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Antitumor effect of *Careya arborea* against Ehrlich ascites carcinoma with reference to lipid peroxidation and enzymatic and non enzymatic antioxidant system in Swiss albino mice

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SUMMARY

The methanol extract of stem barks of *Careya arborea* Roxb. (MECA) (Family- Myrtaceae) was evaluated for antitumor activity and antioxidant status against Ehrlich's Ascites Carcinoma (EAC) bearing Swiss albino mice. After 24 h of tumor inoculation the MECA was administered at the doses of 50, 100 and 200 mg/kg body weight/mice/day for 14 days. After the last dose and 18 h fasting mice were sacrificed. The effect of MECA on the growth of transplantable murine tumor, life span of EAC bearing hosts, hematological profiles, serum and liver biochemical parameters were estimated. The MECA showed significant ($P < 0.01$) decrease in ascites volume, packed cell volume and viable cell count and prolonged the life span of EAC tumor bearing mice. Hematological profiles reverted to more or less normal levels in extract treated mice. The MECA also produced protective effect by decreasing the activity of serum enzymes, bilirubin and increase the protein and uric acid levels. MECA significantly ($P < 0.05$) decreased the levels of lipid peroxidation, while significantly ($P < 0.05$) increased the levels of glutathione content, vitamin C, vitamin E, superoxide dismutase and catalase CAT. The results indicate that MECA exhibited significant antitumor and antioxidant activity in EAC bearing mice.

Key words: *Careya arborea*; Ehrlich's ascites carcinoma; Antitumor activity; Hematological parameters; Antioxidants

INTRODUCTION

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 Sidha. Only a few of them have been scientifically explored. Plant derived natural products such as flavonoids, terpenes, alkaloids etc., (Keith *et al.*, 1990; Osawa *et al.*, 1990; Giulia *et al.*, 1999) have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemopreventive effects (Roja and Heble, 1994).

Careya arborea commonly known as Wild Guava

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belongs to the family Myrtaceae medium sized deciduous tree, dark grey exfoliating in thin strip of bark which is widely available in India, Ceylon, Malay and Peninsula. The plant has been extensively investigated and a number of chemical constituents from the barks, leave and seeds of the plant have previously reported which includes triterpenoids (Mahati *et al.*, 1973; Ramachandra and Prakash, 1976; Das and Mahato, 1982), flavonoids (Gupta *et al.*, 1975), coumarin (Mahato and Dutta, 1972; Basak *et al.*, 1976), saponins (Gedeon and Kinel, 1956) and tannins (Kulakkattolickal, 1987).

Stem barks of *Careya arborea* was traditionally used in the treatment of tumors, anthelmintic, bronchitis, epileptic fits, astringents, antidote to snake-venom and skin disease (Kirtikar and Basu, 1975). It also used as remedy for diarrhea (Sikarwar *et al.*, 1994), dysentery with bloody stools and ear pain (Girach *et al.*, 1994; Bhandary *et al.*, 1995). Antipyretic (Das and Mahato, 1982), leech repellent, fish poison and antivenin activities were also reported in literature (Talapatra *et al.*, 1981; John, 1984; Selvanayahgam *et al.*, 1994). The aqueous extract of fresh root bark used as fish poison (Gedeon and Kinel, 1956). The tribal peoples of Kolli Hills of Tamil Nadu used the stem bark of the plant for the treatment of various tumor and liver disorders. Pervious report from our laboratory showed hepatoprotective and antioxidant (Sambath Kumar *et al.*, 2005a), antimicrobial and *in vitro* antioxidant activity (Sambath Kumar *et al.*, 2006) and anti-inflammatory and analgesic activity of methanol extract of stem barks of Careya arborea Roxb. (MECA) (Sambath Kumar *et al.*, 2005b). Based on the previous report, traditional usage and chemical constituents the MECA was selected for the present study.

