Antitumor activity and antioxident role of *Bauhinia racemosa* against Ehrlich ascites carcinoma in Swiss albino mice[1]

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ABSTRACT

AIM: To study the antitumor effect and antioxidant role of *Bauhinia racemosa.* **METHODS:** Antitumor activity and antioxidant status of methanol extract (50, 100, and 200 mg/kg) of *Bauhinia racemosa* stem bark was evaluated against Ehrlich ascites carcinoma (EAC) tumor in mice. Acute and short-term toxicity studies were performed initially in order to ascertain the safety of methanol extract of *Bauhinia racemosa* (MEBR). After 24 h of tumor inoculation, the extract was administered daily for 14 d. After administration of the last dose followed by 18 h fasting, mice were then sacrificed for observation of antitumor activity. The effect of MEBR 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. **RESULTS:** The MEBR showed decrease in tumor volume, packed cell volume and viable cell count, and increased 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 MEBR decreased the levels of lipid peroxidation and increased the levels of glutathione, superoxide dismutase and catalase. **CONCLUSION:** The methanol extract of *Bauhinia racemosa* stem bark exhibited antitumor effect by modulating lipid peroxidation and augmenting antioxidant defense system in EAC bearing mice.

INTRODUCTION

Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives every year[1]. 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. Plants, vegetables and herbs used in the folk and traditional medicine have been accepted currently as one of the main source of cancer chemoprevention drug discovery and development[2]. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine. The plant *Bauhinia racemosa* Lam belongs to the family, *Caesalpiniaceae* popularly known as Sittacha (Tamil) has a widespread occurrence in India, Ceylon, China, and Timor. It is used in traditional medicine for the treatment of various ailments. The stem bark of the plant is an astringent and is used in the treatment of headache, fever, skin diseases, tumors, diseases of the blood, dysentery and diarrhea[3]. Pharmacological studies of the plant revealed that the ethanol extract of leaves of *B racemosa* presented analgesic, antipyretic, anti-

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inflammatory, antispasmodic[4], and antimicrobial activity[5]. The fresh flower buds of this plant showed antiulcer activity[6]. Cytotoxicity against CA-9 KB in cell culture, hypotensive, and hypothermic activities were also reported from the hydroalcholic extract of *B racemosa*[7]. Several phytochemical constituent of *B racemosa* have been isolated and chiefly include flavonoids (kaempferol and quercetin), coumarins (scopoletin and scopolin)[8], triter-penoids (β-amyrin), steroids (β-sitosterol)[9], and stilbenes (resveratrol)[10].

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 antioxidant and antitumor activity[11,12]. Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against infection and degenerative diseases. From this viewpoint the present study was carried out to evaluate the antitumor activity, lipid peroxidation and antioxidant status of methanol extract of *Bauhinia racemosa* (MEBR) against Ehrlich ascites carcinoma (EAC) in Swiss albino mice.

MATERIALS AND METHODS

Materials The dried powder material of the stem bark of *Bauhinia racemosa* was extracted with methanol (yield 9.25 %), in a soxhlet apparatus. The methanol extract was then distilled, evaporated, and dried in vacuum. The extract was dissolved in propylene glycol for further experiments. All the chemicals were of analytical grade. 1-Chloro-2,4-dinitrobenzene (CDNB), bovine serum albumin (Sigma chemical St Louis, MO, USA), Thiobarbituric acid, Nitrobluetetrazolium chloride (NBT) (Loba Chemie, Bombay India), 5,5'-dithio *bis*-2-nitrobenzoic acid (DTNB) (SICCO Research Laboratory, Bombay). 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. Studies were carried out using male Swiss albino mice weighing 20±2 g were obtained from Indian Institue of Chemical Biology (IICB), Kolkata India. All procedures described were reviewed and approved by the University Animals Ethical Committee.

