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1 ***Research paper***

2 Antioxidant and antitumor activity of *Plumeria acuminata* in 3 Ehrlich Ascites Carcinoma bearing Swiss Albino mice

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29 **ABSTRACT**

30 This study was designed to determine the antitumor and antioxidant properties of
31 crude methanol extract from the leaves of *Plumeria acuminata* (Apocynaceae) (MEPA)
32 against Ehrlich Ascites Carcinoma (EAC) bearing Swiss albino mice. The extract was
33 administered at the doses of 100, 250 and 500 mg/kg per day for 14 days, after 24 hr of
34 tumor inoculation. After the administration of the last dose followed by 18 hr fasting,
35 mice were then sacrificed for observation of antitumor activity. The effect of MEPA on
36 the growth of transplantable murine tumor, life span of EAC bearing host, viable and
37 non-viable cell count, packed cell volume, hematological profile and biochemical
38 parameters such as lipid peroxidation (LPO), reduced glutathione content (GSH),
39 superoxide dismutase (SOD) and catalase (CAT) activities were estimated. MEPA caused
40 significant ($P<0.01$) decrease in tumor volume, packed cell volume and viable count; and
41 it prolonged the life span of EAC-tumor bearing mice. Hematological studies reveal that
42 the Hb content and RBC count were decreased in EAC treated mice, whereas the
43 restoration to near normal levels was observed in extract treated animals. MEPA
44 significantly ($P<0.05$) decreased the levels of LPO and significantly increased the levels
45 of GSH, SOD and CAT. Moreover the MEPA was found to be devoid of conspicuous
46 short-term toxicity in the mice when administered daily for 14 days at the doses of 100,
47 250 and 500 mg/kg. The results suggested that the methanol extract of *Plumeria*
48 *acuminata* leaves exhibited antitumor effect by modulating lipid peroxidation and
49 augmenting antioxidant defense system in EAC bearing Swiss albino mice.

50 **Key words**: *Plumeria acuminata*, Ehrlisch Ascites Carcinoma, Hematological
51 Parameters, Antioxidant activity.

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52 **1. INTRODUCTION**

53 The role of natural products as a source for remedies has been recognized since
54 ancient times (Farnsworth et al., 1985; Cragg et al., 1997). Despite major scientific and
55 technological progre0ss in combinatorial chemistry, drugs derived from natural products
56 still make an enormous contribution to drug discovery today (Balandrin et al., 1993).

57 Cancer continues to represent the longest cause of mortality in the world and
58 claims over 6 million lives every year (Abdullaev et al., 2000). The enhanced generation
59 of ROS in vivo could be quite deleterious, since they are involved in mutagenesis,
60 apoptosis, ageing and carcinogenesis (Halliwell and Gutteridge, 1990). Free radicals also
61 cause DNA strand breaks and chromosome deletions and rearrangements. Further,
62 activated oxygen species most likely play an important role in tumor promotion and
63 progression (Kelloff et al., 1995). A variety of bioactive compounds and their derivatives
64 has been shown to inhibit cancer in a number of experimental systems involving
65 initiation, promotion and progression (Ho et al., 1994; Huang et al., 1994). Plants contain
66 abundant quantities of these substances and have consistently been shown to be
67 associated with a lower risk of cancers at almost every site (Steinmetz and Potter, 1991).
68 Efforts therefore are being made to identify naturally occurring anticarcinogenesis which
69 would prevent, slow and/or reverse the cancer induction and its subsequent development
70 (Chaung et al., 2000). An extremely promising strategy for cancer prevention today is
71 chemoprevention, which is defined as the use of synthetic or natural agents (alone or
72 combination) to block the development of cancer in humans. Several plant derived
73 compounds are currently successfully employed in cancer treatment and some of them
74 like vincristine, taxol and so on are available as a drug of choice (Adriana et al., 2001).

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75 The rich and diverse plant sources of India are likely to provide effective anticancer
76 agent. One of the best approaches in search for anticancer agents from plant resources is
77 the selection of plants based on ethnomedical leads and testing the selected plants
78 efficacy and safety in light of modern science. Exploration of traditional medicinal
79 practices in Tamilnadu brought to light ethnomedical use of young leaves of *Plumeria*
80 *acuminata* (Apocynaceae) to treat certain tumors in a few remote villages in Erode
81 district of Tamilnadu, India. This plant is also known as an antitumor agent in ancient
82 systems of medicine such as Ayurveda (Nadkarni, 1976).