MATERIALS AND METHODS

Plant materials and extraction

The plant *Careya arborea* (Family: Myrtaceae) stem bark was collected in March 2004 from the Kolli Hills, Tamil Nadu, India. The plant material was taxonomically identified by Botanical Survey of India, Kolkata, India, and the Voucher specimen (No. GMS-3) was retained in our laboratory for the future reference. The dried powder material (500 g) of the stem bark of *Careya arborea* was extracted with 2,000 ml of methanol in a soxhlet apparatus. The methanol extract was then distilled, evaporated and dried in vacuum. The resulted extract yield was 7.45%, and the appearance of the extract was dried gum resin in nature. The chemical constituents of the extract were identified by qualitative analysis followed by their confirmation by thin layer chromatography, which indicate the presence of flavonoids, triterpenoids and steroids.

Animals

Studies were carried out using male Swiss albino mice weighing 20 ± 2 g. They were obtained from the animal house, Indian Institute of Chemical Biology (IICB), Kolkata, India. The mice were group housed in polyacrylic cages (38 × 23 × 10 cm) with not more than twelve animals percage and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The mice were acclimatized to laboratory condition for 10 days before commencement of experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee.

Chemicals

The following chemical were obtained from the indicated commercial sources:1-Chloro-2,4-dinitrobenzene (CDNB), Bovine serum albumin (Sigma Chemical, Co St. Louis, MO, USA), Thiobarbituric acid Nitroblue tetrazolium chloride (Loba Chemie, Bombay, India), 5, 5'-dithio *bis*-2-nitrobenzoic acid (DTNB) (Sisco Research Laboratory, Bombay, India). Reduced glutathione, Ascorbic acid, 2,4-dinitrophenylhydrazine (DNPH), ferric chloride 2,2-dipyridyl-*p*-phenylenediamine

hydrochloride (S.D. Fine Chemicals, Mumbai, India). All the chemicals used in the present study are of analytical grade.

Tumor Cells

Ehrlich's ascites carcinoma (EAC) cells were obtained from Chittaranjan National Cancer Institute, Kolkata, India. The EAC cells were maintained by intraperitoneal inoculation of 2×10^6 cells/mouse.

Toxicity study

The toxicity studies of the test extracts in the dose ranges of 100 - 1,600 mg/kg were administered to five groups, each consisting of 10 mice respectively. The mortality rates were observed after 72 h. The determination of LD_{50} was performed by the method of Litchfield and Wilcoxon (Litchfield and Wilcoxon, 1949).

Antitumor activity

Male Swiss albino mice were divided into 6 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. From the first day normal saline 5 ml/kg/mouse/day and 10% propylene glycol 5 ml/kg/mouse/day were administered to normal and EAC control groups respectively for 14 days intraperitoneally. Similarly MECA at different doses (50, 100 and 200 mg/kg/mouse/day) and standard drug 5-Fluorouracil (20 mg/kg) were prepared by dissolving the extract and standard drug in 10% propylene glycol to administered in groups 3, 4, 5 and 6 respectively. After the administration of last dose followed by 18 h fasting six mice from each group were sacrificed for the study of antitumor activity, hematological and biochemical parameters. The remaining animals in each of the groups were kept to check the mean survival time (MST) of the tumor bearing hosts.

Tumor growth response

Antitumor effect of MECA was assessed by change in the body weight, ascites volume, packed cell volume, viable and nonviable tumor cell count (Khanam *et al*., 1997), MST, 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 (Mazumder *et al*., 1997; Gupta *et al*., 2000).

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

ILS (%) = (Mean survival of treated group - Mean survival day of control group)/Mean survival day of control group 100.

Hematological studies

The blood was obtained from all animals by puncturing retro-orbital plexus. One part of the blood was used for the estimation of hemoglobin content, Red blood cell (RBC) and White blood cell (WBC) counts (Wintrobe *et al*., 1961; D'Armour *et al*., 1965). WBC differential leukocyte count was carried out from Leishman stained blood smears (Dacie and Lewis, 1958) of normal, EAC control and MECA treated groups respectively.

