Antioxidant and antitumor activity of *Plumeria acuminata* in Ehrlich Ascites Carcinoma bearing Swiss Albino mice

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ABSTRACT

Aim: This study was designed to determine the antitumor and antioxidant properties of crude methanol extract from the leaves of *Plumeria acuminata* (Apocynaceae) (MEPA) against Ehrlich Ascites Carcinoma (EAC) bearing Swiss albino mice.
Study Design:
Place and duration of study: Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Jadavpur, Kolkata, India between 2006 and 2007.
Methodology: The extract was administered at the doses of 100, 250 and 500 mg/kg per day for 14 days, after 24 hr of tumor inoculation. After the administration of the last dose followed by 18 hr fasting, mice were then sacrificed for observation of antitumor activity. The effect of MEPA on the growth of transplantable murine tumor, life span of EAC bearing host, viable and non-viable cell count, packed cell volume, hematological profile and biochemical parameters such as lipid peroxidation (LPO), reduced glutathione content (GSH), superoxide dismutase (SOD) and catalase (CAT) activities were estimated.
Results: MEPA caused significant ($P<0.01$) decrease in tumor volume, packed cell volume and viable count; and it prolonged the life span of EAC-tumor bearing mice. Hematological studies reveal that the Hb content and RBC count were decreased in EAC treated mice, whereas the restoration to near normal levels was observed in extract treated animals. MEPA significantly ($P<0.05$) decreased the levels of LPO and significantly increased the levels of GSH, SOD and CAT. Moreover the MEPA was found to be devoid of conspicuous short-term toxicity in the mice when administered daily for 14 days at the doses of 100, 250 and 500 mg/kg
Conclusion:. The results suggested that the methanol extract of *Plumeria acuminata* leaves exhibited antitumor effect by modulating lipid peroxidation and augmenting antioxidant defense system in EAC bearing Swiss albino mice.
***Key words**: Plumeria acuminata, Ehrlisch Ascites Carcinoma, Hematological Parameters, Antioxidant activity.*

1. INTRODUCTION

The role of natural products as a source for remedies has been recognized since ancient times [1,2]. Despite major scientific and technological progre0ss in combinatorial chemistry, drugs derived from natural products still make an enormous contribution to drug discovery today [3].

Cancer continues to represent the longest cause of mortality in the world and claims over 6 million lives every year [4]. The enhanced generation of ROS in vivo could be quite deleterious, since they are involved in mutagenesis, apoptosis, ageing and carcinogenesis [5]. Free radicals also cause DNA strand breaks and chromosome deletions and rearrangements. Further, activated oxygen species most likely play an important role in tumor promotion and progression [6]. A variety of bioactive compounds and their derivatives has been shown to inhibit cancer in a number of experimental systems involving initiation, promotion and progression [7,8]. Plants contain abundant quantities of these substances and

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have consistently been shown to be associated with a lower risk of cancers at almost every site [9]. Efforts therefore are being made to identify naturally occurring anticarcinogenesis which would prevent, slow and/or reverse the cancer induction and its subsequent development [10]. An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural agents (alone or combination) to block the development of cancer in humans. Several plant derived compounds are currently successfully employed in cancer treatment and some of them like vincristine, taxol and so on are available as a drug of choice [11]. The rich and diverse plant sources of India are likely to provide effective anticancer agent. One of the best approaches in search for anticancer agents from plant resources is the selection of plants based on ethnomedical leads and testing the selected plants efficacy and safety in light of modern science. Exploration of traditional medicinal practices in Tamilnadu brought to light ethnomedical use of young leaves of *Plumeria acuminata* (Apocynaceae) to treat certain tumors in a few remote villages in Erode district of Tamilnadu, India. This plant is also known as an antitumor agent in ancient systems of medicine such as Ayurveda [12].

Plumeria acuminata belonging to the family Apocynaceae is widely distributed throughout the Southern parts of India. In traditional medicinal system different parts of the plant have been mentioned to be useful in a variety of diseases. The bark has been reported to be useful in hard tumors, diarrhoea and gonorrhea. The leaves are reported to have anti-inflammatory, rubefacient in rheumatism and have strong purgative effect. Its branches are used like those of 'chitraka' to produce abortion [12]. However there is no scientific report or verification of the use of this plant in the treatment of these conditions. Our recent findings revealed that the methanol extract of *P. acuminata* leaves showed significant anti-inflammatory activity [13] and antipyretic and antinociceptive activity [14]. So far no reports are available on in vivo antioxidant status of this plant in EAC tumor bearing mice. Hence we evaluated the in vivo antitumor and antioxidant activity of the methanol extract of *P. acuminata* leaves in EAC tumor bearing mice.

