J Pharmacol Sci **103**, 12 – 23 (2007)

Journal of Pharmacological Sciences

Full Paper

Antitumor Activity and Antioxidant Status of the Methanol Extract of *Careya arborea* Bark Against Dalton's Lymphoma Ascites-Induced Ascitic and Solid Tumor in Mice

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Received August 10, 2006; Accepted October 24, 2006

Abstract. Based on the ethnomedical use of *Careya arborea* Roxb bark in the treatment of tumors, the present study was carried out to evaluate the anticancer potentials against Dalton's lymphoma ascites (DLA)-induced ascitic and solid tumors. The methanol extract of its bark given orally to mice at the dose of 250 or 500 mg/kg body weight for 10 days caused significant reduction in percent increase in body weight, packed cell volume, and viable tumor cell count when compared to the mice of the DLA control group. Restoration of hematological and biochemical parameters towards normal was also observed. Histological observations of liver and kidney also indicated repair of tissue damage caused by tumor inoculation. The extract at the dose of 5 or 25 mg/kg body weight given i.p. daily for 14 days significantly reduced the solid tumor volume induced by DLA cells.

Keywords: *Careya arborea*, antitumor, antioxidant, hepatoprotective, solid tumor

Introduction

Combating cancer is of paramount importance today. Multidisciplinary scientific investigations are making the best efforts to combat this disease, but the sure-shot, perfect cure is yet to be brought into world medicine. An alternative solution to western medicine embodied with severe side effects is the use of medicinal plant preparations to arrest the insidious nature of the disease. Of the 92 anticancer drugs commercially available prior to 1983 in the United States, approved worldwide between 1983 and 1994, approximately 62% can be related to natural origins (1). Many herbs have been evaluated in clinical studies and are currently being investigated phytochemicaly to understand their tumoricidal actions against various cancers (2). The rich and diverse plant sources of India are likely to provide effective anticancer agents. One of the best approaches in the search for anticancer agents from plant resources is the selection of plants based on ethnomedical leads (3).

Careya arborea (Family: Barringtoniaceae) is a medium sized deciduous tree known as *Padmaka* in the ayurvedic system of medicine and reputed in indigenous medicine in India. The stem bark is used as a demulcent in cough and colds, as an antipyretic and antipruritic in eruptive fevers, and as an anthelmintic (4). It is useful in tumors, urinary discharges, piles, leucoderma, skin diseases, epileptic fits, antidote for snake venom, and so on (5). Recently, its antidiarrhoeal (6), hepatoprotective, and in vivo antioxidant activities were reported (7). Lupeol and betulin were isolated from its bark (8). However, so far no antitumor activity has been reported from this plant. Hence, in the present study, based on the ethnomedical claims, we investigated the antitumor properties of the methanol extract of *C. arborea* (MECA) against Dalton's lymphoma ascites (DLA)-induced tumor inoculation along with determining its antioxidant status.

Materials and Methods

Plant material

Careya arborea bark was collected from the forests of Western Ghats, Haridravati, Shimoga District, Karnataka, India in the month of July 2004. The plant was authenticated by Dr. S. Rajan, Survey of Medicinal

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Published online in J-STAGE
doi: 10.1254/jphs.FP0060907

Plants and Collection Unit, Government Arts College (Ootacamund, Tamil Nadu, India), where a voucher specimen is preserved for further reference (voucher no. 8570). The bark was shade dried, powdered, and extracted (1 kg) with methanol (7.5 L) using a Soxhlet extractor for 18 – 20 h. The extract was concentrated and dried under reduced pressure and controlled temperature 40°C – 50°C) in a rotary evaporator. The extract yielded a reddish brown crystalline solid weighing 186 g (18.6%) and was preserved in a refrigerator at 4°C until further use.

Chemicals

2,2-Diphenyl-1-picryl hydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) were obtained from Sigma Aldrich Co. (St. Louis, MO, USA). Ecoline Diagnostic Kits were obtained from E. Merck, Ltd. (Mumbai, India). Thiobarbituric acid, trichloroacetic acid, ethylenediaminetetraacetic acid (EDTA), and adrenaline were procured from Loba Chemie Indo Austranal Co. (Mumbai, India). 5-Fluorouracil (5-FU) was purchased from Ranbaxy Laboratories, Ltd. (New Delhi, India) and cisplatin was obtained from Dabur Pharmaceuticals, Ltd. (Sahidabad, India). All chemicals used were of analytical grade.

Tumor cells and inoculation

Human cervix cancer cell (HeLa), Caucasian male larynx epithelium carcinoma (HEp-2), rhabdomyosarcoma (RD), and normal African green monket (Vero) cell lines were obtained from National Centre for Cell Sciences (Pune, India). The cultures were maintained in Dulbecco's modified eagles medium (DMEM) containing 10% inactivated new born serum (PAA Laboratories, Inc., Pasching, Austria) and were grown in 25-cm^2 tissue culture flasks (Tarson Products, Ltd., Kolkatta, India) until confluent and used for cytotoxicity assays. DLA cells were supplied by Amala Cancer Research Centre (Trissur, Kerala, India). The cells were maintained in vivo in Swiss albino mice, by i.p. transplantation. Tumor cells aspirated from the peritoneal cavity of mice were washed with saline and were given i.p. to develop as ascites tumor to all the animals except the normal group.

Preparation of suspensions and solutions

For the in vitro antioxidant studies, MECA and standards used were dissolved in distilled dimethyl sulfoxide (DMSO) except for the hydrogen peroxide method, where DMSO interferes. For this method, the extracts and standards were dissolved in distilled methanol. The stock solutions were serially diluted with the respective solvents to obtain lower dilutions and used. For cytotoxicity studies, the extracts were dissolved in DMSO and the volume was made up to 10 ml using DMEM to obtain a stock solution of 1 mg/ml concentration, sterilized by filtration, and stored at −20°C.

MECA and the standard 5-FU were suspended in distilled water using sodium carboxy methyl cellulose (0.3%) and administered orally to the animals with the help of an intragastric catheter in the ascitic tumor studies. The suspension of the extract and a solution of standard cisplatin in normal saline were administered i.p. in the solid tumor studies.

In vitro antioxidant studies

The antioxidant activities of MECA and the standards were assessed using scavenging of DPPH, ABTS, nitric oxide, hydrogen peroxides, hydroxyl radical (by bleaching of *p*-NDA and deoxyribose methods), superoxide (by alkaline DMSO), lipid peroxidation, and total antioxidant capacity methods (9 – 11). Total phenol content was estimated by the Folin-Ciocalteu method.

In vitro cytotoxic activity

Stock cells of HEp-2, RD, and Vero cell lines were cultured in RPMI-1640 and DMEM supplemented with 10% sheep serum, penicillin (100 IU/ml), and streptomycin (100 μg/ml) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with 0.2% trypsin, 0.02% EDTA in PBS. The cytotoxic assay was carried out by adding 0.1 ml of cell suspension containing 10,000 cells to each well of a 96-well microtitre plate (Tarson) and fresh medium containing different concentrations of the extracts was added at 24 h after seeding. Control cells were incubated without the test extract and with DMSO (solvent). The very small percentage of DMSO present in the wells (maximal 0.2%) was proved not to affect the experiment. The microtitre plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 3 days. Twelve wells were used for each concentration of the extracts. The cells were observed at different time intervals during incubation in the presence of the extracts. Cellular viability was determined by the standard MTT and SRB assay methods (12).

