on body weight, mean survival time, % ILS, tumor volume, packed cell volume, and viable and nonviable tumor cell count of EAC-bearing mice.

Parameters	EAC Control	MEDP (200 mg/kg) + EAC	MEDP (400 mg/kg) + EAC	Standard 5-fluorouracil (20 mg/kg)+ EAC
Body weight (g)	26.4 \pm 0.13	24.84 \pm 0.17 [¶]	22.3 \pm 0.13 [¶]	21.2 \pm 0.19 [¶]
Mean survival time (days)	18.54 \pm 0.16	28.62 \pm 0.16 [¶]	33.87 \pm 0.14 [¶]	37.58 \pm 0.25 [¶]
Increase life span (%)	-	58.23 [¶]	92.56 [¶]	119.35 [¶]
Tumor volume (ml)	4.64 \pm 0.11	3.15 \pm 0.06 [¶]	1.83 \pm 0.04 [¶]	1.02 \pm 0.03 [¶]
Packed cell volume (ml)	27.2 \pm 1.36	22.8 \pm 0.12 [¶]	18.5 \pm 0.03 [¶]	17.4 \pm 0.35 [¶]
Viable tumor cell count ($\times 10^7$ cells/ml)	11.34 \pm 0.05	5.6 \pm 0.07 [¶]	76 \pm 0.04 [¶]	-
Nonviable tumor cell count ($\times 10^7$ cells/ml)	0.32 \pm 0.03	0.83 \pm 0.03 [¶]	1.48 \pm 0.04 [¶]	-

Data are expressed as the mean of results in 6 mice \pm S.E.M. [¶]*P* < 0.01 , extract-treated groups compared with the EAC control group. Body weight of normal mice: 20.7 \pm 0.17 g.

3.1. Effect on mean survival time

In the EAC control group, the mean survival time was 18.54±0.16 days, while it increased to 28.62±0.16 (200 mg/kg), and 33.87± 0.14 (400 mg/kg) days, respectively, in the MEDP-treated groups, whereas the standard drug 5-fluorouracil (20 mg/kg)-treated group had a mean survival time of 37.58±0.25 days.

3.2. Effect on tumor growth

Treatment with MEDP at the doses of 200 and 400 mg/kg significantly ($P < 0.01$) reduced the tumor volume, packed cell volume, and viable tumor cell count in a dose-dependent manner as compared to that of the EAC control group. Furthermore, nonviable tumor cell count at different doses of MEDP were significantly ($P < 0.01$) increased in a dose-dependent manner.

3.3. Effect on hematological parameters

As shown in Table 2, hemoglobin content and RBC count in the EAC control group was significantly ($P < 0.001$) decreased as compared to the normal group. Treatment with MEDP at the dose of 200 and 400 mg/kg significantly ($P < 0.01$) increased the hemoglobin content and RBC count to more or less normal levels. The total WBC counts and protein was found to be increased significantly in the EAC control group when compared with the normal group ($P < 0.001$). Administration of MEDP at the dose of 200 and 400 mg/kg in EAC-bearing mice significantly ($P < 0.01$) reduced the WBC count and protein as compared with the EAC control. In a differential count of WBC, the presence of neutrophils increased, while the lymphocyte count decreased in the EAC control group. Treatment with MEDP at different doses changed these altered parameters more or less to the normal values.

Table 2. Effect of the methanol extract of MEDP on hematological parameters of EAC-bearing mice.

Parameters	Normal (saline, 5 ml/kg)	EAC Control	MEDP (200 mg/kg) + EAC	MEDP (400 mg/kg) + EAC
Hemoglobin (g %)	14.2 ±0.12	10.7 ±0.15 [§]	11.8±0.14 [*]	13.4±0.13 [*]
RBC (×10 ⁹ /μl)	6.2 ±0.15	3.1 ±0.08 [§]	4.6 ±0.24	5.6±0.45 [*]
WBC (×10 ⁹ /μl)	5.3±0.08	16.9 ±0.21 [§]	9.1±0.05 [*]	5.9±0.03
Monocyte (%)	1.6±0.04	1.0 ±0.04 [§]	1.2±0.03	1.5 ±0.04 [*]
Neutrophil (%)	18.7±1.08	59.4 ±4.12 [§]	41.6±3.14 [*]	29.1±3.15
Lymphocyte (%)	75.3±2.36	37.6 ±2.45 [§]	54.8 ±2.35	63.1±2.18 [*]

Data are expressed as the mean of results in 6 mice ± S.E.M. [§] $P < 0.001$, EAC control group compared with the normal group. ^{*} $P < 0.01$, extract treated groups compared with the EAC control group.

4. Discussion

The present study was carried out to evaluate the antitumor effect of MEDP in EAC-bearing mice. The MEDP-treated animals at the doses of 200 and 400 mg/kg significantly inhibited the tumor volume, packed cell volume, tumor cell count, and brought back the hematological parameters to more or less normal levels. In EAC-bearing mice, a regular rapid increase in ascites tumor volume was noted. Ascites fluid is the direct nutritional source for tumor cells and a rapid increase in ascites fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells [21]. Treatment with MEDP increased the percentage of trypan blue positive stained dead cells in tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the life span of animals [22]. The MEDP decreased the ascites fluid volume, viable cell count, and increased the percentage of life span. It may be concluded that MEDP by decreasing the nutritional fluid volume and arresting the tumor growth, this could be the reason for the increase life span of EAC-bearing mice. Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia [23, 24]. 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 conditions [25]. After the repeated treatment, MEDP able to reverse the changes in hematological parameters hemoglobin content, RBC, and WBC counts near to normal levels. This indicates that MEDP is showing protective action on the hemopoietic system.

Some triterpenoids are found to have promising anticancer and antioxidant activity. MEDP shows the presence of tannins and triterpenes which may act as anticancer and antioxidant principles with MEDP [26, 27]. In our earlier studies, we found that MEDP possess hepatoprotective and antioxidant properties [28]. The free radical hypothesis supported the fact that the antioxidants effectively inhibit the tumor, and the observed properties may be attributed to the antioxidant and antitumor principles present in the extract.

The present study demonstrates that MEDP increased the life span of EAC-tumor bearing mice in the liver. The above parameters are responsible for the antitumor and antioxidant activities of *Diospyros peregrina*.

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