2. MATERIAL AND METHODS

2.1 Plant material

The leaves of the plant *Plumeria acuminata* (Family: Apocynaceae) 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 02/05) has been preserved in our laboratory for future reference. The leaves were dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder material of the leaves 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 (MEPA, yield 12.4 %). Phytochemical screening of the extract revealed the presence of flavonoids, tannins, alkaloids, glycosides and steroids. The dried MEPA was suspended in normal saline and used for the present study.

2.2 Chemicals

The following chemicals were obtained from the indicated commercial sources: Phenazonium methosulphate (PMS), 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-Ciocalteau phenol, reduced Glutathione and 5,5'-dithio bis-2-nitro benzoicacid (DTNB) (SISCO Research Laboratory, Bombay, India). All the reagents used were of analytical reagent grade.

2.3 Animals

Studies were carried out using male Swiss albino mice of either sex weighing 21 $\pm$ 2 g. They were obtained from the animal house of Jadavpur University, Kolkata. The mice were grouped and housed in poly acrylic cages (38x23x10 cm) with not more than 12 animals per cage and maintained under standard laboratory conditions (temperature 25 $\pm$ 2^0C) 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.

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2.4 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 $2x10^6$ cells per mouse after every 10 days. EAC cells 9 days old were used for the screening of antitumor activity of MEPA.

2.5 Acute Toxicity Test

The animals were divided into six groups containing eight animals in each group. MEPA was suspended in normal saline and administered orally as a single dose to groups of mice at different concentrations (500, 750, 1000, 1250, 1500 and 2000 mg/kg). 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 [15].

2.6 Antitumor Activity

Male Swiss Albino mice were divided in to 6 groups (n=12). All the groups were injected with EAC cells (0.2 ml of $2x10^6$ cells per mouse) intraperitonelly 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). MEPA at different doses (100, 250 and 500 mg/kg) and the standard drug 5-Fluorouracil (20 mg/kg) [16] were administered to group 3, 4, 5 and 6 respectively for 14 days orally. After the last dose and 18 hr 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 *P. acuminata* was measured in EAC animals with respect to the following parameters:

2.6.1 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.

2.6.2 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 number of cells in the 64 small squares was counted.

2.6.3 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 x dilution/ Area x thickness of liquid film

2.6.4 Percentage increase life span (% ILS)

The effect of MEPA 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 x 100

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

2.6.5 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 5^{th} day during the treatment period.

2.6.6 Hematological Parameters

At the end of the experimental period, the next day after an over night fasting blood was collected from freely flowing tail vein and used for the estimation Hemoglobin (Hb) content, red blood cell count (RBC) [17] and white blood cell count (WBC) [18] WBC differential count was carried out from Leishman stained blood smears [19].

2.6.7 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 1500 rpm for 15 min at 4 ^{0}C. The

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supernatant thus obtained was used for the estimation of superoxide dismutase (SOD), catalase (CAT) and total protein.

2.6.7.1 Estimation of Lipid Peroxidation (LPO)

The levels of Thiobarbituric acid reactive substances (TBARS) in the liver were measured by the method of Ohkawa et al., 1979 [20] 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), 1.5 ml of 0.8 % TBA solution was heated at 95^0 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.

2.6.7.2 Estimation of Reduced Glutathione (GSH)

The tissue GSH was determined by the method of Beutler and Kelly, 1963 [21]. 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.

2.6.7.3 Estimation of Superoxide dismutase (SOD)

The activity of SOD in tissue was assayed by the method of Kakkar et al., 1984 [22]. 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 30^0 C for 90 sec, 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.

2.6.7.4 Estimation of Catalase (CAT)

Catalase was assayed according to the method of Maehly and Chance, 1954 [23]. 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 4^0 C and centrifuged at 5000 rpm. The reaction mixture contained 0.01 M phosphate buffer (pH 7.0), 2 mM H_2O_2 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.

2.6.7.5 Estimation of Total Proteins

The protein content of tissue homogenates was measured by the method of Lowry et al., 1951 [24]. 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.

2.7 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 MEPA 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 [25]. Urea was estimated by the enzymatic method and calcium was estimated by the O-cresolphthalein complexone method [26]. Phosphorous was estimated by the colorimetric method [27]. Liver biochemical parameters were estimated by the standard methods described above.