Methods

Toxicity study An acute toxicity study relating to the determination of LD_{50} was performed[13]. A short-term toxicity study was also carried out for a period of 14 d which is period of the study of antitumor activity. Healthy Swiss albino mice were divided into 5 group of 8 animals in each. Group 1 (vehicle control) received propylene glycol 5 mL/kg intraperitoneally once daily for 14 d. Group 2, 3, 4, and 5 received methanol extract *Bauhinia racemose* (MEBR) at the doses of 50, 100, 200, and 400 mg/kg intraperitoneally once daily for 14 d. After 24 h of the last dose including 18 h of fasting the mice were sacrificed. Blood collected and hematological parameters were determined as described in hematological studies. Liver and other important internal organs were removed, weighed, and observed for pathological changes. Serum glutamate pyruvate transaminase (ALT) and glutamate oxaloacetate transaminases (AST) were determined[14] using a portion of the blood collected. Urea by enzymatic method (Tietz, 1987)[15], calcium by 0-cresolphthalin complexone method (Tietz, 1987)[15], and phosphorus by colorimetric method[16] was estimated from serum. Further, liver biochemical parameters were estimated by methods described in estimation of biochemical parameters.

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 $mL\cdot kg^{-1}\cdot mouse^{-1}\cdot d^{-1}$ and propylene glycol 5 $mL\cdot kg^{-1}\cdot mouse^{-1}\cdot d^{-1}$ were administered to normal and EAC control groups respectively for 14 d intraperitoneally. Similarly MEBR at different doses (50, 100, and 200 $mg\cdot kg^{-1}\cdot mouse^{-1}\cdot d^{-1}$) and standard drug 5-flurouracil (20 mg/kg)[17] were administered in groups 3, 4, 5, and 6, respectively. After the administration of last dose followed by 18 h fasting 6 mice from each group were sacrificed for the study of antitumor activity, hematological and liver biochemical parameters. The remaining animals in each of the groups were kept to check the mean survival time (MST) of the tumor bearing hosts.

Antitumor effect of MEBR was assessed by observation of changes with respect to body weight, ascetics tumor volume, packed cell volume, viable & non-viable tumor cell count, MST and percentage increase in life span (% ILS). MST of each group containing six mice were monitored by recording the mortality daily for 6 weeks and % ILS was calculated using following equation[18,19]. MST=(Day of first death+Day of last death)/2. ILS (%)=[(Mean survival time of treated group/mean survival time of control group)–1]×100.

Hematological studies Hemoglobin content, red blood cell (RBC) and white blood cell (WBC) counts were measured from freely flowing tail vein blood[20, 21]. Differential WBC leukocyte count[22] was carried out from Leishaman stained blood smears of normal, EAC control, and MEBR treated groups respectively.

Estimation of biochemical parameter After the collection of blood samples the mice were sacrificed and their liver were excised, rinsed in ice-cold normal saline followed by cold 0.15 mol/L Tris-HCl buffer (pH 7.4), blotted dry, and weighed. A 10 % w/v homogenate was prepared in 0.15 mol/L Tris-HCl buffer and a portion utilized for the estimation of lipid peroxidation[23] an other portion of the same after precipitating proteins with TCA was used for the estimation of glutathione[24]. The remaining homogenate was centrifuged at 1500 rpm for 15 min at 4 ºC. The supernatant thus obtained was used for the estimation of superoxide dismutase, catalase, and protein content[25- 27].

Statistical analysis The experimental results were expressed as mean±SEM. Data were assessed by the method of analysis of ANOVA followed by *t*-test. $P<0.05$ was considered as statistically significant.

RESULTS

Short term toxicity studies When the mice were observed for the behavioral changes after intraperitoneally administration of a single dose of the extract, none of the mice exhibited any abnormal behavioral responses at doses of 50, 100, and 200 mg/kg. But mice which received 400 mg/kg or above showed slight toxic symptoms. These include inactiveness, loss of appetite, slow movement, dizziness, erection of hairs, and hypothermia. Administration of repeated daily doses of 50, 100, 200, and 400 mg/kg for 14 d did not influence the body weight 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 50, 100, and 200 mg/kg. But there was a marginal increase in WBC count. The hematological parameters, urea, and transaminase activities increased at the dose of 400 mg/kg (Tab 1).