83 *Plumeria acuminata* belonging to the family Apocynaceae is widely distributed
84 throughout the Southern parts of India. In traditional medicinal system different parts of
85 the plant have been mentioned to be useful in a variety of diseases. The bark has been
86 reported to be useful in hard tumors, diarrhoea and gonorrhea. The leaves are reported to
87 have anti-inflammatory, rubefacient in rheumatism and have strong purgative effect. Its
88 branches are used like those of 'chitraka' to produce abortion (Nadkarni, 1976). However
89 there is no scientific report or verification of the use of this plant in the treatment of these
90 conditions. Our recent findings revealed that the methanol extract of *P. acuminata* leaves
91 showed significant anti-inflammatory activity (Gupta et al., 2007) and antipyretic and
92 antinociceptive activity (Gupta et al., 2006). So far no reports are available on in vivo
93 antioxidant status of this plant in EAC tumor bearing mice. Hence we evaluated the in
94 vivo antitumor and antioxidant activity of the methanol extract of *P. acuminata* leaves in
95 EAC tumor bearing mice.

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96 ## 2. MATERIAL AND METHODS

97 ### 2.1 Plant material

98 The leaves of the plant *Plumeria acuminata* (Family: Apocynaceae) were
99 collected from Erode district of Tamilnadu, India. The plant material was taxonomically
100 identified by Botanical Survey of India, Kolkata. A voucher specimen (No. GMG 02/05)
101 has been preserved in our laboratory for future reference. The leaves were dried under
102 shade and then powdered with a mechanical grinder and stored in airtight container. The
103 dried powder material of the leaves was defatted with petroleum ether and the marc thus
104 obtained was then extracted with methanol in a soxhlet apparatus. The solvent was
105 completely removed under reduced pressure and a semisolid mass was obtained (MEPA,
106 yield 12.4 %). Phytochemical screening of the extract revealed the presence of
107 flavonoids, tannins, alkaloids, glycosides and steroids. The dried MEPA was suspended
108 in normal saline and used for the present study.

109 ### 2.2 Chemicals

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

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118 **2.3 Animals**

119 Studies were carried out using male Swiss albino mice of either sex weighing 21
120 ± 2 g. They were obtained from the animal house of Jadavpur University, Kolkata. The
121 mice were grouped and housed in poly acrylic cages (38x23x10 cm) with not more than
122 12 animals per cage and maintained under standard laboratory conditions (temperature 25
123 ± 2^0C) with dark/ light cycle (14/10 h). They were allowed free access to standard dry
124 pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The mice were
125 acclimatized to laboratory conditions for 10 days before commencement of the
126 experiment. All procedures described were reviewed and approved by the University
127 Animal Ethical Committee.

128 **2.4 Tumor Cells**

129 EAC cells were obtained from Chittaranjan National Cancer Institute (CNCI),
130 Kolkata, India. The EAC cells were maintained *in vivo* in Swiss albino mice by
131 intraperitoneal transplantation of $2x10^6$ cells per mouse after every 10 days. EAC cells 9
132 days old were used for the screening of antitumor activity of MEPA.

133 **2.5 Acute Toxicity Test**

134 The animals were divided into six groups containing eight animals in each group.
135 MEPA was suspended in normal saline and administered orally as a single dose to groups
136 of mice at different concentrations (500, 750, 1000, 1250, 1500 and 2000 mg/kg). These
137 animals were observed for a 72 h period. The number of deaths was expressed as a
138 percentile and the LD_{50} was determined by probit a test using the death percentage versus
139 the log dose (Thompson and Weil, 1952)

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140 **2.6 Antitumor Activity**

141 Male Swiss Albino mice were divided in to 6 groups (n=12). All the groups were
142 injected with EAC cells (0.2 ml of $2x10^6$ cells per mouse) intraperitonelly except the
143 normal group. This was taken as day zero. On the first day, 5 ml/kg of normal saline was
144 administered to group 1 and 2 (normal and EAC control). MEPA at different doses (100,
145 250 and 500 mg/kg) and the standard drug 5-Fluorouracil (20 mg/kg) (Kavimani and
146 Manisenthil Kumar, 2000) were administered to group 3, 4, 5 and 6 respectively for 14
147 days orally. After the last dose and 18 hr fasting, six mice from each group were
148 sacrificed for the study of antitumor activity, hematological and liver biochemical
149 parameters. The rest of the animal groups were kept to check the survival time of EAC-
150 tumor bearing hosts.

