Antitumor and antioxidant status of *Galega purpurea* root in Ehrlich ascites carcinoma bearing Swiss albino mice

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SUMMARY

The present study was designed to determine the antitumor and antioxidant properties of methanol extract from the root of *Galega purpurea* (Papilionaceae) (MEGP) against Ehrlich Ascites Carcinoma (EAC) bearing Swiss albino mice. Acute and short-term toxicity studies were performed initially in order to ascertain the safety of MEGP. The effect of MEGP on the growth of transplantable murine tumor, life span of EAC bearing hosts and simultaneous alterations in the hematological profile and liver biochemical parameters (lipid peroxidation, antioxidant enzymes) were estimated. The MEGP showed decrease in tumor volume, packed cell volume and viable cell count and increases the nonviable cell count and mean survival time thereby increasing life span of EAC tumor bearing mice. Hematological profile reverted to more or less normal levels in extract treated mice. Treatment with MEGP decreased the levels of lipid peroxidation and increased the levels of glutathione, superoxide dismutase and catalase. The results suggested that the methanol extract of *Galega purpurea* root exhibited antitumor effect by modulating lipid peroxidation and augmenting antioxidant defense system in EAC bearing mice.

Key words: *Galega purpurea*; Antitumor; Antioxidant; Ehrlich ascites carcinoma

INTRODUCTION

Galega purpurea (Papilionaceae) a plant popularly known as 'kolinji' in Tamil, which thrives throughout China and Southern parts of India. It grows on hard stony ground too difficult to be rooted. The various parts of the plant are widely used in the folk medicine for the treatment of cough, asthma, bilious febrile attacks, arthritis and rheumatism. Decoction of the root useful in the management of enlargement and obstruction of the liver, spleen and kidney. Also the root has been

reported to be useful in hard tumors, diarrhoea and gonorrhoea (Nadkarni, 1976). In view of this and evidence from the existing information show that this plant may possess some important biological activities. But the lack of scientific information about the traditional use, phytochemical, pharmacological and toxicological activities of this plant prompted us to evaluate the *in vivo* antitumor and antioxidant activity of the methanol extract of *Galega purpurea* root in Ehrlich Ascites Carcinoma (EAC) tumor bearing Swiss albino mice.

MATERIALS AND METHODS

Plant material

The roots of the plant *Galega purpurea* (Family:

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Papilionaceae) were collected from Erode district of Tamilnadu, India. The plant material was taxonomically identified by Botanical Survey of India, Kolkata. A voucher specimen (No. GMG 03/05) has been preserved in our laboratory for future reference. The root were dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder material of the root was defatted with petroleum ether and the marc thus obtained was then extracted with methanol in a soxhlet apparatus. The solvent was completely removed under reduced pressure and a semisolid mass was obtained (MEGP, yield 7.3%). Phytochemical screening of the extract revealed the presence of flavonoids, tannins, alkaloids, glycosides and steroids. The dried MEGP was suspended in normal saline and used for the present study.

Chemicals

The following chemicals were obtained from the indicated commercial sources: Phenazonium methosulphate (PMS) and Nicotinamide adenine dinucleotide (NADH), thiobarbituric acid (TBA), nitroblue tetrazolium chloride (NBT) (Loba Chemie, Bombay, India); 1-chloro-2,4-dinitro benzene (CDNB), bovine serum albumin (Sigma chemical co., St. Luis, MO, USA); Folin-Ciocalteu phenol, reduced Glutathione and 5,5'-dithio bis-2-nitro benzoic acid (DTNB) (SISCO Research Laboratory, Bombay, India). All the reagents used were of analytical reagent grade.

Animals

Studies were carried out using male Swiss albino mice weighing 21 ± 2 g. They were obtained from the animal house of Jadavpur University, Kolkata. The mice were grouped and housed in poly acrylic cages ($38 \times 23 \times 10$ cm) with not more than 12 animals per cage and maintained under standard laboratory conditions (temperature $25 \pm 2^\circ\text{C}$) with dark/ 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 conditions for 10 days before commencement of the experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee.