Effect of MEBR on mean survival time and tumor growth In the EAC control group the mean survival time was 18.0 d, while it increased to 23.1 (50 mg/kg), 30.4 (100 mg/kg), and 35.9 (200 mg/kg) d, respectively in MEBR-treated groups. The group treated with the standard drug 5-fluoruracil (20 mg/kg) showed 39.5 d for the same. Treatment with MEBR at the doses of 50, 100, and 200 mg/kg reduced the body weight, tumor volume, packed cell volume, and viable tumor cell count in a dose-dependent manner as compared to that of EAC control group. Further, nonviable tumor cell counts at different doses of MEBR were increased when compared with the EAC control (Tab 2).

Effect of MEBR on hematological parameters Hemoglobin content and RBC count in the EAC control group was decreased when compared to normal group.

Tab 1. Effect of methanol extract of *Bauhinia racemosa* (MEBR) on hematological, biochemical parameters, and body weight of normal mice. *n*=8. Mean±SEM. $^{b}P<0.05$, $^{c}P<0.01$ *vs* EAC control group.

Parameters	Vehicle control 5 mL/kg	MEBR 50 mg/kg	MEBR 100 mg/kg	MEBR 200 mg/kg	MEBR 400 mg/kg
Hb/g %	11.80±0.12	9.40±0.16	10.00±0.13	10.90±0.24	13.50±0.25^{b}
RBC/10^{6}·mm^{-3}	6.60±0.20	6.00±0.35	6.4±0.4	6.8±0.5	8.2±0.4^{b}
Total WBC/10^{3}·mm^{-3}	5.40±0.19	5.90±0.22	6.9±0.5	8.5±0.5^{c}	7.30±0.21
ALT/U·L^{-1}	65.3±0.5	66.3±0.4	71.1±0.5	74.2±0.6	92.5±0.7^{b}
AST/U·L^{-1}	39.50±0.22	41.8±0.3	43.3±0.4	44.2±0.5	80.1±0.4^{b}
Serum urea/mg·L^{-1}	228.0±2.4	214.0±2.9	225±4	228±3	132±5^{c}
Serum calcium/mg·L^{-1}	103.0±2.3	104±3	107.0±1.2	109±5	82±6
Serum phosphorus/mg·L^{-1}	44±6	46.0±2.5	5.02±2.1	55.0±1.7	50.0±2.8
LPO/nmol MDA·mg^{-1} (protein)	0.96±0.04	0.94±0.02	0.98±0.05	1.01±0.07^{c}	1.09±0.03
GSH/mg·g^{-1}(wet tissue)	2.35±0.12	2.31±0.28	2.45±0.17	2.21±0.29^{c}	2.48±0.25
SOD/U·mg^{-1}(protein)	4.49±0.18	4.53±0.27	4.60±0.37	4.65±0.42	4.56±0.51
CAT/U·mg^{-1}(protein)	26.40±0.29	27.0±0.4	27.50±0.19	28.4±0.4	27.5±0.4
Body weight/g	21.70±0.19	22.10±0.13	22.30±0.15	22.90±0.23	21.20±0.22

Tab 2. Effect of methanol extract of *Bauhinia racemosa* (MEBR) on body weight, mean survival time, % ILS, tumor volume, packed cell volume, viable and non-viable tumor cell count of EAC bearing mice. *n*=6. Mean±SEM. $^{c}P<0.01$ *vs* EAC control group. Body weight of normal mice: 20.70±0.12 g.