151 The antitumor activity of the methanol extract of *P. acuminata* was measured in
152 EAC animals with respect to the following parameters:

153 2.6.1 *Tumor volume*

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

157 2.6.2 *Tumor cell count*

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

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161 2.6.3 *Viable/ non-viable tumor cell count*

162 The cells were then stained with trypan blue (0.4 % in normal saline) dye. The
163 cells that didn't take up the dye were viable and those that took the dye were non-viable.
164 These viable and non-viable cells were counted.

165 Cell count = Number of cells x dilution/ Area x thickness of liquid film

166 2.6.4 *Percentage increase life span (% ILS)*

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

169 % ILS = (Mean survival of treated group/ Mean survival of control group)-1 x 100

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

171 2.6.5 *Body weight*

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

175 *2.6.6 Hematological Parameters*

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

181 2.6.7 *Biochemical Assays*

182 After the collection of the blood samples, the mice were sacrificed. Then their
183 liver was excised, rinsed in ice-cold normal saline solution followed by cold 0.15 M Tris-

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184 HCl (pH 7.4), blotted dry and weighed. A 10 % w/v homogenate was prepared in 0.15 M
185 Tris-HCl buffer and was used for the estimation of lipid peroxidation (LPO) and reduced
186 glutathione (GSH). The rest of the homogenate was centrifuged at 1500 rpm for 15 min at
187 4 ^{0}C. The supernatant thus obtained was used for the estimation of superoxide dismutase
188 (SOD), catalase (CAT) and total protein.

189 2.6.7.1 *Estimation of Lipid Peroxidation (LPO)*

190 The levels of Thiobarbituric acid reactive substances (TBARS) in the liver were
191 measured by the method of Ohkawa et al., 1979 as a marker for lipid peroxidation. A
192 mixture of 0.4 ml of 10 % liver homogenate, 1.5 ml of 8.1 % sodium dodecyl sulphate
193 (SDS), 1.5 ml of 0.8 % TBA solution was heated at 95^{0} C for 1 h. After cooling, 5.0 ml of
194 n-butanol-pyridine (15:1) was added and the absorbance of the n-butanol-pyridine layer
195 was measured at 532 nm.

196 2.6.7.2 *Estimation of Reduced Glutathione (GSH)*

197 The tissue GSH was determined by the method of Beutler and Kelly, 1963.
198 Virtually all the non-protein sulfhydryl groups of tissues are in the form of reduced GSH.
199 0.2 ml of tissue homogenate was mixed with 1.8 ml of EDTA solution. To this 3.0 ml
200 precipitation reagent (after precipitating proteins with TCA) was added, mixed
201 thoroughly and kept for 5 min before centrifugation. To 2.0 ml of the filtrate, 4.0 ml of
202 0.3 M disodium hydrogen phosphate solution and 1.0 ml of DTNB reagent were added
203 and the absorbance read at 412 nm.

204 2.6.7.3 *Estimation of Superoxide dismutase (SOD)*

205 The activity of SOD in tissue was assayed by the method of Kakkar et al., 1984.
206 The assay mixture contained 1.2 ml sodium pyrophosphate buffer (pH 8.3, 0.025 mol/L),

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207 0.1 ml PMS (186 mmol/L), 0.3 ml nitroblue tetrazolium (300 mmol/L, 0.2 ml NADH
208 (780 mmol/L) and diluted enzyme preparation and water in a total volume of 3 ml. After
209 incubation at 30^0 C for 90 sec, the reaction was terminated by the addition of 1.0 ml of
210 glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 ml n-
211 butanol. The color intensity of the chromogen in the butanol layer was measured at 560
212 nm against n-butanol.