2.8 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 value <0.05 was considered as statistically significant.

3. RESULTS

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3.1 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 (LD_{50}) was determined to be higher than the highest dose tested i.e., 2.0 g/kg.

3.2 Antitumor activity

3.2.1 Effect of MEPA on Mean Survival Time

The effects of MEPA at the doses of 100, 250 and 500 mg/kg on the Mean survival time of EAC bearing mice is shown in Table 1. In the EAC control group the mean survival time was 21.03 ± 0.12, while it increased to 27.26 ± 0.21 (100 mg/kg), 31.34 ± 0.16 (250 mg/kg) and 35.00 ± 0.2 (500 mg/kg) days respectively in the MEPA treated groups. The group treated with the standard drug 5-Fluorouracil (20 mg/kg) shows 39.54 ± 0.25 days for the same.

3.2.2 Effect of MEPA 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 MEPA 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 MEPA were 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 MEPA. All these results clearly indicate that the MEPA 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 1).

Table 1. Effect of methanol extract of *Plumeria acuminata* (MEPA) 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 ($2x10^6$ cells/ml/mouse)	EAC + MEPA (100 mg/kg)	EAC + MEPA (250 mg/kg)	EAC + MEPA (500 mg/kg)	EAC + 5-FU (20 mg/kg)
Body weight (g)	27.5±0.25	25.86±0.19*	24.71±0.19	22.84±±1.17*	21.94±0.03*
Mean Survival Time (days)	21.03±0.12	27.26±0.21*	31.34±0.16*	34.00±0.2*	36.54±0.25*
Increase Life Span (%)	-	29.62	49.03	61.63	73.75
Tumor volume (ml)	3.96±0.03	3.42±0.13*	2.51±0.03*	1.3±0.04*	-
Packed cell volume (ml)	2.14±0.06	1.51±0.03	0.94±0.07	0.32±0.02*	-
Viable tumor cell count ($x10^7$ cells/ml)	10.41±0.06	8.94±0.01	4.32±0.16	2.63±0.05	0.9±0.13*
Non-viable tumor cell count ($x10^7$ cells/ml)	0.97±0.05	1.38±0.04*	1.57±0.02*	-	-

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

3.2.3 Effect of MEPA on hematological parameters

The effect of MEPA on hematological parameters of EAC treated animals were shown in Table 2. 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 MEPA 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 MEPA 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

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group. Treatment with MEPA at different doses changed these altered parameters towards more or less normal values.

Table 2. Effect of methanol extract of *Plumeria acuminata* (MEPA) on hematological parameters of EAC bearing mice

Parameters	Normal (0.9 % NaCl, 5 ml/kg)	EAC control ($2x10^6$ cells/ml/mouse)	EAC+MEPA (100 mg/kg)	EAC+MEPA (250 mg/kg)	EAC+MEPA (500 mg/kg)	EAC+5-FU (20 mg/kg)
Hemoglobin (g %)	13.6±0.8	10.65±1.5†	10.91±1.1*	11.64±0.66*	12.98±0.42*	13.1±1.2
RBC (cells/ml $x10^6$)	5.43±1.5	3.96±1.3†	4.28±1.6	4.84±0.35*	5.27±0.37*	4.97±1.5*
WBC (cells/ml $x10^6$)	8.22±1.2	16.54±1.1†	14.72±1.2*	10.2±0.80*	8.01±0.50	8.53±1.2*
Monocytes (%)	2.27±0.5	1.59±1.4†	1.70±0.04*	1.85±0.12	1.97±0.55	2.15±0.5
Lymphocytes (%)	72.63±1.5	37.26±1.6†	44.29±0.08*	56.1±0.40*	64.7±0.22	70.14±0.8*
Neutrophils (%)	25.41±0.1	64.14±1.0†	38.71±0.32	45.5±0.21*	32.3±0.35*	29.52±1.5