Tumor cells

EAC cells were obtained from Chittaranjan National Cancer Institute (CNCI), Kolkata, India. The EAC cells were maintained in vivo in Swiss albino mice by intraperitoneal transplantation of 2×10^6 cells per mouse after every 10 days. EAC cells 9 days old were used for the screening of antitumor activity of MEGP.

Acute toxicity test

The animals were divided into six groups containing eight animals in each group. MEGP was suspended in normal saline and administered orally as a single dose to groups of mice at different concentrations (500, 750, 1000, 1,250, 1,500 and 2,000 mg/kg body weight). These animals were observed for a 72 h period. The number of deaths was expressed as a percentile and the LD_{50} was determined by probit a test using the death percentage versus the log dose (Thompson and Weil, 1952).

Short-term toxicity

To determine short-term (14 days) toxicity, healthy Swiss albino mice were divided into 4 groups of 8 animals in each. Group 1 received normal saline (5 ml/kg), orally once daily for 14 days (vehicle control). Groups 2,3 and 4 received MEGP at the doses of 100, 250 and 500 mg/kg respectively, orally once daily for 14 days. At twenty-four hours after the last dose, and after 18 h fasting, the mice were sacrificed. Blood and liver were collected and important internal organs were removed, weighed and observed for pathological changes. Hematological parameters were determined as described above. Serum glutamate pyruvate transaminase (SGPT) and glutamate oxaloacetate transaminase (SGOT) were determined (Bergmeyer *et al.*, 1978). Urea was estimated by the enzymatic method and calcium

was estimated by the O-cresolphthalein complexone method (Tietz, 1987). Phosphorous was estimated by the colorimetric method (Henry, 1974). Liver biochemical parameters were estimated by the standard methods described above.

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 per mouse) intraperitoneally except the normal group. This was taken as day zero. On the first day, 5 ml/kg of normal saline was administered to group 1 and 2 (normal and EAC control). MEGP at different dose (100, 250 and 500 mg/kg) and the standard drug 5-Fluorouracil (20 mg/kg) (Kavimani and Mani Senthilkumar, 2000) were administered in group 3, 4, 5 and 6 respectively for 14 days orally. After the last dose and 18 h fasting, six mice from each group were sacrificed for the study of antitumor activity, hematological and liver biochemical parameters. The rest of the animal groups were kept to check the survival time of EAC-tumor bearing hosts.

The antitumor activity of the methanol extract of *Galega purpurea* was measured in EAC animals with respect to the following parameters:

Tumor volume

The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and packed cell volume was determined by centrifuging at 100 rpm per 5 min.

Tumor cell count

The ascitic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension was placed on the Neubauer's counting chamber and the numbers of cells in the 64 small squares were counted.

Viable/non-viable tumor cell count

The cells were then stained with trypan blue (0.4%

in normal saline) dye. The cells that didn't take up the dye were viable and those that took the dye were non-viable. These viable and non-viable cells were counted.

Cell count = Number of cells $\times$ dilution / Area $\times$ thickness of liquid film

Percentage increase life span (% ILS)

The effect of MEGP on tumor growth was monitored by recording the mortality daily for a period of 6 weeks and percentage increase in life span (% ILS) was calculated.

% ILS = (Mean survival of treated group / Mean survival of control group) - 1 $\times$ 100

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

Body weight

Body weight of the experimental mice were recorded both in the treated and control group at the beginning of the experiment (day 0) and sequentially on every 5th day during the treatment period.

Hematological parameters

At the end of the experimental period, the next day after an overnight fast blood was collected from freely flowing tail vein and used for the estimation Hemoglobin (Hb) content, red blood cell count (RBC) (D'Armour *et al.*, 1965) and white blood cell count (WBC) (Wintrobe *et al.*, 1961). WBC differential count was carried out from Leishman stained blood smears (Dacie, 1958).