Estimation of biochemical parameters

Another part of the blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2,500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters SGPT, SGOT (Bergmeyer *et al*., 1978), SALP (King, 1965), serum bilirubin (Malloy and Evelyn, 1937), protein content (Lowry *et al*., 1951) and plasma uric acid (Caraway, 1963).

After collection of blood samples the mice were sacrificed and their livers excised, rinsed in ice cold normal saline followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. A 10 w/v% of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of LPO by the method of Ohkawa *et al.* (1973). A part of homogenate after precipitating proteins with Trichloroacetic acid

was used for estimation of glutathione by the method of Ellman (1959). Vitamin E by Quaife and Dju (1948), with slight modification by Baker and Frank (1951) and vitamin C by Omaye *et al.* (1979) were also estimated. The rest of the homogenate was centrifuged at 15,000 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of SOD by the method of Kakkar *et al.* (1984) and CAT activities were measured by the method of Aebi (1974).

Statistical analysis

The experimental results were expressed as mean± S.E.M. Data were assessed by the method of analysis of (ANOVA), P value of < 0.05 was considered as statistically significant.

RESULTS

Toxicity study

The LD_{50} value of intraperitoneal administration of MECA in mice was found to be 955 mg/kg body weight (log 2.98 and antilog 955).

The chemical constituents of the extract were identified by qualitative analysis followed by confirmation by thin layer chromatography. The extract showed a positive Shinoda test for flavonoids, a positive Liebermann-Burchard reaction for steroids, and a positive Noller test for triterpenoids. These results were confirmed by silica gel G thin layer chromatography using the solvent system benzene-ethyl acetate (1 : 1). Further separation of the specific phytochemicals is in progress in our laboratory.

The present investigation indicates that the MECA showed significant antitumor and antioxidant activities in EAC bearing mice. The results are summarized in Tables 1-4. The effects of MECA at the doses of 50, 100 and 200 mg/kg on survival time, percentage increase in life span (% ILS), ascites volume, packed cell volume and tumor cell count (viable and non-viable cell) are shown in Table 1.

Effect on mean survival time

In the EAC control group the mean survival time was 18.24 days, while it increased to 23.53 (50 mg/kg), 28.62 (100 mg/kg) and 34.12 (200 mg/kg) days respectively with MECA treated groups, whereas the standard drug 5-Fluoruracil (20 mg/kg) treated group shows 38.44 days for the same.

Effect on tumor growth

Treatment with MECA at the doses of 50, 100 and 200 mg/kg significantly (P < 0.01) reduced the ascites volume, packed cell volume and viable tumor cell count in a dose dependent manner as

Table 1. Effect of MECA on mean survival time, % ILS, ascites volume, packed cell volume, viable and non-viable tumor cell count of EAC treated mice

Parameters	EAC Control (2×10^6 cells/ mouse/ml)	MECA (50 mg/kg) + EAC	MECA (100 mg/kg) + EAC	MECA (200 mg/kg) + EAC	Standard 5 - flourouracil (20 mg/kg) + EAC
Body weight (g)	28.42 ± 1.18	25.45 ± 1.20	24.72 ± 1.16	23.75 ± 1.14	22.28 ± 1.13
Mean survival time (days)	18.24 ± 0.59	23.53 ± 1.21**	28.62 ± 1.34**	34.12 ± 1.72**	38.44 ± 2.25**
Increase life Span (%)	-	24.44	58.88	87.22	119.49
Ascites volume (ml)	5.11 ± 0.27	4.03 ± 0.24**	3.22 ± 0.12**	1.74 ± 0.09**	Nil
Packed cell volume (ml)	2.45 ± 0.08	1.78 ± 0.09**	1.06 ± 0.05**	0.42 ± 0.02**	Nil
Viable tumor cell count $\times 10^7$ cells/ml	13.72 ± 0.67	9.33 ± 0.46**	5.51 ±0.34**	1.81 ± 0.06**	Nil
Nonviable tumor cell count $\times 10^7$ cells/ml	0.41 ± 0.02	0.74 ± 0.04**	0.89 ± 0.05**	1.54 ± 0.03**	Nil

Values are mean ± S.E.M. (n= 6). Extract treated groups compared with EAC control group (**P < 0.01).