Animals

The ascitic antitumor studies induced by DLA cells were carried out by using healthy adult Swiss albino mice weighing 25 – 30 g. They were obtained from the animal house of J.S.S. College of Pharmacy (Ootacamund, Tamil Nadu, India). The DLA-induced solid tumor study was carried out by using inbred fresh female BALB/c mice weighing 25 – 30 g. They were purchased

from Small Animal Breading Station, Agricultural University (Mannuthy, Trissur, Kerala, India). All the animals were kept under standard laboratory conditions with a 12 ± 1 h light-dark cycle. Animals were provided with normal mouse chow (Sai Durga Feeds and Foods, Bangalore, India) and water ad libitum. The experiments were performed as per the recommendations of CPCSEA, Chennai (No. JSSCP/IAEC/PH.D/Ph.Chem /04/2005-2006).

DLA-induced ascitic antitumor studies

Group I containing 6 animals, served as the normal control, for which inoculation of tumor cells was not done. The remaining animals were inoculated with DLA (1×10^6 cells/mouse) and divided into 4 groups containing 12 mice in each group. Group II, served as the tumor control. Groups I and II received sodium CMC suspension (0.3%). Group III, which served as a positive control, was treated with 5-FU at the dose of 20 mg/kg body weight. Groups IV and V were treated with MECA at 250 and 500 mg/kg body weight, respectively. All the treatments were given orally at 24 h after tumor inoculation and continued once daily for 10 days. On the 11th day, six animals from each group were anaesthetized slightly with anesthetic ether and blood was collected from retro-orbital puncture. The hematological parameters like WBC, RBC, hemoglobin (Hgb), and platelets (PLT) were estimated by a cell analyzer (Medonic CA 530; Boule Medical, AB, Stockholm, Sweden). The differential count of WBC was carried out in the blood smear.

The remaining blood was centrifuged and serum was used for the estimation of hepatoprotective parameters like aspartate amino transferase (ASAT), alanine amino transferase (ALAT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), triglycerides (TGL), creatinine (CR), albumin, total protein (TP), total cholesterol (TC), and total bilirubin (TB) by using Ecoline Kits. The antioxidant parameters, catalase (CAT) (13), superoxide dismutase (SOD) (14), and thio-barbituric acid reactive substances (TBARS) (15) were also estimated. The ascitic fluid was collected from the peritoneal cavity and measured for tumor cell packed volume and viable tumor cell counts (16).

Immediately after collecting the blood samples, the mice were killed by cervical dislocation. The liver and kidney were excised, rinsed in ice-cold normal saline solution followed by ice-cold 10% KCl solution, blotted, dried, and weighed. A 10% w/v homogenate was prepared in ice-cold 10% KCl solution and was centrifuged at 1500 rpm for 15 min at 4°C. The supernatants, thus obtained were used for the estimation of the above biochemical parameters.

A part of the dissected liver and a part of the kidney from all the groups were cleared off of the surrounding tissues and kept in 10% buffered neutral formalin, dehydrated in alcohol, and then embedded in paraffin. The paraffin blocks were sectioned at 5-μm intervals and stained with haematoxylin-eosin for histological examinations. The rest of the animals were kept to check the average life span and percentage increase in life span and body weight analysis (16).

DLA-induced solid tumor studies

DLA cell lines (1.5×10^6 cells/mouse) were injected subcutaneously to the right hind limb of BALB/c mice and divided into 4 groups containing 6 mice in each group. Group I served as tumor control and received sodium CMC suspension (0.3%). Group II was treated with standard cisplatin at a dose of 2 mg/kg body weight dose. Groups III and IV were treated with MECA at 5 and 25 mg/kg body weight doses. All the treatments were given i.p. at 24 h after DLA tumor inoculation and continued once daily for 14 days. Initial diameter of the right hind limb was noted using vernier calipers. From the 7th day onwards, tumor diameter was measured every third day and recorded up to 34 days (17). The tumor volume was calculated by the following formula: $V = 4/3\ \pi\ r_1^2 r_2$, where r_1 and r_2 are the radii of tumors at two different planes.

Statistical analysis

The significance of the in vivo data was analyzed by one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test. $P<0.05$ was considered as statistically significant.

HPTLC profile

In order to standardize the extract, HPTLC finger printing was carried out. MECA was dissolved in distilled methanol, and 5 μl (1 mg/ml) sample was applied onto a commercially available precoated TLC plate of silica gel GF_{254} with a bandwidth of 5 mm using Linomat-IV applicator (CAMAG, Muttenz, Switzerland). The plate was developed in a twin-trough chamber using the solvent system ethyl acetate: formic acid: glacial acetic acid: water (8:4:2:5) and scanned by a densitometer (CAMAG) at 366 nm.

Results

In vitro antioxidant studies

The MECA exhibited potent in vitro antioxidant activity in ABTS, DPPH, nitric oxide, hydrogen peroxides, hydroxyl radical scavenging by deoxy ribose, and TBARS methods. The IC_{50} values found for the

Table 1. In vitro antioxidant activity of MECA determined by different methods

Extract/ Standards	IC_{50} values ± S.E.M. (μg/ml) by methods*						Total antioxidant capacity**	Total phenol content* (mg/g)
	ABTS	DPPH	H_2O_2	Nitric oxide	Deoxy ribose	TBARS		
Extract								
MECA	7.11 ± 0.13	6.21 ± 0.13	21.05 ± 0.27	29.47 ± 1.51	83.66 ± 1.85	81.50 ± 5.30	4.365 ± 0.362	394.00 ± 9.33
Standards								
Ascorbic acid	11.2 ± 0.49	2.69 ± 0.05	—	—	—	—	—	—
Rutin	0.51 ± 0.01	3.91 ± 0.10	36.66 ± 0.22	65.44 ± 2.56	—	—	—	—
BHA	—	—	24.88 ± 0.16	—	86.16 ± 4.04	—	—	—
α-Tocopherol	—	—	—	—	—	91.66 ± 4.92	3.418 ± 0.477	—

*Average of three determinations. **The total antioxidant capacity was expressed as mM equivalent of ascorbic acid by the phosphomolybdenum method.

extract were comparable to those of the standards used (Table 1). The total antioxidant capacity of the extract expressed as mM equivalent of ascorbic acid was found to be 4.365 ± 0.362, slightly less active than the standard α-tocopherol, 3.418 ± 0.477. The total phenol content of the extract was found to be 394.00 ± 9.33 mg/g, indicating that the antioxidant activity may be due to the high phenol content of the bark extract.

Cytotoxicity studies

MECA exhibited potent cytotoxicity against the cancerous HeLa, HEp-2, and RD cell lines with average IC_{50} values of 9.4, 16.0, and <62.5 μg/ml, respectively. However, against the normal Vero cell lines, the average IC_{50} value was found to be 127.2 μg/ml. This indicated that MECA possesses strong cytotoxicity against the cancerous cell lines, but is safe towards the normal cells.