213 2.6.7.4 *Estimation of Catalase (CAT)*

214 Catalase was assayed according to the method of Maehly and Chance, 1954. The
215 estimation was done spectrophotometrically following the decrease in absorbance at 230
216 nm. The tissue was homogenized in M/150 phosphate buffer (pH 7.0) at 4^0 C and
217 centrifuged at 5000 rpm. The reaction mixture contained 0.01 M phosphate buffer (pH
218 7.0), 2 mM $H_2 O_2$ and the enzyme extract. The specific activity of catalase is expressed in
219 terms of units/mg protein. A unit is defined as the velocity constant per second.

220 2.6.7.5 *Estimation of Total Proteins*

221 The protein content of tissue homogenates was measured by the method of Lowry
222 et al., 1951. 0.5 ml of tissue homogenate was mixed with 0.5 ml of 10 % TCA and
223 centrifuged for 10 min. The precipitate obtained was dissolved in 1.0 ml of 0.1 N NaOH.
224 From this an aliquot was taken for protein estimation. 0.1 ml of aliquot was mixed with
225 5.0 ml of alkaline copper reagent and allowed to stand at room temperature for 10 min.
226 0.5 ml of Folin's phenol reagent was added and the blue color developed was read after
227 20 min at 640 nm.

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228 **2.7 Short-term Toxicity**

229 To determine short-term (14 days) toxicity, healthy Swiss albino mice were
230 divided into 4 groups of 8 animals in each. Group 1 received normal saline (5 ml/kg)
231 orally once daily for 14 days (vehicle control). Groups 2, 3 and 4 received MEPA at the
232 doses of 100, 250 and 500 mg/kg respectively, orally once daily for 14 days. At twenty-
233 four hours after the last dose and after 18-h fasting, the mice were sacrificed. Blood and
234 liver were collected and important internal organs were removed, weighed and observed
235 for pathological changes. Hematological parameters were determined as described above.
236 Serum glutamate pyruvate transaminase (SGPT) and glutamate oxaloacetate transaminase
237 (SGOT) were determined (Bergmeyer et al., 1978). Urea was estimated by the enzymatic
238 method and calcium was estimated by the O-cresolphthalein complexone method (Tietz,
239 1987). Phosphorous was estimated by the colorimetric method (Henry, 1974). Liver
240 biochemical parameters were estimated by the standard methods described above.

241 **2.8 Statistical Analysis**

242 The experimental results were expressed as mean ± S.E.M. Data were assessed
243 by ANOVA followed by the Student *t*-test; P value <0.05 was considered as statistically
244 significant.

245 **3. RESULTS**

246 **3.1 Acute Toxicity study**

247 In the acute toxicity assay no deaths were observed during the 72 h period at the
248 doses tested. At these doses, the animals showed no stereotypical symptoms associated
249 with toxicity such as convulsion, ataxy, diarrhoea or increased diuresis. The median
250 lethal dose (LD_{50}) was determined to be higher than the highest dose tested i.e., 2.0 g/kg.

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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).

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

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

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.

3.3.2 *Effect of MEPA on reduced glutathione*

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

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297 mice increased GSH levels by 8.24 %, 16.47 % and 24.71 % respectively, as compared
298 with EAC control group (P<0.05).

299 3.3.3 *Effect of MEPA on SOD level*

300 Figure 3 shows the activity of SOD in liver tissue of experimental groups. The
301 levels of SOD in the liver of EAC bearing mice decreased by 28.87 % (P<0.01) in
302 comparison with normal group. Administration of MEPA at the doses of 100, 250 and
303 500 mg/kg increased the levels of SOD by 14.34 %, 24.05 % and 36.77 % respectively
304 (P<0.05) as compared to EAC control animals.

305 3.3.4 *Effect of MEPA on CAT levels*

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

311 **3.5 Short-term toxicity**

312 When the mice were observed for the behavioural changes after oral
313 administration of a single dose of the extract none of the mice were exhibited any
314 abnormal behavioural responses at the doses of 100, 250 and 500 mg/kg. Administration
315 of repeated daily doses of 100, 250 and 500 mg/kg for 14 days did not influence the
316 bodyweight of the mice. The weights of liver, kidney, brain and spleen were also not
317 altered by the treatment. Hematological parameters like hemoglobin and RBC count
318 remained unaltered at the dose of 100, 250 and 500 mg/kg. But there was a marginal
319 increase in WBC count. The results were summarized in Table 3.

