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Antitumor and antioxidant activity of *Diospyros peregrina* against Dalton's ascites lymphoma in rodents

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ABSTRACT

The methanol extract of *Diospyros peregrina* (Ebenaceae) bark (MEDP) were evaluated for antitumor activity and antioxidant activity against Dalton's ascites lymphoma (DAL)-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 DAL-bearing hosts, hematological profile, biochemical and antioxidant profile. MEDP caused significant decrease in tumor volume, packed cell volume, and viable cell count; and it prolonged the life span of DAL-tumor bearing mice. Hematological profile converted to more or less normal levels in extract-treated mice. The lipidperoxidation was increased in tumor bearing animals, after treatment with MEDP antioxidant levels increased significantly. The results indicate that MEDP exhibited significant antitumor activity in DAL-bearing mice.

Key words: Dalton's ascites lymphoma, *Diospyros peregrina*, Antitumor, Antioxidant, Anticancer

INTRODUCTION

Cancer is the uncontrolled growth of abnormal cells in the body. This results from a series of molecular events that fundamentally alter the normal properties of cells. In cancer cells the normal control systems that prevent cell overgrowth and the invasion of other tissues are disabled. These altered cells divide and grow, display uncontrolled growth, invasion and sometimes metastasis. According to a study by the World Health Organization, one in 12 women in urban India will develop cancer in their lifetime. Approximately 40 per cent of new cases of cancer in India afflict women. Cancer is one of the leading cause to death in the developed and developing countries. [1]. Cancer accounted for 7.1 million deaths in 2003 and it is estimated the overall number of new cases will rise by 50% in the next 20 years [2].

Experimental tumor models have a wide role in anticancer drug discovery. A Dalton's ascites lymphoma (DAL) tumorigenesis model in Balb/c / Swiss albino mice provides a convenient model system to study antitumor activity within a short time [3]. Following transplantation of DAL cells into the abdominal cavity of healthy recipient mice, tumorigenesis begins immediately and aggressively [4, 5]. Free radicals are the chemical species contains at least one 'unpaired of electron'. Reactive oxygen species (ROS) are the free radicals associated with the oxygen atom or their equivalents and have stronger reactivity with other molecules than with molecular oxygen (O₂). ROS usually

indicate the major following four species: (i). Superoxide anion radical (O_2^-); (ii) hydrogen peroxide (H_2O_2); (iii) hydroxyl Radical ($\cdot OH$); and (iv). Singlet oxygen (1O_2). (H_2O_2) and (1O_2) are not free radicals by definition but they behave like free radicals.

Free radicals are implicated in many pathological conditions by irreversibly damaging the structure of biological molecules like cell membranes, DNA, Proteins etc. These free radicals can directly interacting with DNA produce single or double strand DNA breaks, DNA cross linking, purine, pyrimidine, or deoxyribose modifications, and DNA cross-links. Persistent DNA damage can result in either arrest or induction of transcription, induction of signal transduction pathways, replication errors, and genomic instability, all of which to cancer. [6].

Plants are the rich source of medicines from ages. They produce bioactive molecules which can used to ameliorate various types of disorders. Over the last few decades there has been increased interest by pharmaceutical industries to discover the new drugs from the ethnobotanicals to provide new and alternative drugs to synthetic drugs for treatment of dreadful diseases. Potent anticancer drugs like taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, and etoposide derived from plant sources and they are in efficient clinical use.

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, 4'-O- α -L-rhamnopyranoside, leaves contain triterpenes, anthocyanin, 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].

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 [8, 9]. It is also reported to possess, hepatoprotective [10], hypoglycemic, antiviral and antiprotozoal activity [11]. The authors studied the antitumor activity of *D.peregrina* against Ehrlich ascites carcinoma in rodents. The aim of the present study to evaluate the antitumor activity of the methanolic extract of bark of *D.peregrina* against dalton's ascitic lymphoma.

MATERIALS AND METHODS

Plant material

The plant *D. peregrina* (Family: Ebenaceae) was collected in the month of January 2011. from the Talakona forest, Chittor district. The plant material was taxonomically identified by the taxonomist, S.V University, Tirupathi. The dried powder material of the bark of the *D. peregrina* was extracted with methanol (yield 10.5%) 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, saponin, reducing sugar and triterpenes. The methanol extract of *D.peregrina* (MEDP) was used for the present study.

Experimental Animals

The study was carried out after obtaining permission from Institutional animal ethics committee (No. 160/SPIPS/Wgl/IAEC/2011) 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*.