Parameters	EAC control (2×10^6 Cells/mouse)	MEBR (50 mg/kg) +EAC	MEBR (100 mg/kg) +EAC	MEBR (200 mg/kg) +EAC	Standard 5-floururacil (20 mg/kg)+EAC
Body weight /g	26.70±0.16	24.60±0.19	22.50±0.13	21.20±0.14	20.70±0.09
Mean survival time /d	18.00±0.11	23.20±0.17^{c}	30.40±0.16^{c}	35.90±0.19^{c}	39.50±0.15^{c}
Increase life Span/%	–	34.12	68.75	99.50	119.49
Tumor volume/mL	4.51±0.07	3.37±0.07^{c}	2.41±0.03^{c}	1.20±0.01^{c}	–
Packed cell volume/mL	2.11±0.84	1.40±0.04^{c}	0.81±0.01^{c}	0.23±0.01^{c}	–
Viable tumor cell count/10^{10}cells·L^{-1}	12.30±0.07	0.32±0.01	8.84±0.06	0.68±0.04^{c}	5.04±0.04^{c}
Non Viable tumor cell count/10^{10}cells·L^{-1}	0.89±0.06	1.62±0.06^{c}	1.57±0.05^{c}	-	-

Treatment with MEBR at the dose of 50, 100, and 200 mg/kg increased the hemoglobin content and RBC count to more or less normal levels. The total WBC counts and protein were found to be increased in EAC control group when compared with normal group. Administration of MEBR at the dose of 50, 100, and 200 mg/kg in EAC bearing mice reduced both WBC count and protein as compared with EAC control. In the differential count of WBC, increase of neutrophils and the lymphocyte count decreased in EAC control group. Treatment with MEBR at different doses changed these altered parameters more or less normal (Tab 3).

Effect of MEBR on lipid peroxidation and glutathione Tab 4 showed that the levels of lipid peroxidation in liver tissue were increased by 48.9 % in EAC control group as compared to the normal group ($P<0.01$). After administration of MEBR at different doses (50, 100, and 200 mg/kg) to EAC bearing mice the level of lipid peroxidation were reduced by 11.4 %, 23.6 %, and 31.4 %, respectively in comparison to EAC control group ($P<0.05$). Inoculation of EAC drastically decreased the GSH content to 30.6 % ($P<0.01$) in EAC control group when compared with normal group. The administration of MEBR at the dose of 50, 100, and 200 mg/kg to the EAC bearing mice increased GSH levels by 7.4 %, 14.2 %, and 22.0 %, respectively as compared with EAC control group ($P<0.05$).

Effect of MEBR on antioxidant enzymes The effect of MEBR on the antioxidant enzymes was given in Tab 4. The levels of superoxide dismutase (SOD) in the liver of EAC bearing mice decreased by 35.6 % ($P<0.01$) in comparison with normal group. After administration of MEBR at the dose of 50, 100, and 200 mg/kg increased levels of SOD by 13.4 %, 23.9 %, and 27.2 %, respectively as compared to that of EAC control group ($P<0.05$). The catalase (CAT) level in EAC

Tab 3. Effect of methanol extract of *Bauhinia racemosa* (MEBR) on hematological parameters of EAC bearing mice. *n*=6. Mean±SEM. $^{c}P<0.01$ *vs* normal group, $^{f}P<0.01$ *vs* EAC control group.

Parameters	Normal	EAC control (2×10^6 Cells/mouse)	MEBR (50 mg/kg) +EAC	MEBR (100 mg/kg) +EAC	MEBR (200 mg/kg) +EAC
Hemoglobin/g%	13.40±0.14	10.51±1.15^{c}	11.02±0.17^{f}	11.87±0.19	12.95±0.18^{f}
RBC/10^{15}·L^{-1}	6.53±0.11	3.71±0.09^{c}	4.92±0.02^{f}	5.47±0.03^{f}	5.95±0.06^{f}
WBC/10^{12}·L^{-1}	4.73±0.09	17.20±0.03^{c}	12.51±0.08	8.97±0.03^{f}	5.02±0.06^{f}
Monocyte/%	1.80±0.01	1.10±0.02	1.27±0.03^{f}	1.40±0.01^{f}	1.80±0. 01
Neutrophil/%	17.8±0.15	65.4±0.17^{c}	53.5±0.19	39.9±0.12^{f}	25.1±0.12^{f}
Lymphocyte/%	80.4±0.23	32.5±0.42	46.3±0.35^{f}	58.7±0.33	73.1±0.43^{f}

Tab 4. Effect of methanol extract of *Bauhinia racemosa* (MEBR) on lipid peroxidation glutathione content and antioxidant enzymes in the liver of EAC bearing mice. *n*=6. Mean±SEM. $^{c}P<0.01$ *vs* normal group, $^{e}P<0.05$ *vs* EAC control group.

