Antitumor Activity and Antioxidant Status of *Caesalpinia bonducella* Against Ehrlich Ascites Carcinoma in Swiss Albino Mice

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**Abstract.** The methanol extract of *Caesalpinia bonducella* FLEMING (Caesalpiniaceae) leaves (MECB) were evaluated for antitumor activity against Ehrlich ascites carcinoma (EAC)-bearing Swiss albino mice. The extract was administered at the doses of 50, 100, and 200 mg/kg body weight per day for 14 days after 24 h of tumor inoculation. After the last dose and 18 h fasting, the mice were sacrificed. The present study deals with the effect of MECB on the growth of transplantable murine tumor, life span of EAC-bearing hosts, hematological profile, and biochemical parameters such as lipid peroxidation (LPO), glutathione content (GSH), superoxide dismutase (SOD), and catalase (CAT) activities. MECB caused significant ($P<0.01$) decrease in tumor volume, packed cell volume, and viable cell count; and it prolonged the life span of EAC-tumor bearing mice. Hematological profile converted to more or less normal levels in extract-treated mice. MECB significantly ($P<0.05$) decreased the levels of lipid peroxidation and significantly ($P<0.05$) increased the levels of GSH, SOD, and CAT. The MECB was found to be devoid of conspicuous short-term toxicity in the mice when administered daily (i.p.) for 14 days at the doses of 50, 100, 200, and 300 mg/kg. The treated mice showed conspicuous toxic symptoms only at 300 mg/kg. The results indicate that MECB exhibited significant antitumor and antioxidant activity in EAC-bearing mice.

**Keywords:** *Caesalpinia bonducella*, Ehrlich ascites carcinoma, hematological parameter, antioxidant, antitumor activity

**Introduction**

India is a rich source of medicinal plants and a number of plant extracts are used against diseases in various systems of medicine such as *Ayurveda*, *Unani*, and *Siddha*. Only a few of them have been scientifically explored. Plant derived natural products such as flavonoids, terpenes, alkaloids (1 – 3), and so on have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemopreventive effects (4). Several plant products have been tested for anticancer activity and some of them like vincristine, taxol, and so on are now available as a drug of choice. The rich and diverse plant sources of India are likely to provide effective anticancer agents. 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 medical practices in Tamilnadu brought to light ethnomedical use of young leaves of *Caesalpinia bonducella* FLEMING (Caesalpiniaceae) to treat certain tumors in a few remote villages in Kolli Hills, Nammakal District of Tamilnadu, India. This plant is also known as an antitumor agent in ancient systems of medicine such as *Ayurveda* (5).

*Caesalpinia bonducella* commonly known as Nata Karanja (in Hindi) is a prickly shrub found throughout the hotter regions of India, Myanmar, and Sri Lanka. The twigs and young leaves of *Caesalpinia bonducella* are traditionally used for the treatment of tumors, inflammation, and liver disorders (5). In addition, vari-
ous parts of this plant has been reported to possess multiple therapeutic properties like antipyretic, antidiuretic, anthelmintic, antibacterial, anticonvulsant, antiviral, antiasthmatic, antiamebic, and antiestrogenic activities (6–10). Recently hepatoprotective and antioxidant role of *Caesalpinia bonducella* were reported from our laboratory (11). The chemical constituents of the plant include flavonoids, triterpenoids, diterpenoids, and steroids (12–15). A number of reports on flavonoids, triterpenoids, and steroids indicate that they exert multiple biological effects due to their antioxidant and free radical scavenging abilities. These phytoconstituents produced protective effects against tumors, heart disease, and different pathologies (16, 17). Realizing these facts, this work was carried out to evaluate the antitumor activity and antioxidant status of the methanol extract of *Caesalpinia bonducella* leaves (MECB) against Ehrlich ascites carcinoma (EAC) in Swiss albino mice.

**Materials and Methods**

**Plant material**

The plant *Caesalpinia bonducella* (Family: Caesalpiniiaceae) was collected in the month of March 2003 from the Kolli Hills. The plant material was taxonomically identified by the Botanical Survey of India (Shibpur, Kolkata, India), and the Voucher specimen (No. GMS-2) was retained in our laboratory for future reference. The dried powder material of the twigs and young leaves of *Caesalpinia bonducella* were extracted with methanol (yield 8.78%) in a soxhlet apparatus. The methanol extract was then distilled, evaporated, and dried in vacuum. Phytochemical screening of the extracts revealed the presence of alkaloids, saponins, flavonoids, terpenes, tannins, and steroids. The required amount of MECB was dissolved in propylene glycol to obtain the necessary doses employed in the study.