Tumor cells

DAL cells were obtained from Amala Cancer Institute, Thrissur, Kerala, India. . The DAL cells were maintained by intraperitoneally inoculation of 2×10^6 cells /mouse. Ascitic fluid was drawn out from DAL tumor bearing mouse at the log phase (days 10–12 of tumor bearing) of the tumor cells. Each animal received 0.2 ml of tumor cell suspension containing 2×10^6 tumor cells intraperitoneally.

Treatment schedule

60 male Swiss albino mice were divided into five groups (n = 10) and given food and water *ad libitum*. All the animals in each groups except Group-I received DAL cells (2×10^6 cells/mouse i.p.) This was taken as day '0'. Group-I served as normal control (25% Tween 80 per oral) and Group-II served as DAL control. 24-h after DAL transplantation, Group-III and Group-IV received methanol extract of *D.peregrina* (MEDP) at a dose of 200 and 400 mg/kg/oral for 14 consecutive days, respectively. Group-V received reference drug 5-fluorouracil (20 mg/kg oral) for 14 consecutive days (12). 24 hours of last dose, 5 animals of each group were sacrificed to study the tumor growth parameters (mean survival time, viable, non-viable cell, tumor volume, tumor weight and tumor packed cell volume), antioxidant and hematological parameters and the rest were kept with food and water *ad libitum* to check percentage increase in life span of the tumor host.

In Vitro-cytotoxicity study

DLA cells (1×10^6) in phosphate buffer saline (PBS) and different concentrations (50, 100, 200, 400, 600, 800, 1000, 1600 ug/ml) of MEDP were incubated at 37° c for 3 hrs in 5% CO₂ atmosphere in the filtered cap, flat bottom cell culture flasks. The viability of cells was determined by Trypan Blue dye exclusion method (13).

$$\% \text{ cell viability} = \frac{\text{No. of Dead cells}}{\text{No. of viable cells} + \text{No. of dead cells}} \times 100$$

Tumor growth parameters**Tumor volume and weight**

After 14 days of treatment, mice were dissected and the ascetic fluid was collected from peritoneal cavity. The volume was measured by taking it in a centrifuge tube and weighed immediately (14).

Viable and non-viable tumor cell count

The viability and nonviability of the cell were checked by trypan blue dye exclusion assay. The cells were stained with trypan blue (0.4% in normal saline) dye. Live (viable) cells actively pump out the dye by efflux mechanism where as dead (non-viable) cells do not. The number of viable and nonviable cells was counted (15).

$$\text{Cell count} = \frac{\text{Number of cells} \times \text{dilution factor}}{\text{Area} \times \text{thickness of liquid film}}$$

Tumor packed cell volume

The ascetic fluid was collected into Wintrobe's tube and it was centrifuged at the rate of 3000 rpm for a period of one hour. The volume of packed cells read directly as percentage. (15).

Percentage increased in life span

The effect of MEDP on percentage increases in life span was calculated on the basis of mortality of the experimental mice (16).

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

$$\text{Mean survival time}^* = \frac{\text{First death} + \text{last death}}{2}$$

* Time denoted by days.

Hematological parameters

At the end of the experimental period, blood was collected from retroorbital plexus and used for the estimation of hemoglobin (Hb) content, red blood cell (RBC) count, white blood cell (WBC) count, packed cell volume (PCV) and differential count (17, 18, 19) by standard procedures.

Biochemical parameters

The remaining blood was centrifuged and serum was used for the estimation of liver biochemical parameters like Serum glutamic pyruvic transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT), Albumin, Total protein (TP), Total bilirubin, Alkaline phosphatase, Gamma-glutamyl transpeptidase (GGTP) (20).

Antioxidant activity

The liver was excised, rinsed in ice cold normal saline followed by cold 0.15M Tris-HCl (pH 7.4), blotted and weighed. The homogenate was processed for estimation of Lipid peroxidation (LPO) (21), Superoxide Dismutase (SOD) (22), Catalase (CAT) (23), Reduced glutathione (GSH) (24), Glutathione peroxidase (GP_X) (25), Glutathione-S-Transferase (GST) (26).