Biochemical assays

After the collection of the blood samples, the mice were sacrificed. Then their liver was excised, rinsed in ice-cold normal saline solution followed by cold 0.15 M Tris-HCl (pH 7.4), blotted dry and weighed. A 10% w/v homogenate was prepared in 0.15 M Tris-HCl buffer and was used for the

estimation of lipid peroxidation (LPO) and reduced glutathione (GSH). The rest of the homogenate was centrifuged at 1,500 rpm for 15 min at 4°C the supernatant thus obtained was used for the estimation of superoxide dismutase (SOD), catalase (CAT) and total protein.

Estimation of lipid peroxidation (LPO)

The levels of Thiobarbituric acid reactive substances (TBARS) in the liver were measured by the method of Ohkawa (1979) as a marker for lipid peroxidation. A mixture of 0.4 ml of 10% liver homogenate, 1.5 ml of 8.1% sodium dodecyl sulphate (SDS), and 1.5 ml of 0.8% TBA solution was heated at 95°C for 1 h. After cooling, 5.0 ml of n-butanol-pyridine (15: 1) was added, and the absorbance of the n-butanol-pyridine layer was measured at 532 nm.

Estimation of reduced glutathione (GSH)

The tissue GSH was determined by the method of Beutler and Kelly (1963). Virtually all the non-protein sulfhydryl groups of tissues are in the form of reduced GSH. 0.2 ml of tissue homogenate was mixed with 1.8 ml of EDTA solution. To this 3.0 ml precipitation reagent (after precipitating proteins with TCA) was added, mixed thoroughly and kept for 5 min before centrifugation. To 2.0 ml of the filtrate, 4.0 ml of 0.3 M disodium hydrogen phosphate solution and 1.0 ml of DTNB reagent were added and the absorbance read at 412 nm.

Estimation of superoxide dismutase (SOD)

The activity of SOD in tissue was assayed by the method of Kakkar (1984). The assay mixture contained 1.2 ml sodium pyrophosphate buffer (pH 8.3, 0.025 mol/l), 0.1 ml PMS (186 mmol/l), 0.3 ml nitroblue tetrazolium (300 mmol/l, 0.2 ml NADH (780 mmol/l) and diluted enzyme preparation and water in a total volume of 3 ml. After incubation at 30°C for 90 s, the reaction was terminated by the addition of 1.0 ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 ml n-butanol. The color intensity of the chromogen in

the butanol layer was measured at 560 nm against n-butanol.

Estimation of catalase (CAT)

Catalase was assayed according to the method of Maehly and Chance (1954). The estimation was done spectrophotometrically following the decrease in absorbance at 230 nm. The tissue was homogenized in M/150 phosphate buffer (pH 7.0) at 4°C and centrifuged at 5,000 rpm. The reaction mixture contained 0.01 M phosphate buffer (pH 7.0), 2 mM H₂O₂ and the enzyme extract. The specific activity of catalase is expressed in terms of units/mg protein. A unit is defined as the velocity constant per second.

Estimation of total proteins

The protein content of tissue homogenates was measured by the method of Lowry (1951). 0.5 ml of tissue homogenate was mixed with 0.5 ml of 10% TCA and centrifuged for 10 min. The precipitate obtained was dissolved in 1.0 ml of 0.1 N NaOH. From this an aliquot was taken for protein estimation. 0.1 ml of aliquot was mixed with 5.0 ml of alkaline copper reagent and allowed to stand at room temperature for 10 min. 0.5 ml of Folin's phenol reagent was added and the blue color developed was read after 20 min at 640 nm.

Statistical analysis

The experimental results were expressed as mean $\pm$ S.E.M. Data were assessed by ANOVA followed by the Student t-test; $P < 0.05$ was considered as statistically significant.

RESULTS

Acute toxicity study

In the acute toxicity assay no deaths were observed during the 72 h period at the doses tested. At these doses, the animals showed no stereotypical symptoms associated with toxicity, such as convulsion, ataxy, diarrhoea or increased diuresis. The median lethal

dose (LD₅₀) was determined to be higher than the highest dose tested i.e., 2.0 g/kg.