DLA-induced ascitic antitumor studies

Effect on tumor growth: In the DLA tumor control group, the average life span of animals was found to be 20.83 ± 1.424 days (Fig. 1a). MECA at the doses of 250 and 500 mg/kg body weight reduced the average life span to 18.00 ± 1.693 and 17.83 ± 0.833 days, respectively. These values were nonsignificant. However, the average life span of 5-FU treatment was found to be 25.83 ± 1.682 days, indicating its potent antitumor nature. A significant increase in total WBC count in the MECA-treated animals, when compared to the DLA tumor control group, was also observed. The antitumor nature of MECA was evidenced by the significant reduction in percent increase in body weight of animals treated with MECA at the doses of 250 and 500 mg/kg body weight, when compared to DLA-tumor-bearing mice (Fig. 1b). It was also supported by the significant reduction in packed cell volume and viable tumor cell count in both the doses of extract treatment when compared to the DLA tumor control (Fig. 1: c and d).

Effect on hematological parameters: As shown in Table 2, RBC, Hgb, PLT, lymphocyte, and eosinophils were decreased and neutrophils count was significantly increased in the DLA control group as compared to the normal group. Treatment with MECA at both the doses significantly increased the Hgb content, RBC, PLT, lymphocyte, and eosinophils and significantly decreased the neutrophils count to about normal levels. All these results suggest the anticancer nature of the extract and among the two doses, the lower dose of 250 mg/kg body weight was found to be more potent. However, the standard 5-FU at the dose of 20 mg/kg body weight produced better results in all these parameters.

Effect on biochemical parameters: The inoculation of DLA cells caused significant decreases in the levels of ASAT and ALAT and significant increases in the levels of TGL, TP, TB, and TBARS in the serum of tumor control animals, when compared to the normal group. The treatment with MECA at 250 and 500 mg/kg body weight reversed these changes towards the normal levels (Table 3). Most of the values were found to be significant. The treatment with standard 5-FU also gave similar results.

The inoculation of DLA cells to tumor control animals caused significant increases in the levels of ASAT, ALAT, ALP, LDH, CR, TB, albumin, and TBARS in the liver and kidney when compared to normal animals. Significant decreases in the levels of TGL, TC, TP, and CAT was also observed in both the liver and kidney. Although a reduction in the level of SOD was observed in serum, liver, and kidney tissues of the tumor control group, the kidney value was found to be significant. The treatment with MECA at the doses of 250 and 500 mg/kg body weight reversed these changes towards the normal values (Tables 4 and 5). Most of the results were found to be significant. Almost similar results were observed for 5-FU treatment. Values lower than the normal were observed for both the doses of

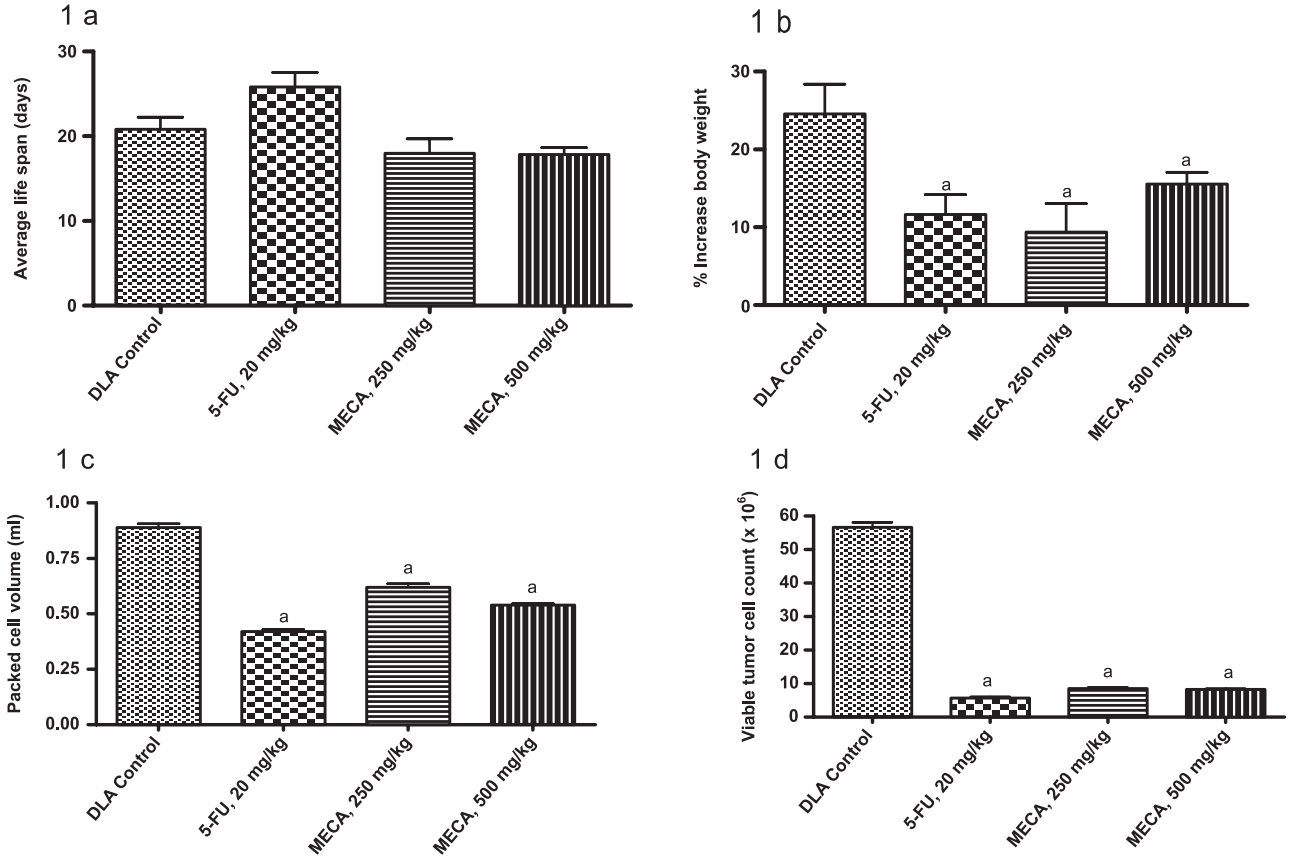

Fig. 1. Effect of MECA on DLA-tumor bearing mice on average life span (a), % increase in body weight on day 15 (b), packed cell volume on day 11 (c), tumor viable cell count on day 11 (d). Data are expressed as the mean ± S.E.M., (n = 6), [a]$P<0.001$ between control and treated groups.