Effect of on normal peritoneal cell count

To evaluate effect of MEDP on normal peritoneal cells, 3 groups of normal mice (n= 4) were taken. One group was treated with 400 mg/kg p.o. of MEDP and the second group received the same treatment for 2 consecutive days. The untreated third group was used as control. Peritoneal exudate cells were collected after 24 h treatment by repeated intraperitoneal wash with normal saline and counted in each of the treated groups and compared with those of the untreated group (27)

Effect of on solid tumor

Mice were divided into two groups (n = 4). DAL cell lines (1x10⁶ cells/mice) were injected into right hind limb (thigh) of all mice intramuscularly. The Group I used as DAL tumor control. The Group II treated with MEDP 400 mg/kg/oral for 14 days. Tumor mass was MEDPured from 15th day of tumor induction. The MEDPurement was carried out every 5th day for a period of 30 days. Tumor mass volume was MEDPured using following formula $V=4/3\pi r^2$, where r is the mean of r₁ and r₂ which are two independent radii of the tumor mass (28).

Statistical analysis

All data are expressed as mean \pm S.E.M. Statistical significance (p) calculated by ANOVA followed by Dennett's (tumor volume, tumor weight, viable, non viable, mean survival time, tumor PCV) and Benferroni tests (hematological, SGPT, SGOT, Total Protein, albumin, Antioxidant parameters). P<0.05 was considered as statistically significant.

RESULTS**In Vitro-cytotoxicity study**

The In vitro cytotoxicity effect of MEDP at various concentrations 50, 100, 200, 400, 600, 800, 1000, 1600 ug/ml on DAL cell lines using trypan blue dye exclusion assay method has shown in the table 1. the percentage of cell viability 10 %, 25 %, 30 %, 42 %, 54 %, 70 %, 85 %, 92 % respectively. The IC₅₀ value was found to be 600 ug/ml.

Effect of MEDP on mean survival time

The effect of MEDP on mean survival time were shown in table 1, On oral treatment of MEDP to the tumor induced DAL mice, the mean survival time of DAL control group was found to be 13.2 \pm 0.96, while it increased to 19.4 \pm 0.58 (MEDP 200 mg/kg), 22.6 \pm 2.24 (MEDP 400 mg/kg) respectively in MEDP treated groups and whereas the standard drug 5-fluorouracil (20 mg/kg)-treated group had a mean survival time 25.6 \pm 2.

Effect of MEDP on tumor growth

The effect of MEDP on tumor growth response were shown in table 1, After treatment with MEDP (200 and 400 mg/kg) significantly (P< 0.01, P<0.001) reduced the tumor volume, viable tumor cell count and (P<0.05, P<0.01) tumor packed cell volume in a dose-dependent manner as compared to that of the DAL Control group. Furthermore, nonviable tumor cell count at different doses of MEDP were significantly (P<0.01) increased in a dose-dependent manner.

Effect of MEDP on hematological parameters

The effect of MEDP on hematological parameters were shown in table 2, Hemoglobin content (P< 0.05) and RBC count in the DAL control group was decreased as compared to the normal control group. After treatment with MEDP at various doses 200 mg/kg, 400 mg/kg significantly (P<0.01) increased the hemoglobin content and RBC count and brought up to the near or less to the normal levels. The total WBC counts and PCV (P<0.001) was found to be increased significantly in the DAL control group when compared with the normal group. Administration of MEDP in DAL-bearing mice significantly reduced the WBC count and PCV (P<0.05) as compared with the DAL group. In a differential count of WBC, the presence of neutrophils increased, while the lymphocyte, eosinophils,

monocytes counts decreased in the DAL. Treatment with MEDP at different doses changed these altered parameters more or less to the normal values.

Table 1: Effect of methanol extract of *Diospyros peregrina* on tumor growth parameters

Parameters	DAL	DAL + MEDP 200 mg/kg	DAL+ MEDP 400 mg/kg	DAL + 5-FU 20 mg/kg
Mean survival time(days)	13.2±0.96	19.4±0.58	22.6±2.24*	25.6±2.06**
Increased life span (%)	—	31	46*	61*
Tumor volume(ml)	18±2.2	10.4±2.08**	4.8±0.91***	4.8±1.29***
Tumor packed cell volume (ml)	50.12±2.9	34.68±2.9**	36.7±1.7**	32.7±1.7**
Viable cell count(x10 ⁷ cells/ml)	19.63±0.96	16.91±0.83*	12.48±0.7**	12.65±0.67**
Nonviable cell count(x10 ⁷ cells/ml)	0.16±0.017	0.26±0.02*	0.53±0.04**	0.72±0.08**

Data are expressed as the mean ± SEM. n= 5. *P<0.05, **P<0.01, ***P<0.001, extract-treated groups compared with the DAL Group.