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320 **4. DISCUSSION**

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

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

331 Myelosupression is a frequent and major complication of cancer chemotherapy.
332 Compared to the EAC control animals, MEPA treatment and subsequent tumor inhibition
333 resulted in appreciable improvements in hemoglobin content, RBC and WBC counts.
334 These observations assume great significance as anemia is a common complication in
335 cancer and the situation aggravates further during chemotherapy since a majority of
336 antineoplastic agents exert suppressive effects on erythropoiesis (Price and Greenfild,
337 1958; Hogland, 1982) and thereby limiting the use of these drugs. Treatment with MEPA
338 brought back the hemoglobin content, RBC and WBC cell count near to normal levels.
339 This indicates that MEPA possess protective action on the hemopoietic system.

340 Decreased levels of lipid peroxidase, SOD and catalase suggest that the extracts
341 possess potent antioxidant activity. Antitumor activity of these antioxidants is either
342 through induction of apoptosis (Ming et al., 1998) or by inhibition of neovascularization

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343 (Putul et al., 2000). The implication of free radicals in tumors is well documented (Ravid
344 and Korean 2003; Feng et al., 2001). In our earlier studies, we found that MEPA possess
345 hepatoprotective and antioxidant properties. The free radical hypothesis supported the
346 fact that the antioxidants effectively inhibit the tumor, and the observed properties may
347 be attributed to the antioxidant and antitumor principles present in the extract.

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

351

352 5. CONCLUSION

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

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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

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485 **Table 2. Effect of methanol extract of *Plumeria acuminata* (MEPA) on hematological**
486 **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

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

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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

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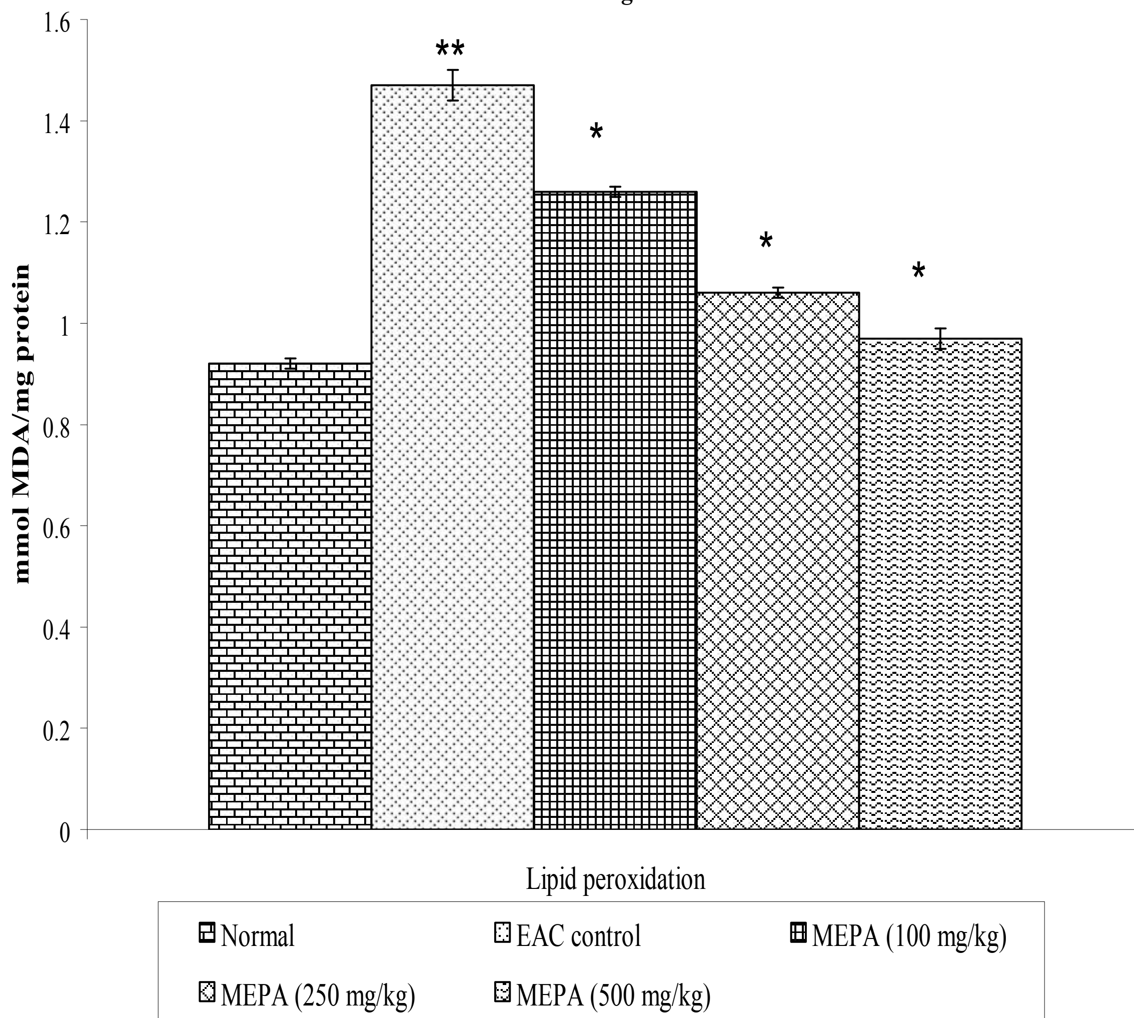