Table 2. Effect of MECA on hematological parameters of DLA-bearing mice

Parameters	Treatment (Dose, mg/kg body weight)				
	Normal	DLA	DLA + 5-FU (20)	DLA + MECA (250)	DLA + MECA (500)
Total WBC (1×10^3/mm^3)	11.18 ± 0.193	14.56 ± 0.419[c]	7.50 ± 0.425[f]	15.74 ± 0.424	17.44 ± 0.624[f]
RBC (1×10^6/mm^3)	10.72 ± 0.148	7.79 ± 0.284[c]	9.75 ± 0.229[f]	10.90 ± 0.221[f]	9.79 ± 0.131[f]
Hgb (g/dl)	15.11 ± 0.342	11.65 ± 0.692[b]	13.91 ± 0.743	15.18 ± 0.828	14.28 ± 0.430
PLT (10^3/mm^3)	184.00 ± 4.626	147.50 ± 3.879[b]	222.83 ± 6.695[f]	170.50 ± 6.238	292.67 ± 10.902[f]
WBC Differential count (%)					
Lymphocytes	61.50 ± 2.661	30.75 ± 2.287[c]	65.00 ± 2.646[f]	60.00 ± 2.121[f]	60.50 ± 2.327[f]
Neutrophils	33.50 ± 1.708	65.25 ± 2.213[c]	42.75 ± 1.702[f]	38.25 ± 1.109[f]	41.50 ± 2.398[f]
Eosinophils	5.75 ± 0.322	4.00 ± 0.408[a]	5.75 ± 0.250[d]	5.00 ± 0.408	5.62 ± 0.375[d]

Values are expressed as the mean ± S.E.M., (n = 6) on day 11 of the experiment; [a]$P<0.05$, [b]$P<0.01$, and [c]$P<0.001$ between normal and tumor control; [d]$P<0.05$, [e]$P<0.01$, and [f]$P<0.001$ between tumor control and treated groups.

MECA treatment in the levels of ASAT, ALP, albumin, and TB in the liver and ASAT, ALAT, ALP, and albumin in the kidney. The increase in CAT levels for the extract treatment was found to be more than the normal levels in liver. The extract treatment at 250 mg/kg body weight was found to be more potent than the higher-dose treatment in restoring these changes towards the normal values.

Histological observations

Histological examination of the liver and kidney tissues under a light microscope was done to observe the

Table 3. Effect of MECA on hepatoprotective and antioxidant status of DLA-bearing mice serum

Parameters	Treatment (Dose, mg/kg body weight)				
	Normal	DLA	DLA + 5-FU (20)	DLA + MECA (250)	DLA + MECA (500)
ASAT (U/I)	93.33 ± 9.888	63.33 ± 7.601[a]	60.00 ± 1.826	85.00 ± 5.627	145.00 ± 5.627[f]
ALAT (U/I)	46.66 ± 2.108	29.83 ± 0.166[c]	24.00 ± 2.000	30.00 ± 2.582	35.00 ± 4.282
ALP (U/I)	94.33 ± 2.654	118.17 ± 3.487[a]	85.50 ± 5.766[e]	103.17 ± 7.087	94.00 ± 5.972[d]
LDH (U/I)	85.50 ± 4.440	117.83 ± 6.534[a]	76.66 ± 11.727[d]	114.17 ± 2.982	98.33 ± 7.397
TGL (mg/dl)	148.50 ± 15.716	218.67 ± 20.600[a]	136.00 ± 7.398[e]	158.50 ± 16.105[d]	149.17 ± 7.613[d]
CR (mg/dl)	1.06 ± 0.128	1.30 ± 0.159	1.03 ± 0.117	0.96 ± 0.122	1.11 ± 0.060
Albumin (mg/dl)	2.68 ± 0.253	2.70 ± 0.131	3.75 ± 0.232[d]	3.15 ± 0.319	2.54 ± 0.132
TP (g/dl)	6.66 ± 0.494	11.66 ± 1.256[b]	9.00 ± 0.816	6.33 ± 0.802[e]	6.00 ± 0.577[f]
TC (U/I)	229.83 ± 11.191	288.67 ± 8.413	190.00 ± 8.509[e]	212.83 ± 23.009[d]	226.50 ± 21.427
TB (mg/dl)	0.88 ± 0.090	2.26 ± 0.084[c]	0.83 ± 0.084[f]	0.76 ± 0.076[f]	0.98 ± 0.070[f]
CAT (U/ml serum)	0.16 ± 0.013	0.32 ± 0.010	0.22 ± 0.022	0.23 ± 0.072	0.16 ± 0.037
SOD (U/ml serum)	0.64 ± 0.017	0.43 ± 0.019	0.63 ± 0.045	1.03 ± 0.091[f]	0.83 ± 0.048[f]
TBARS (MDA nM/ml)	2.02 ± 0.200	4.16 ± 0.388[c]	2.71 ± 0.296[d]	2.56 ± 0.268[e]	2.58 ± 0.206[e]

Values are expressed as the mean ± S.E.M., (n = 6) on day 11 of the experiment; [a]$P<0.05$, [b]$P<0.01$, and [c]$P<0.001$ between normal and tumor control; [d]$P<0.05$, [e]$P<0.01$, and [f]$P<0.001$ between tumor control and treated groups.

Table 4. Effect of MECA on hepatoprotective and antioxidant status of DLA-bearing mice liver

Parameters	Treatment (Dose, mg/kg body weight)				
	Normal	DLA	DLA + 5-FU (20)	DLA + MECA (250)	DLA + MECA (500)
ASAT (U/I)	60.50 ± 1.857	100.37 ± 6.209[c]	49.93 ± 2.559[f]	58.13 ± 4.479[f]	65.80 ± 3.289[f]
ALAT (U/I)	89.55 ± 5.815	161.19 ± 11.750[c]	54.11 ± 3.779[f]	38.68 ± 7.469[f]	78.84 ± 6.729[f]
ALP (U/I)	179.31 ± 7.479	284.19 ± 21.516[c]	140.32 ± 7.320[f]	36.41 ± 2.019[f]	44.88 ± 4.646[f]
LDH (U/I)	124.12 ± 4.514	215.86 ± 11.946[c]	159.20 ± 15.168[e]	124.12 ± 9.030[f]	113.33 ± 2.956[f]
TGL (mg/dl)	63.05 ± 0.657	16.42 ± 1.077[c]	67.34 ± 3.259[f]	42.65 ± 1.251[f]	27.32 ± 2.468[e]
CR (mg/dl)	3.38 ± 0.217	6.06 ± 0.310[c]	4.48 ± 0.248[f]	2.60 ± 0.089[f]	3.27 ± 0.167[f]
Albumin (mg/dl)	3.48 ± 0.149	4.36 ± 0.083[a]	3.27 ± 0.106[e]	3.36 ± 0.043[e]	3.19 ± 0.331[f]
TP (g/dl)	1.24 ± 0.019	0.64 ± 0.190[b]	1.13 ± 0.047[d]	1.29 ± 0.083[f]	1.16 ± 0.039[e]
TC (U/I)	19.03 ± 0.531	9.06 ± 0.312[c]	14.58 ± 1.060[f]	16.22 ± 0.331[f]	11.05 ± 0.685
TB (mg/dl)	0.81 ± 0.076	1.23 ± 0.067[c]	0.82 ± 0.062[f]	0.68 ± 0.050[f]	0.73 ± 0.029[f]
CAT (U/mg tissue)	5.95 ± 0.196	3.47 ± 0.211[a]	7.11 ± 0.617[e]	6.73 ± 0.699[e]	6.82 ± 0.866[e]
SOD (U/mg tissue)	0.42 ± 0.013	0.29 ± 0.003	0.46 ± 0.111	0.43 ± 0.055	0.32 ± 0.039
TBARS (MDA nM/g)	4.68 ± 0.253	6.88 ± 0.296[b]	4.35 ± 0.418[e]	4.68 ± 0.526[d]	4.89 ± 0.401[d]

