Anti-tumor effect of *Euphorbia hirta* on Ehrlich’s ascites carcinoma in mice

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**SUMMARY**

Anti-tumor activity of *Euphorbia hirta* (50 mg/kg and 100 mg/kg) has been evaluated against Ehrlich’s ascites carcinoma (EAC) in Swiss albino mice. Intraperitoneal (i.p) administration of *Euphorbia hirta* was effective in reducing solid tumor mass development induced by EAC cells. It exhibited significant anti-tumor activity in mice, when used at the dose of 100 mg/kg/day i.p., for 14 days. The administration of *Euphorbia hirta* (100 mg/kg/day i.p.) resulted in an increase (*P < 0.001*) of the life span (59.9%) of ascites tumor bearing mice as compared to the control group. After 14 days, on developed tumor masses, *Euphorbia hirta* administration brought about significant reduction in tumor volume and it reverse the changes in the hematological parameters, responding to tumor inoculation. The results are indicative of the anti-tumor activity of *Euphorbia hirta* against EAC induced tumor in a dose dependent manner.

**Key words:** *Euphorbia hirta*; Ehrlich’s ascites carcinoma; Anti-tumor activity; Median survival time; Hematological parameters

**INTRODUCTION**

Cancer is the second leading cause of death after heart disease in the world. It involves fundamental biological process concerning disordered cell replication death, disorganization of organ structure (Polasa, 2000) and ability to invade various tissues to form malignant tumors (Cotran et al., 1994, Workman and Koy, 2001). Plant products have been contributed several novel compounds promising anti-tumor activity. Many naturally occurring tested for anticancer activity on experimental animals in the present availability of some effective anticancer drugs (Refsnes et al., 1974; Siegas et al., 1999; Ramsev et al., 2000; Filleur et al., 2001; Ramnath et al., 2002). Herbs have been used as food and for medicinal purposes for centuries. Research interest has been focussed on various herbs that posses antiplatelet, anti-tumor, or immune- stimulating properties that may be useful adjuncts in helping reduce the risk of cardiovascular disease and cancer. Many herbs contain potent antioxidant compounds that provide significant protection against chronic diseases and may also have antiviral or anti-tumor activity. The volatile essential oils of commonly used culinary herbs, spices, and herbal teas inhibit mevalonate synthesis and thereby suppress cholesterol synthesis and tumor growth.

*Euphorbia hirta* (Family: Euphorbiaceae) is an
erect or climbing ascending annual herb with hairy stems and opposite or long lanceolate leaves found throughout India (Ambasta, 1986). This plant is very well known for its various treatments in the ancient traditional system of medicine, it is administered in the form of liquid extract or tincture with lobelia or senega in the treatment of cough and asthma. It is also applied topically to ulcers, edemas, phlegmons, abscesses and inflamed glands (Kirtikar and Basu, 1975). Anti-diarrheal effect (Galvez et al., 1993), anti-amoebic effect (Nadir et al., 1981) ACE inhibitory effect (Williams et al., 1997), anti-bacterial effect (Ajao et al 1985), diuretic effect (Johnson et al., 1999), anti-inflammatory effect (Martinez - Vazquez et al., 1999) of E. hirta have been reported earlier. The present study was undertaken to evaluate the anti-tumor effect of the ethanol extract of Euphorbia hirta against Ehrlich’s ascites carcinoma in mice.

MATERIALS AND METHODS

Plant materials
Leaves of Euphorbia hirta, which was reported to be used against cancer by folk doctors in TamilNadu and leaves, were collected in the month of June 2002. The twigs of the plant along with flowers were submitted and Chief botanist at Department of Botany, St Joseph’s college, Trichirappalli, authenticated it. A voucher specimen (Voucher No: PPRL/HS/1/2005) was preserved in our laboratory herbarium, Department of Pharmaceutical Technology, Jadavpur University, Kolkata.