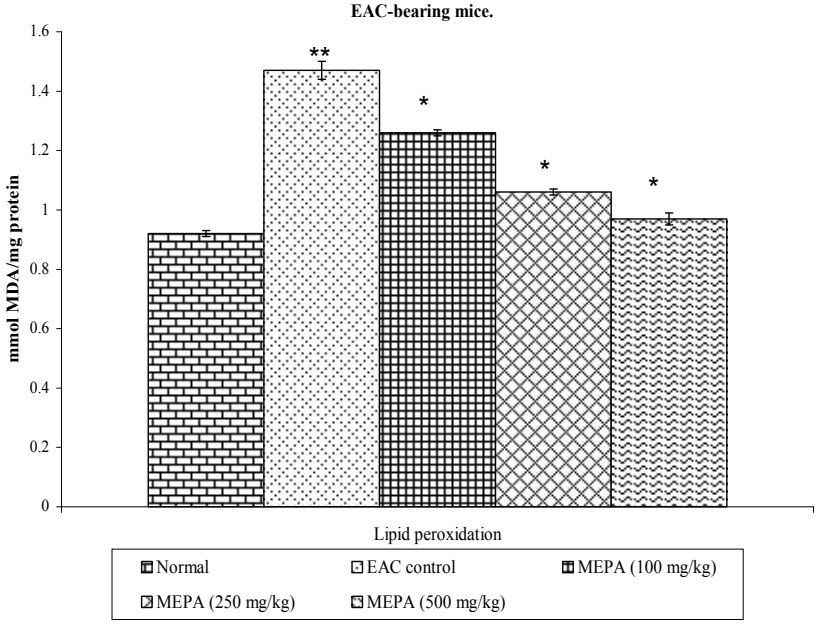
Data are expressed as the mean of results in 6 mice ± S.E.M.
*P<0.01 Experimental groups compared with the EAC control group
†P<0.001 Experimental groups compared with the normal group

3.3 Biochemical assays

3.3.1 Effect on lipid peroxidation

As shown in Figure 1, the levels of lipid peroxidation in liver tissue were significantly increased by 37.41 % in the EAC control group as compared to the normal group (P<0.001). After administration of MEPA at different doses (100, 250 and 500 mg/kg) to EAC bearing mice, the level of lipid peroxidation was reduced by 14.28 %, 27.89 % and 34.01 % respectively in comparison to the EAC control group (P<0.05). Results were expressed as nmoles MDA/mg protein/ml.

Fig 1. Effect of methanol extract of *Plumeria acuminata* (MEPA) on hepatic lipid peroxidation level in EAC-bearing mice.



Data are expressed as the mean of results in 6 mice (+) or (-) S.E.M.
†P<0.001, EAC control group compared with the normal group.
*P<0.05, Experimental groups compared with the EAC control group.

3.3.2 Effect of MEPA on reduced glutathione

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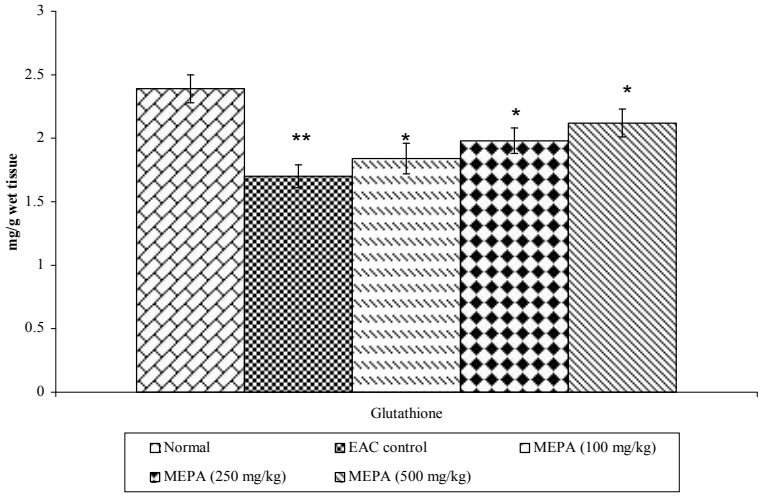
The effect of MEPA on reduced glutathione content of EAC bearing mice were summarized in Figure 2. Inoculation of EAC drastically decreased the GSH content to 28.87 % in the EAC control group when compared with the normal group (P<0.001). The administration of MEPA at the doses of 100, 250 and 500 mg/kg to the EAC bearing mice increased GSH levels by 8.24 %, 16.47 % and 24.71 % respectively, as compared with EAC control group (P<0.05).