Values are expressed as the mean ± S.E.M., (n = 6) on day 11 of the experiment; [a]$P<0.05$, [b]$P<0.01$, and [c]$P<0.001$ between normal and tumor control; [d]$P<0.05$, [e]$P<0.01$, and [f]$P<0.001$ between tumor control and treated groups.

effects of MECA on the structural integrity of the cells. The liver of normal animals showed normal histological appearance (Fig. 2a). The tumor control animal liver showed slight enlargement of hepatocytes, dilated sinusoidal spaces containing lymphocytes, and portal triads showing collections of lymphocytes (Fig. 2b). The animals treated with standard 5-FU at 20 mg/kg body weight or MECA at 250 or 500 mg/kg body weight exhibited almost normal histological appearance of liver cells, except for a few lymphocytic collections in the portal area (Figs. 2: c and d). The animals treated with MECA at 500 mg/kg body weight also showed normal histology with no lymphocytes in the portal area, indicating its potent hepatoprotective action when compared to standard 5-FU treatment (Fig. 2e).

The kidney of normal animals showed normal histological appearance (Fig. 3a). The tumor control animals exhibited atrophied glomeruli and dilated renal tubules

Table 5. Effect of MECA on hepatoprotective and antioxidant status of DLA-bearing mice kidney

Parameters	Treatment (Dose, mg/kg body weight)				
	Normal	DLA	DLA + 5-FU (20)	DLA + MECA (250)	DLA + MECA (500)
ASAT (U/I)	66.89 ± 3.856	180.77 ± 10.839[c]	53.15 ± 1.473[f]	57.63 ± 2.819[f]	64.24 ± 2.861[f]
ALAT (U/I)	81.22 ± 7.727	147.37 ± 6.343[c]	40.19 ± 2.016[f]	35.41 ± 3.966[f]	60.82 ± 1.191[f]
ALP (U/I)	162.00 ± 8.304	205.37 ± 6.960[c]	111.27 ± 1.224[f]	90.63 ± 2.890[f]	81.43 ± 2.759[f]
LDH (U/I)	124.12 ± 6.153	250.85 ± 17.979[c]	175.39 ± 4.514[f]	124.12 ± 11.191[f]	180.78 ± 9.030[e]
TGL (mg/dl)	101.47 ± 3.127	65.93 ± 0.655[c]	111.40 ± 3.780[f]	87.40 ± 1.138[f]	91.33 ± 1.153[f]
CR (mg/dl)	2.58 ± 0.300	4.85 ± 0.167[c]	4.51 ± 0.199	3.33 ± 0.059[f]	2.28 ± 0.255[f]
Albumin (mg/dl)	3.90 ± 0.072	4.45 ± 0.132[a]	3.50 ± 0.148[f]	2.91 ± 0.084[f]	3.23 ± 0.084[f]
TP (g/dl)	4.03 ± 0.034	3.64 ± 0.033[c]	4.10 ± 0.031[f]	3.93 ± 0.080[e]	4.06 ± 0.064[f]
TC (U/I)	14.33 ± 0.187	3.96 ± 0.216[c]	4.67 ± 0.760	5.78 ± 0.905	5.77 ± 0.353
TB (mg/dl)	0.68 ± 0.122	1.53 ± 0.041[c]	0.76 ± 0.091[f]	0.65 ± 0.081[f]	0.70 ± 0.029[f]
CAT (U/mg tissue)	7.06 ± 0.680	3.32 ± 0.102[c]	6.36 ± 0.307[f]	5.49 ± 0.334[e]	5.39 ± 0.409[d]
SOD (U/mg tissue)	0.28 ± 0.022	0.10 ± 0.012[c]	0.26 ± 0.023[f]	0.20 ± 0.020[e]	0.27 ± 0.021[f]
TBARS (MDA nM/g)	2.17 ± 0.269	4.91 ± 0.251[c]	2.75 ± 0.246[f]	2.94 ± 0.163[f]	1.88 ± 0.258[f]

Values are expressed as the mean ± S.E.M., (n = 6) on day 11 of the experiment; [a]$P<0.05$, [b]$P<0.01$, and [c]$P<0.001$ between normal and tumor control; [d]$P<0.05$, [e]$P<0.01$, and [f]$P<0.001$ between tumor control and treated groups.

(Fig. 3b). However, the kidney of MECA treatment at both the doses and standard 5-FU treatment showed normal histological appearance. These findings clearly indicate that the liver and kidney tissues that were damaged by DLA inoculation showed recovery with MECA and 5-FU treatments (Fig. 3: c – e).

Effect on DLA-induced solid tumor

In the control animals, the solid tumor volume induced by DLA cells was found to be about 10 times increased from day 0 to day 34. However, the tumor volume was found to be only six, four, and two times increased in animals treated with MECA at 5 mg/kg body weight, MECA at 25 mg/kg body weight doses, and standard cisplatin, respectively, during these days. Significant reduction in solid tumor volumes was observed for the MECA treatment at 5 mg/kg body weight from day 16 onwards until the end of the experiment, when compared to the tumor control group. However, for treatment with the MECA dose of 25 mg/kg body weight and for the standard cisplatin at 2 mg/kg body weight, the significant reduction in solid tumor volume was seen from day 7 to the end of the experiment. About 30% and 50% reduction in tumor volume was observed for the lower and higher doses treatment of the extract, respectively, on day 34 of the experiment (Fig. 4). No toxic symptoms and death were observed in animals for both the doses of extract treatment.

HPTLC profile

Three compounds with R_f values of 0.02, 0.64, and 0.80 were found to be major components with percent areas of 58.79, 10.61, and 28.37, respectively. Reproducibility of peaks was observed.

Discussion

Plants have served as a good source of antitumor agents. Several studies have been conducted on herbs under a multitude of ethnobotanical grounds. A large number of plants possessing anticancer properties have been documented (3, 16, 18 – 21). Stem bark of *Careya arborea* was traditionally used in the treatment of tumors. The present investigation was carried out to evaluate the antitumor activity of the MECA in DLA-tumor-bearing mice.