Table 2. Effect of MECA on hematological parameters of EAC treated mice

Parameters	Normal (saline5 ml/ kg)	EAC Control (2×10^6cells / mouse/ml)	MECA (50 mg/kg) + EAC	MECA (100 mg/kg) +EAC	MECA (200 m g/kg) + EAC	Standard 5-flourouracil (20 mg/kg) + EAC
Hemoglobin (%)	13.7 ± 0.78	10.9 ± 0.41***	11.7 ± 0.57** (28.57)	12.8 ± 0.59** (67.85)	13.9 ± 0.68** (107.52)	13.4 ± 0.72 (89.28)
RBC ($\times 10^9/\mu l$)	6.9 ± 0.31	3.3 ± 0.19***	4.2 ± 0.22** (25.00)	5.2 ± 0.23** (52.77)	6.8 ± 0.35** (97.22)	6.7 ± 0.35 (94.44)
WBC ($\times 10^6/\mu l$)	5.2 ± 0.29	19.2 ± 1.13***	14.2 ±. 0.78** (35.71)	10.1 ± 0.43** (65.00)	5.9 ± 0.26** (95.00)	± 0.24 (97.85)
Monocyte (%)	1.7 ± 0.03	1.2 ± 0.01***	1.4 ± 0.02** (40.00)	1.6 ± 0.03** (80.00)	1.8 ± 0.04** (120.00)	± 0.02 (80.00)
Neutrophil (%)	18.9 ± 0.66	64.1 ± 3.17***	46.6 ± 2.19** (38.71)	40.3 ± 2.14** (52.62)	35.5 ± 1.82** (63.27)	19.1 ± 0.45 (99.55)
Lymphocyte (%)	79.4 ± 4.23	34.7 ± 2.42***	52.0 ± 3.35** (39.37)	58.1 ±. 3.33** (52.34)	63.7 ± 3.26** (64.87)	79.3 ± 3.21 (90.82)

The data in the parenthesis indicate percent protection in individual hematological parameters from their elevated values caused by the EAC. The % of protection is calculated as 100 × (values of EAC control - values of sample)/(values of EAC control - values of vehicle control). Values are mean ± S.E.M. (n = 6). EAC control group compared with normal group (***$P < 0.001$). Extract treated groups compared with EAC control group (**$P < 0.01$).

compared to that of EAC control group. Further, nonviable tumor cell count at different doses of MECA was significantly increased ($P < 0.01$), when compared with the EAC control. The animals treated with standard drug (5 - Fluorouracil) showed complete absence of tumor volume on 14th day and, hence the viable cell and non-viable cell count could not be performed.

Effect on hematological parameter

As shown in Table 2, hemoglobin content and RBC count in the EAC control group was significantly ($P < 0.001$) decreased as compared to normal group. Treatment with MECA at the doses of 50, 100 and 200 mg/kg significantly ($P < 0.01$) increased and brought back the hemoglobin content and RBC count to near normal levels. The total WBC count was found to be increased significantly in EAC control group when compared with normal group ($P < 0.001$). Administration of MECA at the doses of 50, 100 and 200 mg/kg in EAC bearing mice were significantly ($P < 0.01$) reduced the WBC count as compared with EAC control. In a differential count of WBC, the presence of neutrophils increased, while the lymphocyte count decreased in EAC control group, treatment with MECA at different doses and 5-Fluorouracil changed these altered parameters more or less near normal.