Fig 2. Effect of methanol extract of *Plumeria acuminata* (MEPA) on hepatic glutathione content in EAC-bearing mice

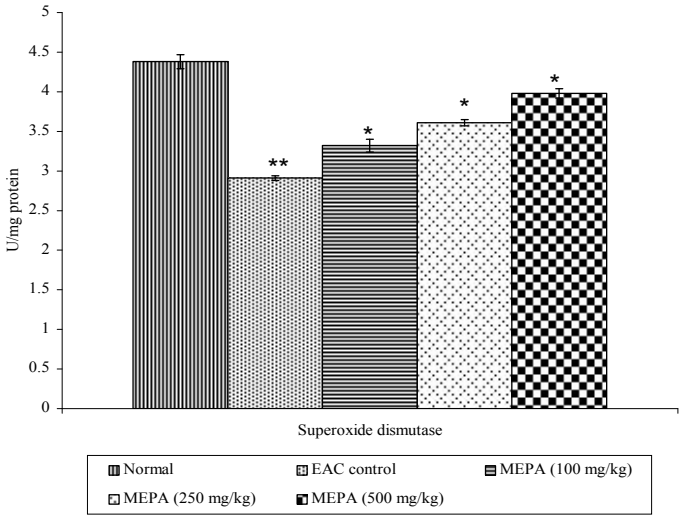


Data are expressed as the mean of results in 6 mice (+) or (-) S.E.M.
†P<0.001, EAC control group compared with the normal group.
*P<0.05, Experimental groups compared with the EAC control group.

3.3.3 Effect of MEPA on SOD level

Figure 3 shows the activity of SOD in liver tissue of experimental groups. The levels of SOD in the liver of EAC bearing mice decreased by 28.87 % (P<0.01) in comparison with normal group. Administration of MEPA at the doses of 100, 250 and 500 mg/kg increased the levels of SOD by 14.34 %, 24.05 % and 36.77 % respectively (P<0.05) as compared to EAC control animals.

Fig 3. Effect of methanol extract of *Plumeria acuminata* (MEPA) on hepatic SOD activity in EAC-bearing mice



Data are expressed as the mean of results in 6 mice (+) or (-) S.E.M.

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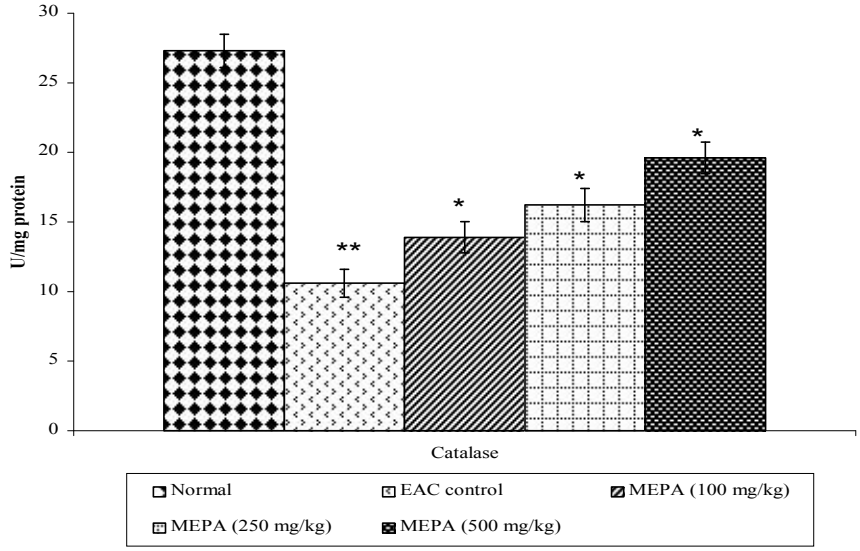
†P<0.001, EAC control group compared with the normal group.
*P<0.05, Experimental groups compared with the EAC control group.

3.3.4 Effect of MEPA on CAT levels

Figure 4 illustrates the activity of catalase in experimental animals. The CAT levels in EAC control group decreased by 61.17 % (P<0.01) compared with normal group. Treatment with MEPA at the doses of 100, 250 and 500 mg/kg increased the CAT levels significantly (P<0.05) by 23.74 %, 34.65 % and 45.92 % respectively when compared to that of EAC control mice.

Fig 4. Effect of methanol extract of *Plumeria acuminata* (MEPA) on hepatic CAT activity in EAC-bearing mice



Data are expressed as the mean of results in 6 mice (+) or (-) S.E.M.
†P<0.001, EAC control group compared with the normal group.
*P<0.05, Experimental groups compared with the EAC control group

3.5 Short-term toxicity

When the mice were observed for the behavioural changes after oral administration of a single dose of the extract none of the mice were exhibited any abnormal behavioural 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 3.