Table 2: Effect of methanol extract of *Diospyros peregrina* on hematological parameters

Parameters	Control	DAL	DAL + MEDP 200 mg/kg	DAL+ MEDP 400 mg/kg	DAL + 5-FU 20 mg/kg
Hemoglobin (%)	14.52±0.46	6.8±1.25 ^a	7.98±0.49 ^e	10.54±0.69 ^e	9.96±0.99 ^e
RBC(x10 ⁶ cell/mm ³)	13.14±0.56	7.5±0.5 ^a	8.4±0.5 ^e	9.52±0.39 ^e	11.4±0.60 ^e
PCV (%)	29.84±4.13	43.16±3.61 ^c	35.5±3.45 ^d	25.98±1.32 ^d	23.62±1.67 ^d
WBC(x10 ⁴ cells/mm ³)	0.628±0.13	5.74±0.7 ^c	2.58±0.23 ^d	1.8±0.19 ^d	1.51±0.17 ^d
Neutrophils (%)	37.6±1.74	54.8±7.01 ^b	52.2±1.88	40.6±0.7 ^e	38.4±1.69 ^e
Lymphocytes (%)	60.4±2.33	46±5.07 ^a	39.6±1.28	46.2±1.06	46.2±2.43
Eosinophils (%)	3.4±1.03	1.6±0.4	2.6±0.67	3.4±5.0	4.4±1.03
Monocytes (%)	1.8±0.37	0.2±0.2	0.2±0.2	0.2±0.2	0.8±0.37

Data are expressed as the mean ± SEM, n=5. ^aP<0.05, ^bP<0.01, ^cP<0.001, Control Vs DAL. ^dP<0.05, ^eP<0.01 and ^fP<0.001, DAL Vs extract treated groups

Effect of MEDP on biochemical parameters:

As shown in table 3, SGOT, SGPT, albumin levels were increased significantly (P<0.01) and total protein levels were decreased (P<0.05) as when DAL control group compared to the normal control group. After treatment with MEDP at the dose of 200 mg/kg, 400mg/kg and 5 FU significantly decreased the elevated SGOT, SGPT, albumin to normal levels and increased total protein levels.

Table 3: Effect of methanolic extract of *Diospyros peregrina* on biochemical parameters

Parameters	Control	DAL	DAL + MEDP 200 mg/kg	DAL+ MEDP 400 mg/kg	DAL + 5-FU 20 mg/kg
SGPT(U/L)	28.6±2.7	52.8±2.49 ^b	37.4±7.9 ^c	31.6±0.78 ^d	30±0.70 ^d
SGOT(U/L)	102.6±27.6	174.8±15.1 ^b	159.8±3.2 ^c	122.8±1.24 ^c	83.4±1.86 ^e
Albumin (gm %)	2.1±0.64	10.2±0.2 ^b	3.4±0.32 ^e	2.5±0.17 ^c	2.32±0.128 ^e
Total Protein (gm %)	5.08±0.64	2.7±0.41 ^a	3.61±0.95 ^a	4.72±0.84 ^b	6.3±0.57 ^b

Data are expressed as the mean of results in 5 mice ± SEM. ^aP<0.05 and ^bP<0.01, DAL Vs normal control, ^cP<0.05 and ^dP<0.01, ^eP< 0.001 extract treated Groups Vs DAL group.

Effect of MEDP on antioxidant activity:

As shown in Table 4, in the DAL group, the LPO level was increased and SOD, CAT, reduced GSH, GP_x, GST levels were decreased significantly (P<0.001) as compared to the normal control. After treatment with MEDP at different doses (200 mg/kg, 400mg/kg and 5-FU significantly decreased the LPO and increased the SOD, CAT, reduced GSH, GP_x, GST levels.

Table 4: Effect of methanolic extract of *Diospyros peregrina* on antioxidant parameters

Parameters	Control	DAL	DAL + MEDP 200 mg/kg	DAL+ MEDP 400 mg/kg	DAL + 5-FU 20 mg/kg
LPO (ng of MDA/mg protein)	0.23±0.02	2.015±0.29 ^a	0.87±0.06 ^b	1.51±0.15 ^c	1.7±0.15 ^c
SOD (U/mg protein)	0.14±0.01	0.014±0.01 ^a	0.05±0.004	0.09±0.01 ^b	0.12±0.01 ^b
CAT (U/mg protein)	0.98±0.13	0.11±0.01 ^a	0.34±0.02	0.63±0.07 ^c	0.7±0.07 ^c
GSH (mg/g wet tissue)	0.72±0.15	0.32±0.08 ^a	0.45±0.17 ^d	0.55±0.16 ^c	0.58±0.17 ^c
GP _x (U/mg protein)	0.027±0.003	0.0016±0.005 ^a	0.005±0.0003	0.015±0.001 ^d	0.019±0.003 ^d
GST (U/mg protein)	0.027±0.003	0.002±0.0006 ^a	0.010±0.000 ^d	0.015±0.004 ^c	0.018±0.003 ^c