Effect on biochemical parameters

Alteration in the activities of SGPT, SGOT, SALP, bilirubin, uric acid and total protein content in the serum of EAC bearing mice as evidence from Table 3. The level of serum marker enzymes SGPT, SGOT, SALP, bilirubin and uric acid were found to be significantly increased and protein content significantly decreased in EAC control group when compared with the normal group ($P < 0.01$). Where as treatment with MECA at the dose of 50, 100 and 200 mg/kg and 5-Fluorouracil showed decreased the activity of SGPT, SGOT, SALP, uric acid, bilirubin and increased the protein content in extract treated mice when compared to that of control group ($P < 0.05$).

The liver biochemical parameter of EAC treated mice are summarized in Table 4, levels of lipid peroxidation (n moles of MDA/mg of protein) in liver tissue were significantly increased in EAC control group as compared to the normal group (*P*

Table 3. Effect of MECA on serum enzymes (SGPT, SGOT and SALP), bilirubin, protein and uric acid in EAC treated mice

Parameters	Normal (Normal saline 5 ml/kg, b.wt)	EAC Control (0.2 ml of 2 × 10^6 cells/ml)	MECA (50 mg/kg) + EAC	MECA (100 mg/kg) + EAC	MECA (200 mg/kg) + EAC	Standard 5 - flourouracil (20 mg/kg) + EAC
SGPT (U/l)	52.15 ± 2.21	107.51 ± 5.92**	92.15 ± 4.41* (27.74)	69.53 ± 3.78* (68.60)	55.54 ± 2.12* (93.87)	53.13 ± 2.42 (98.22)
SGOT (U/l)	62.52 ± 3.28	138.21 ± 7.12**	113.51 ± 8.53* (32.63)	82.52 ± 4.42* (73.57)	67.41 ± 3.24* (93.53)	64.53 ± 3.12 (97.34)
SALP (U/l)	67.42 ± 2.35	118.51 ± 6.02**	106.31 ± 5.85* (23.87)	88.75 ± 4.90* (58.25)	72.54 ± 3.84* (89.97)	69.52 ± 3.65 (95.88)
Bilirubin (mg/dl)	0.95 ± 0.04	2.54 ± 0.15**	2.12 ± 0.12* (26.41)	1.53 ± 0.04* (88.67)	1.02 ± 0.03* (95.59)	0.98 ± 0.02 (98.11)
Protein (mg/dl)	7.03 ± 0.31	5.45 ± 0.22**	5.99 ± 0.34* (36.24)	6.43 ± 0.33* (65.77)	6.98 ± 0.37* (95.97)	7.01 ± 0.31 (98.73)
Uric acid (mg/dl)	2.81 ± 0.14	1.43 ± 0.10**	2.02 ± 0.05* (64.49)	2.41 ± 0.15* (71.01)	2.68 ± 0.16* (90.57)	2.74 ± 0.14 (94.92)

The data in the parenthesis indicate percent protection in individual biochemical parameters from their elevated values caused by the EAC. The % of protection is calculated as 100 × (values of EAC control - values of sample) / (values of EAC control - values of vehicle control). Values are mean ± S.E.M. (n = 6). Control group compared with normal group (** $P < 0.01$). Extract treated groups compared with EAC control group (* $P < 0.05$).

Table 4. Effect of the MECA on lipid peroxidation (LPO), antioxidant enzymes (SOD and CAT) and non enzymatic antioxidant (GSH, vitamin C and vitamin E) in EAC treated mice