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UNDER PEER REVIEW

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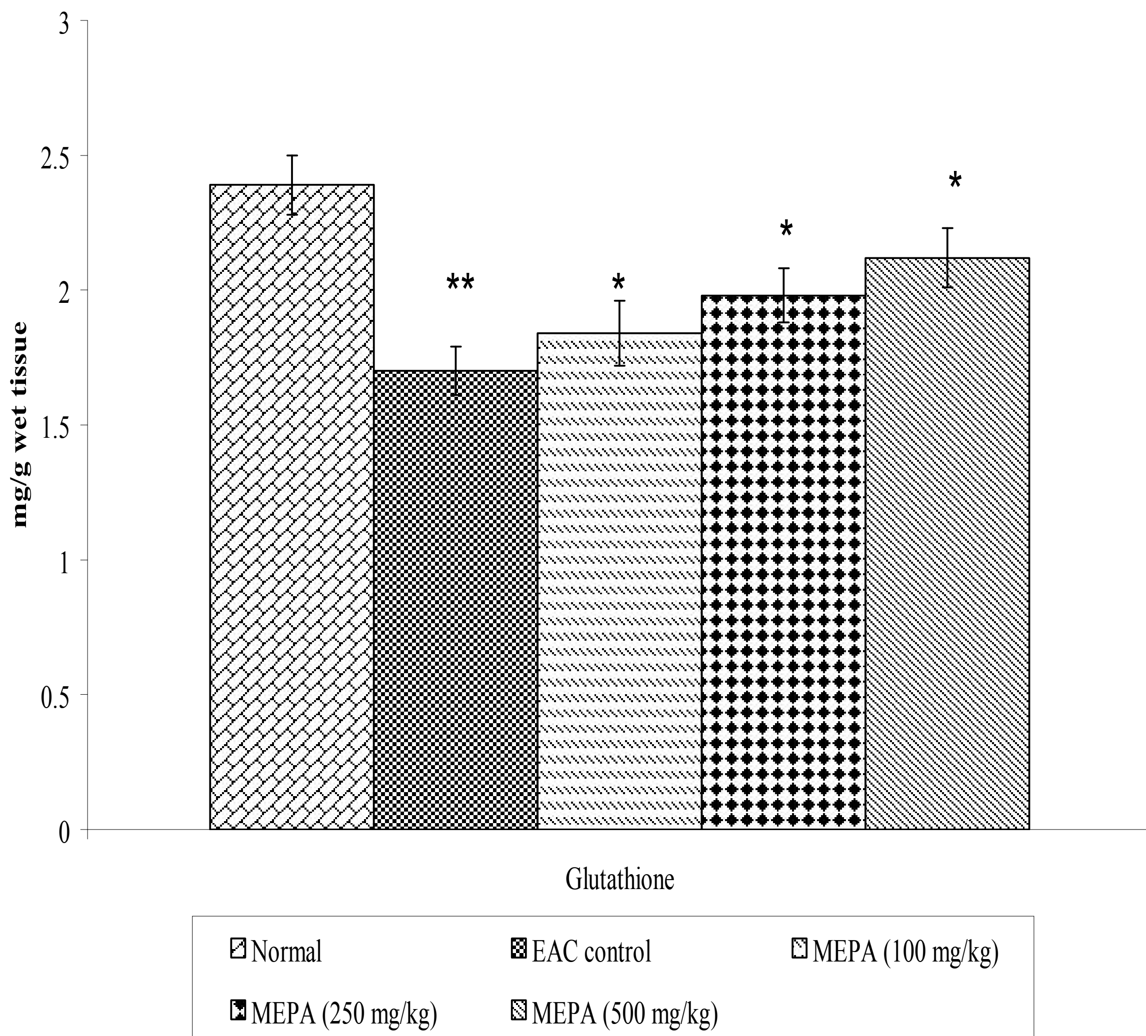