Preparation of plant extracts
The leaves were shade dried and powdered, passed in sieve 22. The powered material was extracted using 95% ethanol in Soxhlet apparatus and concentrated to dryness under reduced pressure by means of rotary-vacuum evaporator (Buchi, Mumbai, India). The dried ethanol extract of Euphorbia hirta (EEEH) was stored in a desiccator for the pharmacological and phytochemical screening.

Animals
Adult Swiss male albino mice (5 - 6 weeks) weighing, 20 - 24 g procured from Venkateswara Enterprises, Bangalore were housed in microlon boxes with controlled temperature (22 ± 2°C) on a 12:12 h light/dark cycle, animals had free access to standard laboratory diet and water ad libitum.

Reagents and chemicals
5-fluorouracil (5-FU) was purchased from Biochem Pharmaceuticals Ltd (Biochem, Mumbai). Trypan blue was purchased from Sigma Chemicals (Sigma, U.S.A.). All other chemicals used were of analytical grade.

Cell lines
Ehrlich’s ascites carcinoma (EAC) cells were obtained from the courtesy of Amala Cancer Research Centre, Thrissur. EAC cells maintained by weekly intraperitoneal (i.p.) inoculation of $10^6$ cells/mouse (Clarkson et al., 1965; Gothoskar et al., 1971).

Treatment schedule
Effect of EEEH on tumor growth and host survival were determined by evaluation tumor volume, tumor cell count and percentage increase in lifespan (% ILS) of the tumor hosts, respectively. For calculating the survival time four groups of mice were used and given food and water ad libitum. Tumor was induced to all the groups by injecting 0.2 ml of $2.3 \times 10^5$/ml of EAC cells in to the peritoneal cavity of mice, except normal group. This was taken as day ‘0’. On the first day the normal saline (0.9% w/v, NaCl 5 ml/kg/mouse/day) administered in to normal group. Two different doses of EEEH (50 mg/kg and 100 mg/kg i.p.) and standard drug 5-fluorouracil (5-FU, 20 mg/kg i.p.) were subsequently administered for 9 days. On the 9th day, after the last dose and 18 h fasting six from each group were sacrificed for the anti-tumor activity and the rest of the animals were kept for check the mean survival time (MST) and increase in the lifespan of the tumor bearing mice.
Tumor growth response
Antitumor growth effect of EEEH was assessed by observation of changes with respect to ascites tumor volume, packed cell volume, viable and nonviable tumor cell count; MST and percentage increase in the lifespan (% ILS). Transplantable murine tumor was carefully collected with the help of sterile 3 ml syringe and measured the tumor volume and the ascetic fluid with was withdraw in a graduated centrifuge tube and packed cell volume was determined by centrifuging at 1,000 × g for 5 min. viable and nonviable cell count of ascitic cell were stained by the tryban blue dye (0.4% in normal saline) exclusion test and count was determined in a neubauer counting chamber. The effect of EEEH on tumor growth was monitored daily by recoding the mortality and percentage increase in life span (5 ILS) was calculated using the following formula ILS (%) = (Mean survival of treated group/ Mean survival of control group) - 1 × 100 (Raikapoor et al., 2003).

Hematological studies
On the 14 day after tumor inoculation blood was obtained through tail vein from normal mice, tumor bearing mice and tumor bearing mice treated with EEEH (100 mg/kg/mouse/day i.p. for the 9 days. For the total count blood was drawn into RBC or WBC pipettes, diluted and counted in a neubauer counting chambers. Sahli’s Hemoglobinimeter was used for the determination of hemoglobin concentration. Differential count of leukocytes was done on a freshly drawn blood film using Leishman’s stain. Hemoglobin content (D’ Amour et al., 1965), RBC and WBC count (Wintrobe et al., 1961) and differential leukocyte count (Dacie and Lewis, 1958) was estimated from the peripheral blood of normal, EAC control and treated groups.

Measurement of normal peritoneal cells
Three groups of normal mice (n = 6) were used for this study. One group was treated with 100 mg/kg, i.p. of EEEH only once for a single day, while the second group received the same treatment for two consecutive days. The untreated third group was used as control and treated with equivalent volume of normal saline. After 24 h intraperitoneal fluid was collected by repeated intraperitoneal wash with cold normal saline and intraperitoneal cells were counted using hemocytometer in each of the treated groups and compared with untreated control group (Hudson and Hay, 1989; Sur and Ganguly, 1994).