Parameters	Normal (Normal saline 5 ml/kg, b.wt)	EAC Control (0.2 ml of 2 × 10^6 cells/ml)	MECA (50 mg/kg) + EAC	MECA (100 mg/kg) + EAC	MECA (200 mg/kg) + EAC	Standard 5 - flourouracil (20 mg/kg) + EAC
Lipid peroxidation (n mole of MDA/ mg protein)	0.98 ± 0.04	1.71 ± 0.06**	1.66 ± 0.04* (6.84)	1.42 ± 0.05* (39.72)	1.22 ± 0.07* (67.12)	1.07 ± 0.04 (87.67)
Glutathione content (μg/mg of oprotein)	5.40 ± 0.29	2.70 ± 0.05**	2.90 ± 0.08* (7.40)	3.45 ± 0.12* (27.77)	4.15 ± 0.28* (53.70)	5.32 ± 0.21 (97.03)
Vitamine C (mg/g/wet tissue)	1.59 ± 0.03	0.52 ± 0.03**	0.81 ± 0.04* (27.10)	1.14 ± 0.08* (56.21)	1.48 ± 0.09* (89.71)	1.53 ± 0.03 (94.39)
Vitamine E (mg/g/wet tissue)	4.17 ± 0.21	2.24 ± 0.16**	2.75 ± 0.18* (26.42)	3.42 ± 0.15* (61.13)	3.91 ± 0.17* (86.52)	4.02 ± 0.21 (92.22)
Superoxide dismutase (U/mg protein)	91.85 ± 4.39	57.35 ± 3.22**	60.21 ± 3.21* (8.37)	67.42 ± 3.24* (29.48)	85.35 ± 4.24* (81.99)	89.25 ± 4.5 (92.46)
Catalase (U/mg protein)	354.51 ± 17.07	266.82 ± 12.07**	282.17 ± 13.76* (17.50)	305.25 ± 14.09* (43.82)	334.35 ± 15.05* (77.00)	349.37 ± 16.23 (94.13)

The data in the parenthesis indicate percent protection in individual biochemical parameters from their elevated values caused by the EAC. The % of protection is calculated as 100 × (values of EAC control -values of sample) / (values of EAC control - values of vehicle control). Values are mean ± S.E.M. (n = 6). Control group compared with normal group (** $P < 0.01$). Extract treated groups compared with EAC control group (* $P < 0.05$).

< 0.01). After administration of MECA at different doses (50, 100 and 200 mg/kg) and 5 -Fluorouracil to EAC bearing mice the level of lipid peroxidation were reduced when compared to EAC control group ($P < 0.05$). Inoculation of EAC drastically decreased the GSH, vitamin C and vitamin E

content ($P < 0.01$) in the EAC control group. The administration of MECA at the doses of 50, 100 and 200 mg/kg and 5-Fluorouracil to the EAC-induced mice increased GSH, vitamin C and vitamin E as compared with the EAC control group ($P < 0.05$). SOD and CAT levels (U/mg of protein) in the liver of EAC bearing mice significantly decreased when compared with normal group ($P < 0.01$). After administration of MECA at the doses of 50, 100 and 200 mg/kg and 5-Fluorouracil increased levels of SOD and CAT as compared to that of EAC control group ($P < 0.05$).

DISCUSSION

The present study deals with the evaluation of the antitumor activity and antioxidant role of MECA on EAC bearing mice. The MECA treated animals at the doses of 50, 100 and 200 mg/kg significantly inhibited the ascites volume, packed cell volume, tumor cell count and reverts the hematological and serum biochemical parameters to normal levels. The extract also restored the lipid peroxidation and non enzymatic and enzymatic antioxidant in tumor bearing mice to near normal levels.

In EAC bearing mice a regular rapid increase in ascites volume was noted. Ascites fluid is the direct nutritional source to tumor cells and a rapid increase in ascites fluid with tumor growth would be a means to meet the nutritional requirements of tumor cells (Prasad and Giri, 1994). Treatment with MECA inhibited the ascites volume, tumor cell count and increased the percentage of trypan blue positive dead cells in tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of life span of animals (Clarkson, and Burchenal, 1965). The inhibition of tumor cell growth was observed after treatment with different doses of MECA appear to correlate with the findings of enhanced survival time with respect to EAC control group and thereby suggests the antitumor activity of MECA against EAC bearing mice.

In cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia (Price and Greenfield, 1958; Hogland, 1982). 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 (Fenninger and Mider, 1954). Treatment with MECA brought back the hemoglobin content, RBC and WBC cell count near to normal values.