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Table 3. Effect of methanol extract of *Plumeria acuminata* (MEPA) on hematological, biochemical parameters and body weight of normal mice

Parameters	Normal (0.9 % NaCl, 5 ml/kg)	MEPA (100 mg/kg)	MEPA (250 mg/kg)	MEPA (500 mg/kg)
Hemoglobin (g %)	11.6±0.22	9.0±3.4	10.1±0.19	10.8±0.13
RBC (10^6/mm^3)	6.4±0.41	6.1±0.50*	6.3±0.12	6.6±0.40
Total WBC (10^6/mm^3)	5.2±0.51	5.7±0.58	6.6±0.29	7.4±0.48
SGPT (U/L)	65.1±0.25	68.1±0.53#	72.8±0.20	75.1±0.51#
SGOT (U/L)	39.5±0.03	42.6±0.52	44.1±0.18	45.4±0.42
Serum urea (mg/dl)	22.6±2.8	20.2±0.24	21.3±0.19**	23.4±3.7
Serum calcium (mg/dl)	10.1±5.9	10.3±4.8	10.4±0.12	10.7±1.2
Serum phosphate (mg/ml)	4.2±4.7	4.6±1.7	4.8±0.49	5.2±0.15#
LPO (nmol MDA/mg protein)	0.94±0.41	0.89±0.07	0.96±0.04	0.90±0.19
GSH (mg/g wet tissue)	2.33±0.68	2.40±0.29	2.44±0.22†	2.52±0.37
SOD (U/mg protein)	4.47±0.21	4.51±0.42	4.60±5.9	4.72±0.17
CAT (U/mg protein)	26.2±0.44	26.98±0.42#	27.7±2.3	28.1±0.05#
Body weight (g)	21.5±0.25	21.4±0.23	21.8±2.4	21.7±2.1

Data are expressed as the mean of results in 8 mice ± S.E.M.
*P<0.05; #P<0.01 and †P<0.001 Experimental groups compared with the normal group

4. DISCUSSION

The use of chemotherapeutic drugs in cancer therapy involves the risk of life threatening host toxicity. The search, therefore, goes on to develop the drugs which selectively act on tumor cells. The plants belonging to the family Apocynaceae have high medicinal properties. The present study also revealed the potential antitumor properties of *Plumeria acuminata* belonging to the family Apocynaceae.

The results of the present study clearly demonstrated the tumor inhibitory activity of methanolic extract of *Plumeria acuminata* transplantable murine tumor cells. In the EAC bearing mice, cells are present in the peritoneal cavity and the extracts were administered directly in to the peritoneum. Thus the tumor inhibition might be due to the direct effect of the extracts on the tumor cells.

Myelosupression is a frequent and major complication of cancer chemotherapy. Compared to the EAC control animals, MEPA treatment and subsequent tumor inhibition resulted in appreciable improvements in hemoglobin content, RBC and WBC counts. These observations assume great significance as anemia is a common complication in cancer and the situation aggravates further during chemotherapy since a majority of antineoplastic agents exert suppressive effects on erythropoiesis [28,29]) and thereby limiting the use of these drugs. Treatment with MEPA brought back the hemoglobin content, RBC and WBC cell count near to normal levels. This indicates that MEPA possess protective action on the hemopoietic system.

Decreased levels of lipid peroxidase, SOD and catalase suggest that the extracts possess potent antioxidant activity. Antitumor activity of these antioxidants is either through induction of apoptosis [30] or by inhibition of neovascularization [31]. The implication of free radicals in tumors is well documented [32,33]. In our earlier studies, we found that MEPA

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possess hepatoprotective and antioxidant properties. The free radical hypothesis supported the fact that the antioxidants effectively inhibit the tumor, and the observed properties may be attributed to the antioxidant and antitumor principles present in the extract.

In the short-term toxicity study, MEPA at the high dose level (500mg/kg) increased the urea content and transaminase activity, indicating that it causes hepatorenal dysfunction and alters metabolism

5. CONCLUSION

The results of the present study are encouraging as these extracts exhibit significant reduction in the tumor burden and caused prolongation of lifespan of the tumor hosts. Improvements, rather than aggravation, or tumor associated hematological complications such as anemia and bone marrow suppression were also noticed. All these parameters suggest that the methanol extract of *Plumeria acuminata* leaves exhibits potential antitumor and antioxidant activities. Further investigations are in progress in our laboratory to identify the active principles involved in this antitumor and antioxidant activity.

CONSENT

Not applicable

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTEREST

Authors have declared that no competing interests exist.

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