Parameters	Normal	EAC control (2×10^6 Cells/mouse)	MEBR (50 mg/kg) +EAC	MEBR (100 mg/kg) +EAC	MEBR (200 mg/kg) +EAC
Lipid peroxidation/nmol MDA·mg^{-1} (protein)	0.94±0.02	1.40±0.01^{c}	1.20±0.01^{e}	1.07±0.02	0.96±0.01^{e}
Glutathione content/mg·g^{-1} (wet tissue)	2.35±0.09	1.63±0.07^{c}	1.75±0.06^{e}	1.90±0.09^{e}	2.09±0.25^{e}
Superoxide dismutase/U·mg^{-1} (protein)	4.49±0.35	2.89±0.26^{c}	3.27±0.23^{e}	3.58±0.29	3.97±0.32
Catalase/U·mg^{-1} (protein)	26.4±0.07	10.8±0.07^{c}	12.5±0.03^{e}	14.2±0.09^{e}	16.8±0.05^{e}

control group decreased by 59.1 % ($P<0.01$) compared with normal group. Treatment with MEBR at the dose of 50, 100, and 200 mg/kg increased CAT levels by 15.7 %, 31.5 %, and 55.6 %, respectively when compared to that of EAC control ($P<0.05$).

DISCUSSION

The present study was carried out to evaluate the toxicity, antitumor activity, lipid peroxidation and antioxidant status of MEBR on EAC bearing mice. In short-term toxicity study the MEBR at the high dose level (400 mg/kg) increased the urea and transaminase activity indicating its hepatorenal dysfunction and metabolism. The MEBR treated animals at the doses of 50, 100, and 200 mg/kg inhibited the body weight, tumor volume, packed cell volume, tumor cell count and also 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 at doses of 50, 100, and 200 mg/kg for 14 d MEBR did not exhibit any adverse effect.

A reliable criteria for judging the value of any anticancer agents is the prolongation of life span of animals[28]. 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.

In cancer chemotherapy the major problem are of myelosuppression and anemia[29,30]. 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[31]. Treatment with MEBR brought back the hemoglobin content, RBC and WBC cell count near to normal values. This indicates that MEBR posses protective action on the heamoto-poietic 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*[32]. Increased lipid peroxidation would cause degenaration of tissues. Lipid peroxide formed in the primary site would be transferred through the circulation and provoke damage by propagating the process of lipid peroxidation[33]. MDA, the end product of lipid peroxidation was reported to be higher in carcinomatous tissue than in non diseased organs[32]. Glutathione, a potent inhibitor of neoplastic process plays an important role as an endogenous antioxidant system that is found particularly in high concentration in liver and is known to have key function in the protective process[33]. MEBR reduced the elevated levels of lipid peroxidation and increased the glutathione content in EAC bearing mice.

On the other hand the free radical scavenging system, SOD and catalase 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. Sun *et al*[34] reported 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. The inhibition of SOD and CAT activities as a result of tumor growth was also reported[35]. Similar findings were observed in the present investigation with EAC bearing mice. The administration of MEBR 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 MEBR.

Plant derived extracts containing antioxidant principles showed cytotoxicity towards tumor cells[36] and antitumor activity in experimental animals[37]. The lowering of lipid peroxidation and increase in levels of GSH, SOD and catalase in MEBR-treated group indicates its potential as an inhibitor of EAC induced intracellular oxidative stress. We propose that the additive and synergistic antioxidant activity of phytochemicals such as flavonoids, triterpenoids, steroids, *etc*, present in MEBR are responsible for the its potent antitumor activity which can be inferred from the increased the 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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