Short-term toxicity

When the mice were observed for the behavioral changes after oral administration of a single dose of the extract none of the mice were exhibited any abnormal behavioral responses at the doses of 100, 250 and 500 mg/kg. Administration of repeated daily doses of 100, 250 and 500 mg/kg for 14 days did not influence the bodyweight of the mice. The weights of liver, kidney, brain and spleen were also not altered by the treatment. Hematological parameters like hemoglobin and RBC count remained unaltered at the dose of 100, 250 and 500 mg/kg. But there was a marginal increase in WBC count. The results were summarized in Table 1.

Effect of MEGP on mean survival time

The effect of MEGP at the doses of 100, 250 and 500 mg/kg on the mean survival time of EAC bearing mice is shown in Table 2. In the EAC control group the mean survival time was 21.03 ± 0.12 , while it increased to 28.35 ± 0.02 (100 mg/kg), 32.43 ± 0.6

(250 mg/kg) and 35.22 ± 0.4 (500 mg/kg) days respectively in the MEGP treated groups. The group treated with the standard drug 5-Fluorouracil (20 mg/kg) shows 39.54 ± 0.25 days for the same.

Effect of MEGP on tumor growth

The tumor volume, packed cell volume and viable cell count were found to be significantly ($P < 0.01$) increased and non-viable cell count was significantly ($P < 0.01$) low in EAC control animals when compared with normal control animals. Administration of MEGP at the doses of 100, 250 and 500 mg/kg significantly ($P < 0.01$) decreased the tumor volume, packed cell volume and viable cell count. Further more, non-viable tumor cell count at different doses of MEGP was significantly ($P < 0.01$) increased in a dose dependent manner. Finally, the change in body weights of the animals suggests the tumor growth inhibiting property of MEGP. All these results clearly indicate that the MEGP has a remarkable capacity to inhibit the growth of solid tumor induced by EAC cell line in a dose dependent manner in experimental animals (Table 2).

Table 1. Effect of methanol extract of Galega purpurera (MEGP) on hematological, biochemical parameters and body weight of normal mice

Parameters	Normal (0.9 % NaCl, 5 ml/kg)	MEGP (100 mg/kg)	MEGP (250 mg/kg)	MEGP (500 mg/kg)
Hemoglobin (g %)	11.6 ± 0.22	10.2 ± 0.03	10.3 ± 0.01	10.8 ± 0.03
RBC ($10^6/\text{mm}^3$)	6.4 ± 0.41	6.5 ± 0.2^a	6.1 ± 0.1	6.7 ± 0.04
Total WBC ($10^6/\text{mm}^3$)	5.2 ± 0.51	6.2 ± 0.05	6.9 ± 0.2	7.1 ± 0.14
SGPT (U/L)	65.1 ± 0.25	68.3 ± 0.3^b	73.8 ± 0.02	75.1 ± 0.15^b
SGOT (U/L)	39.5 ± 0.03	43.6 ± 0.02	45.3 ± 0.08	48.4 ± 0.23
Serum urea (mg/dl)	22.6 ± 2.8	20.9 ± 2.04	21.4 ± 0.09^b	23.0 ± 0.06
Serum calcium (mg/dl)	10.1 ± 5.9	10.1 ± 0.04	10.4 ± 0.12	10.6 ± 0.04
Serumphosphate (mg/ml)	4.2 ± 4.7	4.6 ± 0.01	4.8 ± 0.03	5.3 ± 0.15^b
LPO (nmolMDA/mg protein)	0.94 ± 0.41	0.89 ± 0.7	0.92 ± 0.04	0.90 ± 0.11
GSH (mg/g wet tissue)	2.33 ± 0.68	2.48 ± 0.29	2.52 ± 0.26^c	2.57 ± 0.24
SOD (U/mg protein)	4.47 ± 0.21	4.55 ± 0.4	4.60 ± 0.5	4.71 ± 0.17
CAT (U/mg protein)	26.2 ± 0.44	27.68 ± 0.2^b	28.7 ± 0.23	29.1 ± 0.5^b
Body weight (g)	21.5 ± 0.25	21.8 ± 0.3	22.8 ± 0.2	21.7 ± 0.3

Data are expressed as the mean of results in 8 mice $\pm$ S.E.M. ^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.001$ Experimental groups compared with the normal group.