The reliable criteria for judging the value of an anticancer drug is the prolongation of the life span of animals. In DLA-tumor-bearing mice, a regular rapid increase in ascitic tumor volume was observed (22). The DLA-bearing mice orally administered MECA at 250 or 500 mg/kg body weight showed no significant change in the average life span compared to animals of the tumor control group. However, the percent increase in body weight, packed tumor cell volume, and number of viable tumor cells were found to be significantly less than the tumor control animals, indicating the anticancer nature of the extract. These results could indicate either a direct cytotoxic effect of MECA on tumor cells as evidenced by the in vitro studies or an indirect local

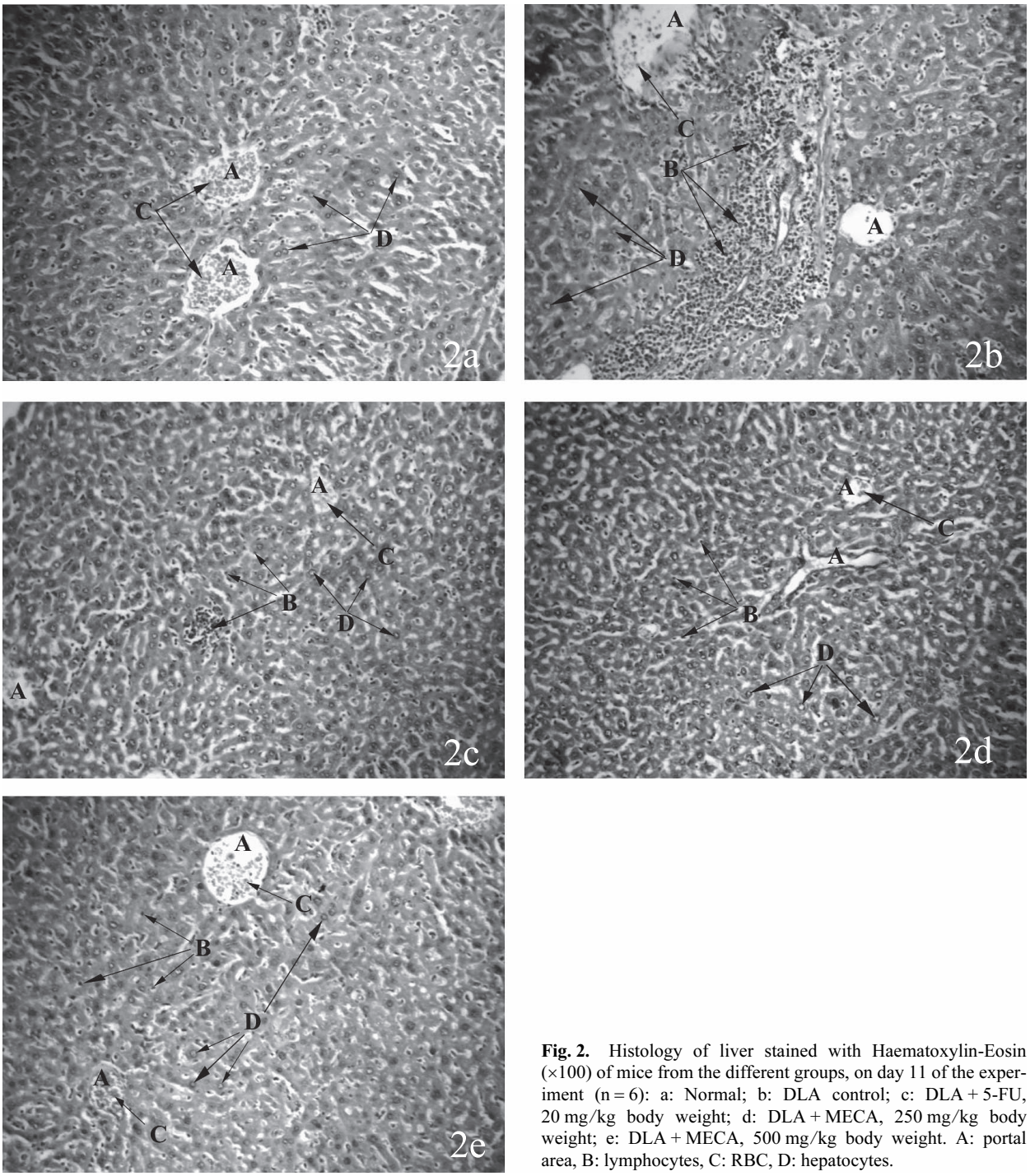

Fig. 2. Histology of liver stained with Haematoxylin-Eosin (×100) of mice from the different groups, on day 11 of the experiment (n = 6): a: Normal; b: DLA control; c: DLA + 5-FU, 20 mg/kg body weight; d: DLA + MECA, 250 mg/kg body weight; e: DLA + MECA, 500 mg/kg body weight. A: portal area, B: lymphocytes, C: RBC, D: hepatocytes.

effect, which may involve macrophage activation and vascular permeability inhibition. Hence, the observed antitumor nature of MECA may be due to the cytotoxic properties.

To investigate if the inhibitory effect of MECA on DLA tumor was local or systemic, the effect of i.p. injection of MECA in another experimental system, DLA-induced solid tumor, was tested. The solid tumor was inhibited by treatment with MECA, suggesting that the inhibitory effect is related not only to a local cytotoxic effect but also with the systemic effect of MECA.

The reversal of Hgb content, RBC, platelets, and differential count of WBC by the MECA treatment towards the values of the normal group clearly indicates that MECA possessed protective action on the haemopoietic system. However, the elevation of WBC