**Animals**

Studies were carried out using male Swiss albino mice weighing 20 ± 2 g. They were obtained from the animal house of Jadavpur University, Kolkata. The mice were grouped and housed in polyacrylic cages (38 x 23 x 10 cm) with not more than twelve animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°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 Animals Ethical Committee.

**Chemicals**

The following chemicals were obtained from the indicated commercial sources: 1-chloro-2,4-dinitrobenzene (CDNB), bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA); thiobarbituric acid, nitroblue tetrazolium chloride (NBT) (Loba Chemie, Bombay, India); 5,5'-dithio bis-2-nitro benzoic acid (DTNB) (SISCO Research Laboratory, Bombay, India). All the reagents used were of analytical reagent grade.

**Tumor cells**

EAC cells were obtained from Chittaranjan National Cancer Institute (CNCI) (Kolkata, India). The EAC cells were maintained by intraperitoneal inoculation of 2 x 10⁶ cells/mouse.

**Toxicity study**

An acute toxicity study relating to the determination of LD₅₀ was performed (18).

**Antitumor activity**

Male Swiss albino mice were than divided into 6 groups (n = 12). All the groups were injected with EAC cells (0.2 ml of 2 x 10⁶ cells/mouse) intraperitoneally except the normal group. This was taken as day zero. On the first day, 5 ml/kg of normal saline was administered in group 1 (Normal). Propylene glycol, 5 ml/kg per day, was administered in group 2 (EAC control). MECB at different doses (50, 100, and 200 mg/kg per day) and the standard drug 5-fluorouracil (19) (20 mg/kg) were administered in groups 3, 4, 5, and 6 respectively for 14 days intraperitoneally. 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.

**Tumor growth response**

The antitumor effect of MECB was assessed by change in the body weight, ascites tumor volume, packed cell volume, viable and nonviable tumor cell count, mean survival time (MST), and percentage increased life span (% ILS). MST of each group containing six mice was monitored by recording the mortality daily for 6 weeks and % ILS was calculated using following equation (20, 21):

\[
\text{MST} = (\text{Day of first death} + \text{Day of last death}) / 2
\]

\[
\text{ILS} (%) = [(\text{Mean survival time of treated group} / \text{mean survival time of control group}) - 1] \times 100
\]

**Hematological studies**

Hemoglobin content, red blood cell (RBC), and white...
blood cell (WBC) counts were measured from freely flowing tail vein blood (22, 23). Differential leukocyte count of WBC was carried out from Leishman stained blood smears (24) of normal, EAC control, and MECB-treated groups, respectively.

Biochemical assays

After the collection of blood samples, the mice were sacrificed. Then their liver was excised, rinsed in ice-cold normal saline 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; a portion was utilized for the estimation of lipid peroxidation (25) and a second portion, after precipitating proteins with TCA, was used for the estimation of glutathione (26). The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of superoxide dismutase, catalase, and protein (27 – 29).

Short-term toxicity

To determine short-term (14 days) toxicity, healthy Swiss albino mice were divided into 5 group of 8 animals in each. Group 1 received propylene glycol, 5 ml/kg, i.p. once daily for 14 days (vehicle control). Groups 2, 3, 4, and 5 received MECB at the doses of 50, 100, 200, and 300 mg/kg, i.p. once daily for 14 days. After 24 hours after the last dose, and after 18-h fasting, the mice were sacrificed. Blood and liver was 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 (30). Urea was estimated by the enzymatic method (Tietz, 1987) (31). Calcium was estimated by the O-cresolphthalein complexone method (Tietz, 1987) (31). Phosphorous was estimated by the colorimetric method (32). Liver biochemical parameters were estimated by the standard methods described above.