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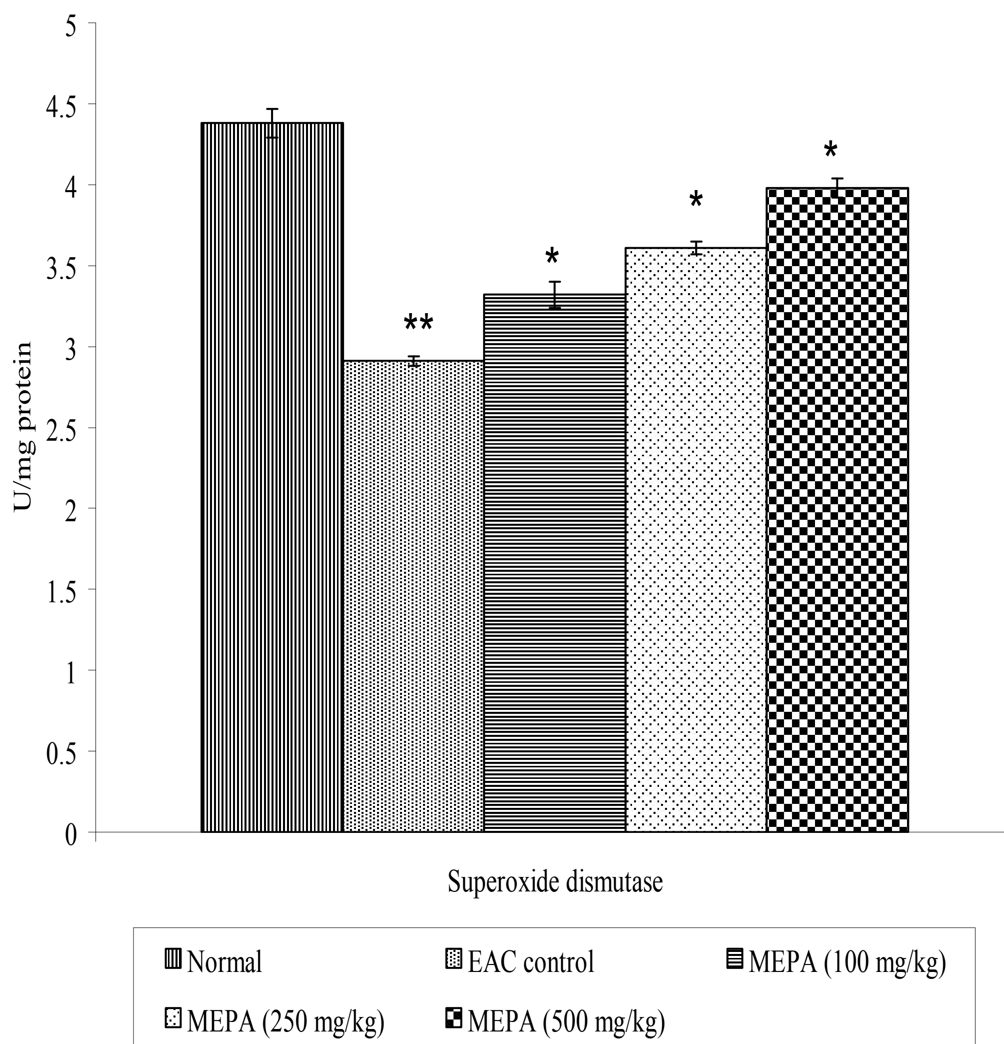