Table 2. Effect of methanol extract of *Galega purpurea* (MEGP) on body weight, mean survival time, % ILS, tumor volume, packed cell volume and viable and non-viable tumor cell count of EAC bearing mice

Parameters	EAC control (2×10^6 cells/ml/mouse)	EAC + MEGP (100 mg/kg)	EAC + MEGP (250 mg/kg)	EAC + MEGP (500 mg/kg)	EAC + 5-FU (20 mg/kg)
Body weight (g)	27.5 $\pm$ 0.25	26.48 $\pm$ 0.01 ^b	25.73 $\pm$ 0.09	22.41 $\pm$ 0.07 ^b	21.94 $\pm$ 0.3 ^b
Mean Survival Time (days)	21.03 $\pm$ 0.12	28.35 $\pm$ 0.02 ^b	32.43 $\pm$ 0.6 ^b	35.22 $\pm$ 0.4 ^b	36.54 $\pm$ 0.25 ^b
Increase Life Span (%)	-	34.81	54.21	67.48	73.75
Tumor volume (ml)	3.96 $\pm$ 0.03	3.31 $\pm$ 0.1 ^b	2.23 $\pm$ 0.31 ^b	1.0 $\pm$ 0.42 ^b	-
Packed cell volume (ml)	2.14 $\pm$ 0.06	1.26 $\pm$ 0.3	0.82 $\pm$ 0.21	0.27 $\pm$ 0.2 ^b	-
Viable tumor cell count ($\times 10^7$ cells/ml)	10.41 $\pm$ 0.06	6.35 $\pm$ 0.03	3.21 $\pm$ 0.12	2.30 $\pm$ 0.5	0.9 $\pm$ 0.13 ^b
Non-viable tumor cell count ($\times 10^7$ cells/ml)	0.97 $\pm$ 0.05	1.76 $\pm$ 0.01 ^b	1.12 $\pm$ 0.03 ^b	-	-

Data are expressed as the mean of results in 6 mice $\pm$ S.E.M. ^b $P < 0.01$ Experimental groups compared with the EAC control group. Body weight of normal mice is 21.8 $\pm$ 0.19.

Table 3. Effect of methanol extract of *Galega purpurea* (MEGP) on hematological parameters of EAC bearing mice

Parameters	Normal (0.9 % NaCl, 5 ml/kg)	EAC control (2×10^6 cells/ ml/mouse)	EAC+MEGP (100 mg/kg)	EAC+MEGP (250 mg/kg)	EAC+MEGP (500 mg/kg)	EAC+5 -FU (20 mg/kg)
Hemoglobin (g %)	13.6 $\pm$ 0.8	10.65 $\pm$ 1.5 ^c	11.61 $\pm$ 1.1 ^b	12.34 $\pm$ 0.66 ^b	12.99 $\pm$ 0.42 ^b	13.1 $\pm$ 1.2
RBC (cells/ml $\times 10^6$)	5.43 $\pm$ 1.5	3.96 $\pm$ 1.3 ^c	4.30 $\pm$ 1.6	4.42 $\pm$ 0.35 ^b	5.33 $\pm$ 0.37 ^b	4.97 $\pm$ 1.5 ^b
WBC (cells/ml $\times 10^6$)	8.22 $\pm$ 1.2	16.54 $\pm$ 1.1 ^c	13.52 $\pm$ 1.2 ^b	10.4 $\pm$ 0.80 ^b	8.15 $\pm$ 0.50	8.53 $\pm$ 1.2 ^b
Monocytes (%)	2.27 $\pm$ 0.5	1.59 $\pm$ 1.4 ^c	1.73 $\pm$ 0.04 ^b	1.91 $\pm$ 0.12	2.08 $\pm$ 0.55	2.15 $\pm$ 0.5
Lymphocytes (%)	72.63 $\pm$ 1.5	37.26 $\pm$ 1.6 ^c	47.92 $\pm$ 0.08 ^b	60.01 $\pm$ 0.40 ^b	66.17 $\pm$ 0.22	70.14 $\pm$ 0.8 ^b
Neutrophils (%)	25.41 $\pm$ 0.1	64.14 $\pm$ 1.0 ^c	39.34 $\pm$ 0.32	42.27 $\pm$ 0.21 ^b	30.53 $\pm$ 0.35 ^b	29.52 $\pm$ 1.5