Table 1. Effect of the methanol extract of Caesalpinia bonducella leaves (MECB) on body weight, mean survival time, % ILS, tumor volume, packed cell volume, and viable and nonviable tumor cell count of EAC-bearing mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>EAC Control</th>
<th>MECB (50 mg/kg) + EAC</th>
<th>MECB (100 mg/kg) + EAC</th>
<th>MECB (200 mg/kg) + EAC</th>
<th>Standard 5-fluorouracil (20 mg/kg) + EAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>27.7 ± 0.12</td>
<td>24.84 ± 0.17**</td>
<td>23.5 ± 0.13**</td>
<td>22.3 ± 0.13**</td>
<td>21.2 ± 0.19**</td>
</tr>
<tr>
<td>Mean survival time (days)</td>
<td>18.02 ± 0.19</td>
<td>22.43 ± 0.17**</td>
<td>28.62 ± 0.16**</td>
<td>33.72 ± 0.19**</td>
<td>39.54 ± 0.25**</td>
</tr>
<tr>
<td>Increase life span (%)</td>
<td>—</td>
<td>24.44</td>
<td>58.88</td>
<td>87.22</td>
<td>119.49</td>
</tr>
<tr>
<td>Tumor volume (ml)</td>
<td>4.51 ± 0.07</td>
<td>3.53 ± 0.03**</td>
<td>2.62 ± 0.03**</td>
<td>1.34 ± 0.03**</td>
<td>—</td>
</tr>
<tr>
<td>Packed cell volume (ml)</td>
<td>2.11 ± 0.06</td>
<td>1.52 ± 0.05</td>
<td>0.93 ± 0.02**</td>
<td>0.31 ± 0.01**</td>
<td>—</td>
</tr>
<tr>
<td>Viable tumor cell count (&lt;10&lt;sup&gt;6&lt;/sup&gt; cells/ml)</td>
<td>12.32 ± 0.07</td>
<td>9.33 ± 0.06**</td>
<td>5.51 ± 0.04</td>
<td>1.81 ± 0.06**</td>
<td>—</td>
</tr>
<tr>
<td>Nonviable tumor cell count (&lt;10&lt;sup&gt;6&lt;/sup&gt; cells/ml)</td>
<td>0.32 ± 0.03</td>
<td>0.63 ± 0.05**</td>
<td>0.84 ± 0.06**</td>
<td>1.42 ± 0.05**</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are expressed as the mean of results in 6 mice ± S.E.M. **P<0.01, extract-treated groups compared with the EAC control group. Body weight of normal mice: 20.7 ± 0.17 g.

Statistical analyses

The experimental results were expressed as the mean ± S.E.M. Data were assessed by ANOVA followed by Student’s t-test; P value of <0.05 was considered as statistically significant.

Results

The present investigation indicates that the MECB showed significant antitumor and antioxidant activities in EAC-bearing mice. The effects of MECB at the doses of 50, 100, and 200 mg/kg on survival time, % ILS, tumor volume, packed cell volume, and tumor cell count (viable and nonviable cell) are shown in Table 1.

Effect on mean survival time

In the EAC control group, the mean survival time was 18.0 ± 0.19 days, while it increased to 22.4 ± 0.17 (50 mg/kg), 28.6 ± 0.16 (100 mg/kg), and 33.7 ± 0.19 (200 mg/kg) days, respectively, in the MECB-treated groups, whereas the standard drug 5-fluorouracil (20 mg/kg)-treated group had a mean survival time of 39.5 ± 0.25 days.

Effect on tumor growth

Treatment with MECB at the doses of 50, 100, and 200 mg/kg significantly (P<0.01) reduced the tumor volume, packed cell volume, and viable tumor cell count.
in a dose-dependent manner as compared to that of the EAC control group. Furthermore, nonviable tumor cell count at different doses of MECB were significantly \((P<0.01)\) increased in a dose-dependent manner.

**Effect on hematological parameters**

As shown in Table 2, hemoglobin content and RBC count in the EAC control group was significantly \((P<0.001)\) decreased as compared to the normal group. Treatment with MECB at the dose of 50, 100, and 200 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 the normal group \((P<0.001)\). Administration of MECB at the dose of 50, 100, and 200 mg/kg in EAC-bearing mice significantly \((P<0.01)\) reduced the WBC count and protein as compared with the EAC control. In a differential count of WBC, the presence of neutrophils increased, while the lymphocyte count decreased in the EAC control group. Treatment with MECB at different doses changed these altered parameters more or less to the normal values.