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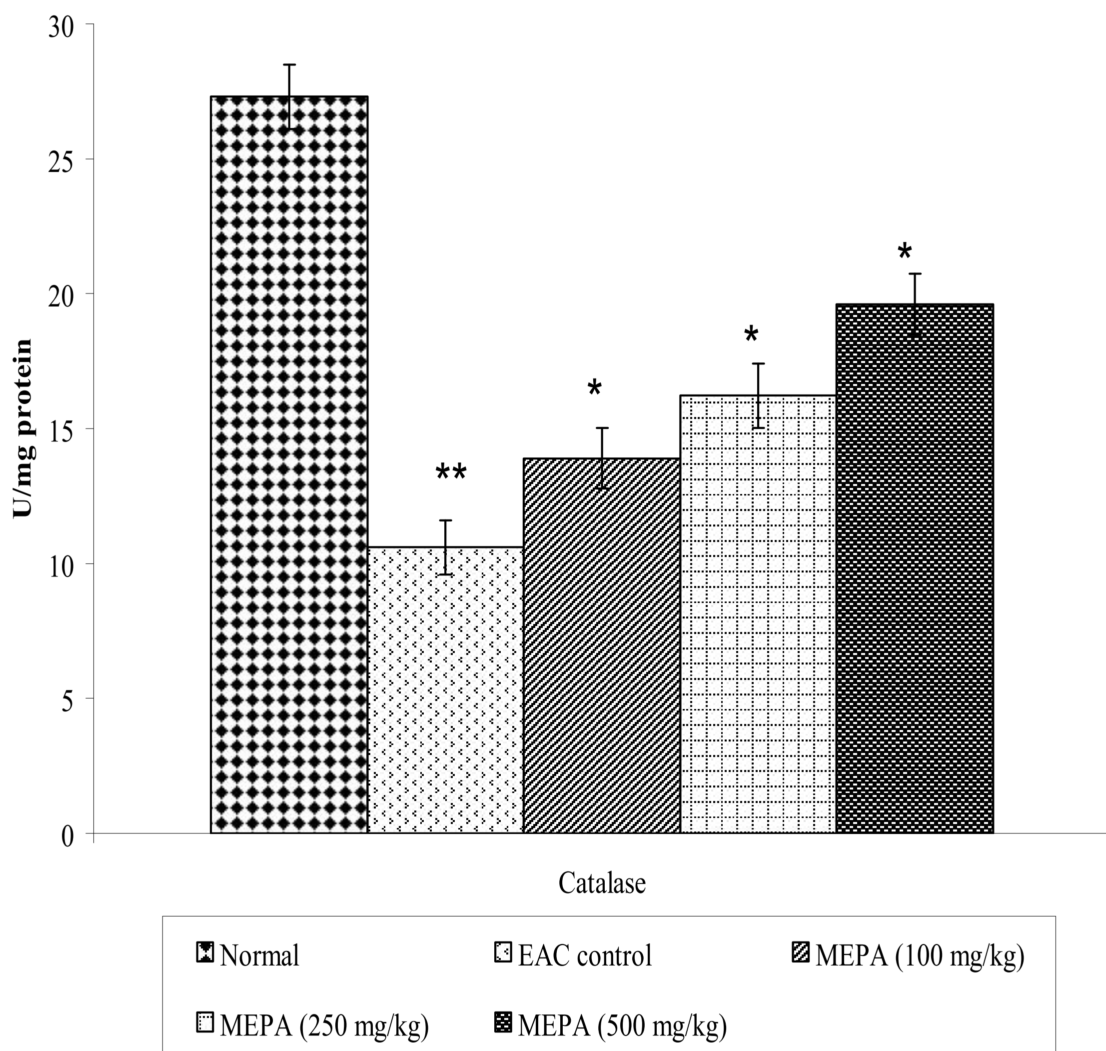

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555 **LEGENDS**

556 **Figure 1, 2, 3 & 4**

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

558 †P<0.001, EAC control group compared with the normal group.

559 *P<0.05, Experimental groups compared with the EAC control group.