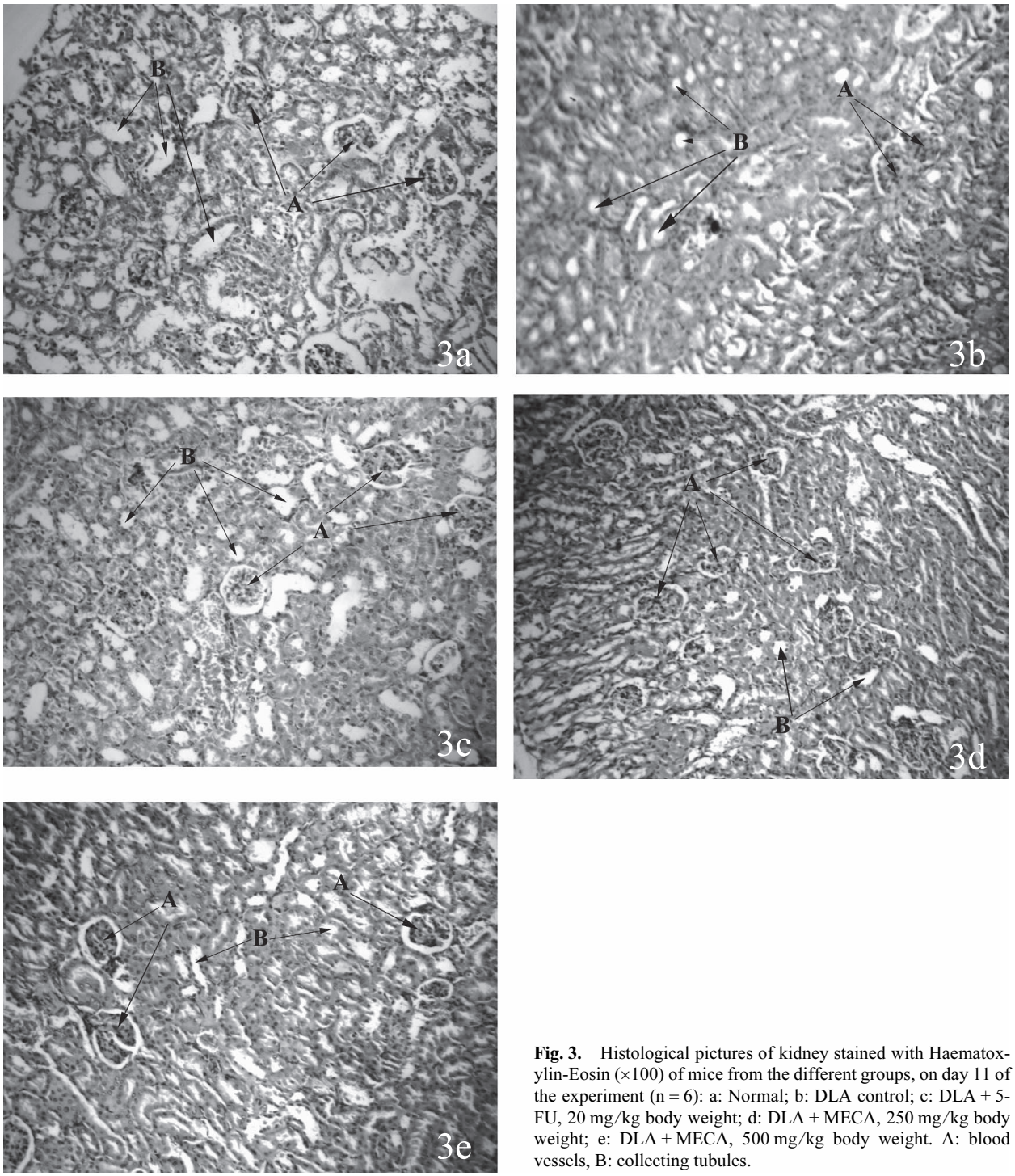

Fig. 3. Histological pictures of kidney stained with Haematoxylin-Eosin (×100) of mice from the different groups, on day 11 of the experiment (n = 6): a: Normal; b: DLA control; c: DLA + 5-FU, 20 mg/kg body weight; d: DLA + MECA, 250 mg/kg body weight; e: DLA + MECA, 500 mg/kg body weight. A: blood vessels, B: collecting tubules.

levels may be due to its adverse effect on the haemopoietic system (23).

It was reported that the presence of tumors in the human body or in experimental animals is known to affect many functions of the liver and kidney. The significantly elevated levels of ALP, LDH, TB, and TBARS and decreases in the levels of SOD in serum, liver, or kidney of tumor-inoculated animals indicated liver damage and loss of functional integrity of cell membranes (16). This was also indicated by the significant increase in the levels of ASAT, ALAT, CR, and albumin and decreases in TGL, TP, TC, and CAT in the liver and kidney tissues of the tumor control group. The significant reversal of these changes towards the normal

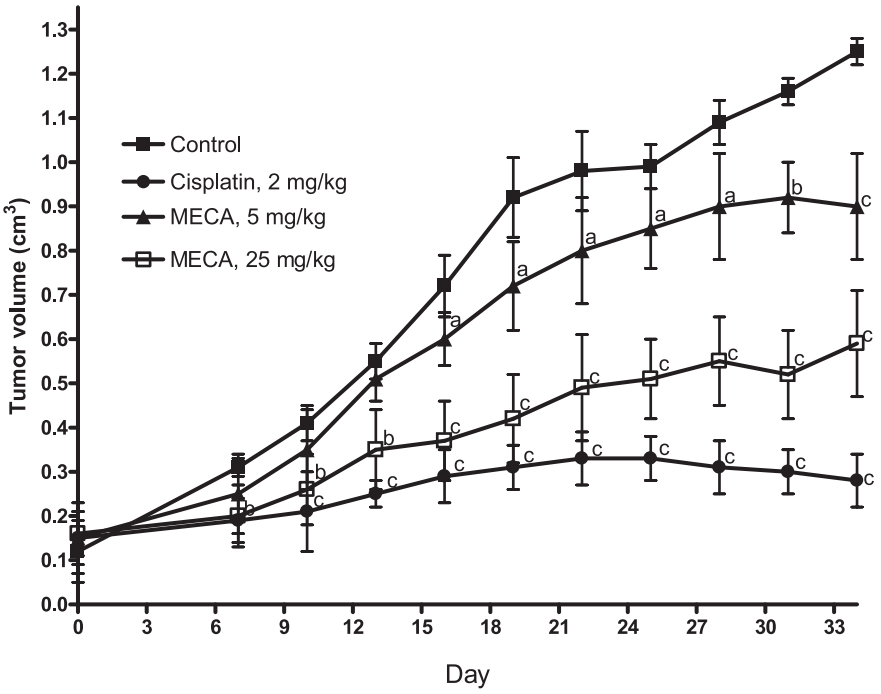

Fig. 4. Effect of MECA on DLA-induced solid tumor. Values are expressed as the mean ± S.E.M., (n = 6) group. [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$: treated group compared with the DLA control group.

by MECA treatment in most of the cases demonstrated the potent hepatoprotective and antioxidant nature of MECA. The antioxidant nature of MECA was also evident by the in vitro studies. Plants with high total phenol content are known to possess strong antioxidant properties (24). The observed antioxidant activity may be due to the high phenolic content of the extract.

Hepatocellular necrosis leads to high levels of ASAT and ALAT, which are released from liver into the blood. ALP activity, on the other hand, is related to the functioning of hepatocytes. Increase in its activity is due to increased synthesis in the presence of increased biliary pressure (25). Reduction in the levels of these towards the respective normal values in liver and kidney tissues is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by tumor inoculation.

Liver damage leads to the accumulation of fat and necrosis in the centrilobular region of the liver. As a consequence, the microsomal enzyme activities are found to decrease and due to lipid peroxidation, the water soluble enzymes leak into plasma from the liver. It is shown by the significant decrease in triglycerides and proteins in the liver and kidney of the tumor-inoculated animals (22). These changed parameters were restored towards the normal levels by MECA treatment, indicating its hepatoprotective nature. The decreased bilirubin level brought about by the MECA treatment indicates the normal functional conditions of the liver.

It was observed that tumor cells produced more peroxides when they proliferate actively after inoculation of tumor. This rise in peroxides indicated the occurrence of intensification of oxygen free radical production (26). Cells which are equipped with enzymatic antioxidant mechanisms play an important role in the elimination of free radicals. High levels (up to 0.05 μmol/h per 10^4 cells) of H_2O_2 are constitutively released from a wide variety of human tumors (27). SOD and CAT are involved in the clearance of superoxide and H_2O_2. Decrease in SOD and CAT activities described in tumors is regarded as markers of malignant transformation. Lowered activities of SOD and CAT were reported in several cancers (28). The significant elevation of SOD and CAT by the extract treatment confirms the potent antioxidant activity of the bark.

The elevation of lipid peroxidation is known to be associated with cancer. MDA, the end product of lipid peroxidation, was reported to be higher in cancer tissue than in normal tissues (29). TBARS levels in the blood and the tested tissues in tumor control animals were found to be higher than those in normal animals. The MECA treatment caused a significant reduction in TBARS levels in all the samples tested. This indicates the reduction in free radical yield and subsequent decrease in harm and damage to the cell membrane and decrease in MDA production.

In the present study, the histological examination of the liver and kidney of DLA-inoculated animals showed marked changes indicating the toxic effects of this tumor. The normalization of these effects observed in the tissues treated with MECA supported the potent hepatoprotective and antioxidant effects of the extract.