**Effect on lipid peroxidation and glutathione**

As shown in Fig. 1, the levels of lipid peroxidation in liver tissue were significantly increased by 48.9\% in the EAC control group as compared to the normal group \((P<0.001)\). After administration of MECB at different doses (50, 100, and 200 mg/kg) to EAC-bearing mice, the level of lipid peroxidation was reduced by 8.6\%, 17.9\%, and 29.3\%, respectively, in comparison to the EAC control group \((P<0.05)\). Inoculation of EAC drastically decreased the GSH content to 30.6\% in the EAC control group when compared with the normal group \((P<0.001)\). The administration of MECB at the dose of 50, 100, and 200 mg/kg to the EAC-bearing mice increased GSH levels by 7.9\%, 17.2\%, and 28.2\%, respectively, as compared with the EAC control group \((P<0.05)\) (Fig. 2).

**Effect on antioxidant enzymes (SOD and CAT)**

As shown in Fig. 3. SOD level in the liver of EAC-bearing mice significantly decreased by 35.6\% in comparison with the normal group \((P<0.001)\). Administration of MECB at the dose of 50, 100, and 200 mg/kg increased levels of SOD by 9.0\%, 19.7\%, and 33.2\%, respectively, as compared to that of the EAC control group \((P<0.05)\). The CAT level in the EAC control

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**Table 2.** Effect of the methanol extract of *Caesalpinia bonducella* leaves (MECB) on hematological parameters of EAC-bearing mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal (saline, 5 ml/kg)</th>
<th>EAC Control (2 × 10^6 cells/ml per mice)</th>
<th>MECB (50 mg/kg) + EAC</th>
<th>MECB (100 mg/kg) + EAC</th>
<th>MECB (200 mg/kg) + EAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g %)</td>
<td>13.4 ± 0.14</td>
<td>10.5 ± 0.15***</td>
<td>10.8 ± 0.17**</td>
<td>11.6 ± 0.19**</td>
<td>12.7 ± 0.18**</td>
</tr>
<tr>
<td>RBC (×10^6/µl)</td>
<td>6.5 ± 0.11</td>
<td>3.7 ± 0.09***</td>
<td>4.2 ± 0.32</td>
<td>4.7 ± 0.38</td>
<td>5.4 ± 0.45**</td>
</tr>
<tr>
<td>WBC (×10^6/µl)</td>
<td>4.7 ± 0.09</td>
<td>17.2 ± 0.13***</td>
<td>13.2 ± 0.08</td>
<td>9.1 ± 0.03**</td>
<td>5.1 ± 0.06</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>1.8 ± 0.05</td>
<td>1.1 ± 0.04***</td>
<td>1.2 ± 0.03</td>
<td>1.4 ± 0.05</td>
<td>1.7 ± 0.06**</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>17.9 ± 1.05</td>
<td>65.1 ± 5.17***</td>
<td>35.5 ± 3.41**</td>
<td>42.5 ± 3.14**</td>
<td>33.6 ± 1.82</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>80.3 ± 2.23</td>
<td>33.8 ± 2.42***</td>
<td>43.3 ± 2.35</td>
<td>56.1 ± 2.33</td>
<td>64.7 ± 4.26**</td>
</tr>
</tbody>
</table>

Data are expressed as the mean of results in 6 mice ±S.E.M. ***\(P<0.001\), EAC control group compared with the normal group. **\(P<0.01\), extract-treated groups compared with the EAC control group.
Antitumor Activity of *C. bonducella*

Group significantly decreased by 59.1% in comparison with the normal group (\(P<0.001\)). Treatment with MECB at the dose of 50, 100, and 200 mg/kg increased CAT levels by 14.8%, 28.7%, and 52.8%, respectively, when compared to that of the EAC control (\(P<0.05\)) (Fig. 4).

**Effect of extract on normal mice**

When the mice were observed for behavioral changes after i.p. administration of a single dose of the extract, none of the mice exhibited abnormal behavioral responses at a doses of 50, 100, and 200 mg/kg. However, the mice that received 300 mg/kg or above showed slight toxic symptoms, which included inactivity, loss of appetite, slow movement, dizziness, erection of hairs, and hypothermia.