Data are expressed as the mean ± SEM. ^aP<0.001, DAL Vs Control. ^bP<0.001, ^cP<0.01 and ^dP<0.05, DAL Vs extract treated groups.

Effect of MEDP on normal peritoneal cells:

The average number of peritoneal exudate cells per normal mouse was found to be 4.9×10^6 . MEDP (400 mg/kg) single treatment enhanced peritoneal cells to 9.4×10^6 while two consecutive treatments enhanced the number to 13.57×10^6 .

Effect of MEDP on solid tumor growth:

There was reduction in the tumor volume of mice treated with MEDP 400 mg/kg from 15th day to 30th day. On 30th day tumor volume of DAL control animals was 6.4 ± 1.13 ml, whereas for the extract-treated group it was found to be 0.03 ± 0.22 ml as shown in Table 5.

Table 5: Effect of *Diospyros peregrine* on solid tumor growth

Groups	Solid tumor volume in ml			
	15 th day	20 th day	25 th day	30 th day
DAL control	0.086 ± 0.01	2.0 ± 0.3	3.9 ± 0.7	6.4 ± 1.19
MEDP 400	$0.19 \pm .007$	0.16 ± 0.16	$0.08 \pm 0.09^{**}$	$0.03 \pm 0.22^{***}$

Data are expressed as the mean of results in 4 mice \pm SEM. $^{**}P < 0.01$, $^{***}P < 0.001$, MEDP 400 Vs DAL Group.

DISCUSSION

The present study was carried out to evaluate the antitumor effect of MEDP in DAL-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 DAL-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 [29]. 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 [30]. 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 DAL-bearing mice. Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia [31, 32]. 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 [33]. 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.

The generation of free radicals in vivo is a constant phenomenon due either to physiological metabolism or pathological alterations. These generated free radicals are the main cause of lipid peroxidation which is an autocatalytic free radical chain propagating reaction, is known to be associated with pathological conditions of a cell [34]. The oxidation of proteins, lipids, nucleotides and carbohydrates causes chemical modification, leads to damage of above. Malondialdehyde is the end product of lipid peroxidation, was reported to be more in cancerous cells when compared to non cancerous cells [35]. Cells have developed enzymatic systems (antioxidant enzymes) like SOD, catalase and glutathione which convert oxidants into non-toxic molecules, thus protecting the organism from the deleterious effects of oxidative stress. Glutathione (a tripeptide), usually the most prevalent intracellular thiol, functions directly or indirectly in a variety of cellular processes. Reduced glutathione (GSH) plays an important role in defense mechanisms by acting as an antioxidant or by reacting with electrophiles (36) and toxic agents to form conjugates that are eliminated from the cell (37). SOD, CAT, and glutathione peroxides are involved in the clearance of superoxide and hydrogen peroxide (H_2O_2). SOD catalyses the diminution of superoxide into H_2O_2 , which has to be eliminated by glutathione peroxidase and/ or catalase (38). The inhibition of SOD and CAT activities as a result of tumor growth was also reported (39). Similar findings were observed in the present investigation with DAL-bearing mice. MEDP significantly reduced the lipid peroxidation and increased the glutathione levels in the DAL bearing drug treated mice. As above stated like the decreased levels of SOD, Catalase and GPx levels were observed in the present study, after drug treatment with different doses the levels of these enzymes were significantly increased.

Natural antioxidants are playing a great role in free radical scavenging activity. Some triterpenoids and flavonoids are found to have promising anticancer and antioxidant activity. [40]

MEDP shows the presence of triterpenes and flavonoids which may act as anticancer and antioxidant principles in the diseased condition [41]. In our earlier studies, we found that MEDP possess antidiabetic and antioxidant properties [42]. 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.

CONCLUSION

The present study demonstrates that MEDP increased the life span of DAL-tumor bearing mice . and decreased the lipid peroxidation and thereby augmented the endogenous antioxidant enzymes in the liver. The above parameters are responsible for the antitumor and antioxidant activities of *Diospyros peregrina*.

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