Data are expressed as the mean of results in 6 mice $\pm$ S.E.M. ^b $P < 0.01$ Experimental groups compared with the EAC control group, ^c $P < 0.001$ Experimental groups compared with the normal group.

Effect of MEGP on hematological parameters

The effect of MEGP on hematological parameters of EAC treated animals were shown in Table 3. Hematological parameters of tumor bearing mice on day 14 were found to be significantly altered compared to the normal group. The hemoglobin content and RBC count in the EAC control group was significantly ($P < 0.001$) decreased as compared to the normal group. Treatment with MEGP at the dose of 100, 250 and 500 mg/kg significantly ($P < 0.01$) increased the hemoglobin content and RBC count to more or less normal levels. The total WBC counts and protein was found to be increased significantly in the EAC control group when compared with normal group ($P < 0.001$). Administration of MEGP at the doses of 100, 250

and 500 mg/kg to EAC bearing mice significantly ($P < 0.01$) reduced the WBC count and protein as compared with the EAC control animals. In differential count of WBC the percentage of neutrophils increased while the lymphocyte count decreased in the EAC control group. Treatment with MEGP at different doses changed these altered parameters towards more or less normal values.

Effect of MEGP on lipid peroxidation

The localization of radical formation resulting in lipid peroxidation, measured as malondialdehyde (MDA) in mice liver homogenate is shown in Figure 1. The increased MDA levels in EAC treated animals (1.47 nmol MDA/mg protein) were reduced by MEGP at the dose of 100, 250 and 500 mg/kg

Fig. 1. Effect of methanol extract of *Galega purpurea* (MEGP) on hepatic lipid peroxidation level in EAC-bearing mice.

Fig. 2. Effect of methanol extract of *Galega purpurea* (MEGP) on hepatic glutathione content in EAC-bearing mice.

by 1.36, 1.14 and 0.95 nmol MDA/mg protein respectively.

Effect of MEGP on glutathione level

The effect of MEGP on glutathione content in the liver is shown in Fig. 2. GSH level in normal group was 2.39 mg/g of liver whereas that of EAC treated group was 1.7 mg/g wet tissue. GSH level increased by MEGP at the dose of 100, 250 and 500 mg/kg were 1.95, 2.01 and 2.23 mg/g of liver respectively.

Fig. 3. Effect of methanol extract of *Galega purpurea* (MEGP) on hepatic SOD activity in EAC-bearing mice.

Fig. 4. Effect of methanol extract of *Galega purpurea* (MEGP) on hepatic CAT activity in EAC-bearing mice.

Effect of MEGP on SOD activity

The effect of MEGP on SOD activity in liver is shown in Fig. 3. The SOD level was decreased in EAC treated animals to 2.91 U/mg protein. The SOD levels were increased to 3.21, 3.53 and 4.07, 39.65 U/mg protein respectively by the treatment of MEGP at the dose of 100, 250 and 500 mg/kg.

Effect of MEGP on catalase activity

CAT activities in the liver homogenate were shown in Fig. 4. CAT activity of total liver homogenate of EAC treated group and normal group were 10.6 and 27.3 U/mg protein respectively. Treatment of

MEGP at the dose of 100, 250 and 500 mg/kg increased the liver CAT activities to 13.84, 18.63 and 23.44 U/mg protein respectively.