![Fig. 2. Effect of the methanol extract of *Caesalpinia bonducella* leaves (MECB) on hepatic glutathione content in EAC-bearing mice. Data are expressed as the mean of results in 6 mice ±S.E.M. ***\(P<0.001\), EAC control group compared with the normal group. *\(P<0.05\), extract-treated group compared with the EAC control group.](image)

![Fig. 3. Effect of the methanol extract of *Caesalpinia bonducella* leaves (MECB) on hepatic catalase activity in EAC-bearing mice. Data are expressed as the mean of results in 6 mice ±S.E.M. ***\(P<0.001\), EAC control group compared with the normal group. *\(P<0.05\), extract-treated group compared with the EAC control group.](image)
Administration of repeated daily doses of 50, 100, 200, and 300 mg/kg for 14 days 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 (Table 3). The hematological profile, urea, and transaminase activities increased significantly at a dose of 300 mg/kg (Table 3).

Discussion

The present study was carried out to evaluate the antitumor effect and antioxidant status of MECB in EAC-bearing mice. The MECB-treated animals at the doses of 50, 100, and 200 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. In short-term toxicity studies, the administration of MECB at the dose of 50, 100, and 200 mg/kg for 14 days did not exhibit any adverse effect.

In EAC-bearing mice, a regular rapid increase in ascites tumor volume was noted. Ascites fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells (33). Treatment with MECB inhibited the tumor volume, tumor cell count, and increased the percentage of tryphan blue positive stained dead cells in tumor-bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the life span of animals (34). The MECB decreased the ascites fluid volume, viable cell count, and increased the percentage of life span. It may be concluded that MECB by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of EAC-bearing mice.

Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia (35, 36). 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 (37). Treatment with MECB brought back the hemoglobin content, RBC, and WBC count more or less to normal levels. This indicates that MECB possess protective action on the hemopoietic system.

Lipid peroxidation, an autocatalytic free radical chain propagating reaction, is known to be associated with pathological conditions of a cell. Malondialdehyde (MDA), the end product of lipid peroxidation, was reported to be higher in cancer tissues than in non-diseased organ (38). Glutathione, a potent inhibitor of the neoplastic process, plays an important role in the endogenous antioxidant system. It is found in particularly high concentration in the liver and is known to have a key function in the protective process. Excessive production of free radicals resulted in oxidative stress, which leads to damage of macromolecules, for example,
lipid peroxidation in vivo (39). It was also reported that the presence of tumors in the human body or in experimental animals is known to affect many functions of the vital organs, especially in the liver, even when the site of the tumor does not interfere directly with organ function (40). MECB significantly reduced the elevated levels of lipid peroxidation and increased the glutathione content in EAC-treated mice. The antitumorigenic effect of MECB may be due to the antioxidant and the free radical quenching property of the phytoconstituents of MECB.

Cells are also equipped with enzymatic antioxidant mechanisms that play an important role in the elimination of free radicals. SOD, CAT, and glutathione peroxides are involved in the clearance of superoxide and hydrogen peroxide (H$_2$O$_2$). SOD catalyses the diminution of superoxide into H$_2$O$_2$, which has to be eliminated by glutathione peroxidase and/or catalase (41). Consistent with this, it has been reported that a decrease in SOD activity in EAC-bearing mice may be due to loss of Mn$^{2+}$-containing SOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver (42). A small amount of catalase in tumor cells was reported (43). The inhibition of SOD and CAT activities as a result of tumor growth were also reported (42). Similar findings were observed in the present investigation with EAC-bearing mice. The administration of MECB at different doses significantly increased the SOD and CAT levels in a dose-dependent manner.

It was reported that plant-derived extracts containing antioxidant principles showed cytotoxicity towards tumor cells (44) and antitumor activity in experimental animals (45). Antitumor activity of these antioxidants is either through induction of apoptosis (46) or by inhibition of neovascularization (47). The implication of free radicals in tumors is well documented (48, 49). In our earlier studies, we found that MECB possess hepatoprotective and antioxidant properties (11). 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, MECB at the high dose level (300 mg/kg) increased the urea content and transaminase activity, indicating that it causes hepatorenal dysfunction and alters metabolism.

The present study demonstrates that MECB increased the life span of EAC-tumor bearing mice and decreased the lipid peroxidation and thereby augmented the endogenous antioxidant enzymes in the liver. The above parameters are responsible for the antitumor and antioxidant activities of *Caesalpinia bonducella*.

Further investigations are in progress in our laboratory to identify the active principles involved in this anticancer and antioxidant activity.

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