In an earlier study (7), MECA was found to be nontoxic and didn't cause any death of mice up to 1.6 g/kg body weight, indicating the safety of the treatment. The hepatoprotective and in vivo antioxidant effects of MECA against CCl_4-induced liver damage were also reported (7). Similar results were also obtained in the present study. MECA may cause firstly an antitumor effect and then influence biochemical parameters. Plant-derived extracts containing antioxidant principles with cytotoxicity towards tumor cells and antitumor activity in experimental animals were reported (17). Plants with potent antitumor, hepatoprotective, and antioxidant properties were also reported (16). The preliminary phytochemical studies of MECA indicated the presence of several triterpenoids, saponins, flavonoids, tannins, glycosides, and so on. The observed antitumor, hepatoprotective, and antioxidant activities may be due to the presence of any of these compounds in MECA.

In conclusion, the methanol extract of *Careya arborea* bark was effective in inhibiting the tumor growth in ascitic and solid tumor models. The biochemical and histological studies supported its antioxidant and hepatoprotective properties. The plant merits further investigation in an ascitic model at low doses and to elucidate its mechanism of action and isolation of its active constituents.

Acknowledgments

The authors thank Dr. Ramadasan Kuttan, Director, Amala Cancer Research Centre, Thrissur, Kerala. One of the authors, SKN thanks the All India Council of Technical Education, New Delhi for a fellowship awarded under the Quality Improvement Programme; and SHD thanks the Department of Biotechnology, Government of India for the awards of Junior and Senior Research Fellowships.

References

1 Gordon MC, Newman DJ. Natural product drug discovery in the next millennium. Pharm Biol. 2001;39:8–17.
2 Premalatha B, Rajgopal G. Cancer – an ayurvedic perspective. Pharmacol Res. 2005;51:19–30.
3 Spiridon KE. Terrestrial plant-derived anticancer agents and plants used in anticancer research. Crit Rev Plant Sci. 2006; 25:79–113.
4 Anonymous. The wealth of India – A dictionary of Indian raw materials and industrial products. New Delhi: CSIR; 1992. p. 273–276.
5 Kirtikar KR, Basu BD, editors. Indian medicinal plants. 2nd ed. Dehradun, India: Bishen Sing Mahendra Pal Sing; 1987. p. 1061–1063.
6 Rahman MT, Khan OF, Saha S, Alimuzzaman M. Antidiarrhoeal activity of the bark extract of *Careya arborea* Roxb. Fitoterapia, 2003;74:116–118.
7 Sambath Kumar R, Sivakumar T, Sivakumar P, Nethaji R, Vijayabaskar M, Perumal P, et al. Hepatoprotective and *in vivo* antioxidant effects of *Careya arborea* against carbon tetrachloride induced liver damage in rats. Int J Mol Med Adv Sci. 2005;1:418–424.
8 Row LR, Sastry CSP. Chemical examination of *Careya arborea* Roxb. Ind J Chem. 1964;2:510–514.
9 Badami S, Prakash O, Dongre SH, Suresh B. *In vitro* antioxidant properties of *Solanum pseudocapsicum* leaf extracts. Ind J Pharmacol. 2005;37:251–252.
10 Sudheer Kumar M, Jagadish PC, Sridhar Reddy B, Kiran Babu S, Unnikrishnan MK. *In vitro* evoluation of antioxidant properties of *Cocus nucifera* L. extracts. Nahrung/Food. 2003;47:126–131.
11 Huong NTT, Matsumato K, Kasai R, Yamasaki K, Watanabe H. *In vitro* antioxidant activity of Vietnamese ginseng saponin and its components. Biol Pharm Bull. 1998;21:978–981.
12 Vijayan P, Vinod Kumar S, Dhanaraj SA, Mukherjee PK, Suresh B. *In vitro* cytotoxicity and antitumour properties of *Hypericum mysorense* and *Hypericum patulum*. Phytother Res. 2003;17: 952–956.
13 Beers RF Jr, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem. 1952;195:133–140.
14 Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and simple assay for superoxide dismutase. J Biol Chem. 1972;247:3170–3175.
15 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissue by of thiobarbituric acid reaction. Anal Biochem. 1979;95:351–358.
16 Gupta M, Mazumder UK, Sambath kumar R, Sivakumar T, Vamsi MLM. Antitumor activity and antioxidant status of *Caesalpinia bonducella* against *Ehrlich ascites carcinoma* in Swiss albino mice. J Pharmacol Sci. 2004;94:177–184.
17 Kumar KBH, Kuttan R. Chemoprotective activity of an extract of *Phyllanthus amarus* against cyclophosphamide induced toxicity in mice. Phytomedicine. 2005;12:494–500.
18 Kim JB, Koo HN, Joeng HJ, Lyu YS, Park SG, Won JH, et al. Induction of apoptosis by Korean medicine *Gagam-whanglyun-haedoktang* through activation of caspase-3 in human leukemia cell line, HL-60 cells. J Pharmacol Sci. 2005;97:138–145.
19 Aoki K, Yamakuni T, Yoshida M, Ohizumi Y. *Ephedrae herba* decreases lipopolysaccharide-induced cyclooxgenase-2 protein expression and NF-κB-dependent transcription in C6 rat glioma cells. J Pharmacol Sci. 2005;98:327–330.
20 Saito SY, Maruyama Y, Kamiyama S, Nakahata N, Ohizumi Y. *Ephedrae herba* in *Mao-Bushi-Saishin-To* inhibits IgE-mediated histamine release and increases cAMP content in RBL-2H3 cells. J Pharmacol Sci. 2004;95:41–46.
21 Shimizu M, Azuma C, Taniguchi T, Murayama T. Expression of cytosolic phospholipase $A_2\alpha$ in murine C12 cells, a variant of L929 cells, induces arachidonic acid release in response to

phorbol myristate acetate and Ca^{2+} ionophores, but not to tumor necrosis factor-*α*. J Pharmacol Sci. 2004;96:324–332.

22 Badami S, Reddy SAM, Kumar EP, Vijayan P, Suresh B. Antitumor activity of total alkaloid fraction of *solanum pseudocapsicum* leaves. Phytotherapy Res. 2003;17:1001–1004.

23 Bansal AK, Bansal M, Soni G, Bhanagar D. Protective role of vitamin E pretreatment on N-nitrosodiethylamine induced oxidative stress in rat liver. Chem Biol Interact. 2005;156:101–111.

24 Lee J, Koo N, Min DB. Reactive oxygen species, aging and antioxidant nutraceuticals. Comp Rev Food Sci Food Saf. 2004;3:21–32.

25 Moss DW, Butterworth DJ. Editors. Enzymology: biochemistry biophysics and medicine. London: Pitman Medical; 1974. p. 139.

26 Navarro J, Obrador E, Pellicer JA, Asensi M, Vina J, Estrela JM. Blood glutathione as an index of radiation-induced oxidative stress in mice and humans. Free Radic Biol Med. 1997;22:1203–1209.

27 Szatrowski TP, Nathan CF. Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Res. 1991;51:794–798.

28 Kavitha K, Manoharan S. Anticarcinogenic and antilipidperoxidative effects of *Tephrosia purpurea* (Linn) pers. in 7,12-dimethyl benz(a)anthracene (DMBA) induced hamster buccal pouch carcinoma. Ind J Pharmacol. 2006;38:185–189.

29 Valenzuela A. The biological significance of malondialdehyde determination in the assessment of tissue oxidative stress. Life Sci. 1990;48:301–309.