DISCUSSION

In the present manuscript we have shown the *in vivo* antitumor and antioxidant status of *Galega purpurea* extract in EAC bearing mice. The MEGP treated animals at the doses of 100, 250 and 500 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. The extract also restored the hepatic lipid peroxidation and free radical scavenging enzyme GSH as well as antioxidant enzymes such as SOD and CAT in tumor bearing mice to near normal levels. Short-term toxicity studies indicate that MEGP at the doses of 100, 250 and 500 mg/kg for 14 days did not exhibit any adverse effects.

In EAC bearing mice a regular rapid increase in ascites tumor volume was noted. Ascites fluid is the direct nutritional source to tumor cells and rapid increase in ascitic fluid with tumor growth could be a means to meet the nutritional requirements of tumor cells (Prasad and Giri, 1994). Treatment with MEGP inhibited the tumor volume, tumor cell count and 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 life span of animals (Clarkson and Burchenal, 1965) and disappearance of leukemic cells from blood (Feninger and Mider, 1954). A decrease in tumor volume and viable tumor cell count as mentioned above finally reduced the tumor burden and enhanced the life span of EAC bearing mice.

The major problems encountered in cancer chemotherapy 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 (Feninger and Mider, 1954). Perturbation of hematological parameters in tumor bearing animals is partly due to the toxic effects produced in them. In addition myelosuppression in cancer chemotherapy is a common phenomenon, which is responsible for poor prognosis (Donehower, 1990). Treatment with MEGP brought back the hemoglobin content, RBC and WBC cell count near to normal levels. This indicates that MEGP possess protective action on the hemopoietic system.

Excessive production of free radicals resulted in oxidative stress, which leads to damage of macromolecules such as lipids can induce lipid peroxidation *in vivo* (Yagi, 1987). Increased lipid peroxidation would cause degeneration of tissues. Lipid peroxidase formed in the primary site would be transferred through the circulation and provoke damage by propagating the process of lipid peroxidation (Sinclair, 1990). Malondialdehyde (MDA), the end product of lipid peroxidation was reported to be higher in cancer tissues than in non-diseased organs (Yagi, 1987). Glutathione, a potent inhibitor of neoplastic process plays an important role as an endogenous antioxidant system. It is found particularly in high concentration in liver and is known to have a key function in the protective process (Sinclair, 1990). MEGP reduced the elevated levels of lipid peroxidation and increased the glutathione content in EAC bearing mice.

SOD and CAT, the free radical scavenging system are present in all oxygen-metabolizing cells and their function is to provide a defense against the potentially damaging reactivities of superoxide and hydrogen peroxide. A decrease in SOD activity in EAC bearing mice which might be due to loss of Mn 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).

The inhibition of SOD and CAT activities as a result of tumor growth was also reported (Marklund *et al.*, 1982). Similar findings were observed in the present investigation with EAC bearing mice. The

administration of MEGP at different doses increased the SOD and CAT levels in a dose dependent manner, which may indicate the antioxidant and free radical scavenging property of MEGP.

It was reported that plant-derived extracts containing antioxidant principles showed cytotoxicity towards tumor cells (Jiau-Jian and Larry, 1977) and antitumor activity in experimental animals (Ruby *et al.*, 1995). Antitumor activity of these antioxidants in either through induction of apoptosis (Ming *et al.*, 1998) or by inhibition of neovascularization (Putul *et al.*, 2000). The lowering of lipid peroxidation and increase in levels of GSH, SOD and CAT in MEGP treated group indicates its potential as an inhibitor of EAC induced intracellular oxidative stress.

The present study demonstrates the MEGP increased the life span of EAC tumor bearing mice and decreased lipid peroxidation and thereby augmented the endogenous antioxidant enzymes in the liver. All these parameters suggest that the methanol extract of *Galega purpurea* root exhibits potential antitumor and antioxidant activities. Further investigation are in progress in our laboratory to identify the active principles involved in this antitumor and antioxidant activity.

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