In the assessment of changes in the enzymes levels during tumor, the determination of enzyme levels such as SGPT and SGOT is largely used. Necrosis or membrane damage releases the enzyme into circulation. High levels of SGOT indicate liver damage. Therefore, SGPT is more specific to the liver, and is thus a better parameter for detecting liver injury (Willianson *et al.,* 1996). MECA at the different doses caused significant inhibition of SGPT and SGOT levels in EAC bearing mice. Serum ALP and bilirubin levels on the other hand, are related to the function of hepatic cell. MECA at different doses caused significant inhibition of SALP and bilirubin levels in EAC-induced tumor in mice.

The reduced level of uric acid in cancer conditions may be due to the increased utilization of uric acid against increased production of the free radicals, which is a characteristic feature of cancer condition. The reversal of altered uric acid level to near normal in MECA-treated mice could be due to strong antioxidant activity of MECA. Malondialdehyde, the end product of lipid peroxidation was reported to be higher in cancer tissues than in non-diseased organ (Yagi, 1991). The present study indicates that the EAC control group produced elevation in the levels of lipid peroxidation, whereas the treatment with MECA significantly reduced the elevated levels of lipid peroxidation, may be due to the antioxidant and free radical scavenging activity of the MECA. GSH, a potent inhibitor of neoplastic process plays an

important role in endogenous antioxidant system that is found particularly in high concentration in liver and it is known to have key function in protective process. The present study indicates that the EAC control group produced depletion in GSH content, whereas the treatment with MECA significantly increase in that by comparison with EAC control group may be due to the antioxidant and free radical mechanism.

The availability of vitamin C is a determined factor in controlling and potentiating many aspects of host resistance to cancer. The decreased level of vitamin C was found EAC control animals. Vitamin C can protect cell membranes and lipoprotein particles from oxidative damage by regenerating the antioxidant form of vitamin E (Buettner, 1993). The recoupment of vitamin C to near normal level in drug treated mice was found to be due to the potent anticancer activity of the extract which may induce the regeneration of ascorbic acid. Vitamin E is the most significant antioxidant in animal cells and it can protect against carcinogenesis and tumor growth (Das, 1994). Significantly decreased vitamin E levels in ECA control animals might be due to the excessive utilization of this antioxidant for quenching enormous free radicals produced in these conditions. The increased level of vitamin E in drug treated mice reveals the antioxidative nature of the MECA. Cells are also equipped with enzymatic antioxidant mechanisms that play an important role in the elimination of free radicals. 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 (Rushmore and Picket, 1993). In correlation, it has been reported a decrease in SOD activity in EAC bearing mice which might be due to loss of Mn^{++} contain SOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver (Sun *et al*., 1989). A small amount of catalase in tumor cell was reported (Marklund *et al*., 1982). The inhibition of SOD and CAT activities as a result of tumor growth was also reported (Sun *et al*., 1989). Similar findings were observed in the present investigation with EAC bearing mice.

Plant derived natural products such as flavonoids, terpenoids and steroids etc. have received considerable attention in recent years due to their diverse pharmacological properties including antitumor and antioxidant activity (DeFeudis *et al*., 2003; Takeoka and Dao, 2003). There has been growing interest in the analysis of certain flavonoids, triterpenoids and steroids stimulated by intense research in to their potential benefits to human against infection and degenerative diseases.

The lowering of lipid peroxidation and increase in levels of GSH, vitamin C, vitamin E, SOD and catalase in MECA treated group indicates its potential as an suppressor of EAC induced intracellular oxidative stress. The presences of phytochemicals in MECA such as flavonoids, triterpenoids, steroids, etc., are responsible for its potent antitumor and antioxidant activities, which can be inferred from the increased life span of EAC tumor bearing mice. Further investigations are in progress in our laboratory to identity the active principles involved in this antitumor and antioxidant activity.

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