Statistical analysis
All the values are expressed in mean ± SEM. The data were statistically analyzed using one way analysis of ANOVA followed by Dunnett’s multiple comparison test using Graphat Instat Software (San Diego, U.S.A.) and student’s t-test (Armitage et al., 1971). Difference in means were considered to be significant when P < 0.01.

RESULTS

Effect of EEEH on survival time of tumor bearing mice
The effect EEEH on the survival of tumor bearing mice is shown in Table 1 and Fig. 1. Median survival time for the control group was 19.7 ± 1.0 days, while it was 27.0 ± 0.9, 31.5 ± 1.1, and 36.1 ± 0.8, days for the groups treated with Euphorbia hirta 50 mg/kg, 100 mg/kg and 5 FU 20 mg/kg, respectively. Significant (P < 0.001) increases in the lifespan of

<table>
<thead>
<tr>
<th>Table 1. Effect of EEEH treatment on the survival of EAC bearing mice</th>
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<tbody>
<tr>
<td>Experiment</td>
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<tr>
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<tr>
<td>EAC Control</td>
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<tr>
<td>EEEH (50 mg/kg)</td>
</tr>
<tr>
<td>EEEH (100 mg/kg)</td>
</tr>
<tr>
<td>5 FU (20 mg/kg)</td>
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</tbody>
</table>

Values are expressed as mean ± SEM (n = 10). Days of drug treatment = 9; ∗P < 0.001, Significantly differ from control group.
tumor bearing mice treated with EEEH 50, 100 mg/kg
and 5 FU-20 mg/kg was found to be 41.6%, 59.6%
and 83.2%, respectively, as compared to the control
group.

**Effect of EEEH on normal peritoneal cells**
The average number of peritoneal exudates cells
per normal mouse was found to be 5.98 ± 0.1. EEEH
(100 mg/kg/day i.p.) treatment increased the number
of peritoneal cells as shown in Table 2. Single
treatment with EEEH (100 mg/kg) enhance the
number to 8.2 ± 0.1, while two consecutive treatments
enhanced the number to 8.9 ± 0.1 (P < 0.001).

**Effect of EEEH on tumor growth**
Intraperitoneal administration of EEEH (100 mg/kg)
to tumor bearing mice was found to be effective in
controlling the tumor volume development was
shown in Table 3. The untreated control mice
showed a tumor volume of 4.0 ± 0.09 ml. A tumor
volume of 1.8 ± 0.14 ml, 1.6 ± 0.1 ml and 1.4 ± 1.12 ml
(induced by EAC cells) were recorded for the
animals treated with the dose of EEEH 50 mg/kg,
100 mg/kg, and 5 FU 20 mg/kg, respectively. Tumor
volumes in mice for the treated groups were lesser
as compared to controls and statistically it was
significant (P < 0.001).

The viable tumor cell counts were shown in
Table 3. The viable tumor cell counts for the control
group 8.4 ± 0.13, while it was 5.77 ± 0.13, 3.51 ± 0.22
and 2.5 ± 0.28 for the groups treated with EEEH
50 mg/kg, 100 mg/kg and 5 FU 20 mg/kg, respectively.
The reduction of viable tumor cell count was found
to be effective (P < 0.001) as compared to the control
group.

**Effect of EEEH on hematological parameters**
Hematological parameters of tumor bearing mice
on 14th day were found to be significantly altered
from group was shown in Table 4. The total WBC
count, protein and PCV were found to be increased
along with the hemoglobin content of RBC.

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**Table 2. Effect of EEEH (100 mg/kg/day i.p.) on peritoneal cells**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Number of peritoneal cells (x10⁶) per mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.98 ± 0.1</td>
</tr>
<tr>
<td>Once treated</td>
<td>8.2 ± 0.1</td>
</tr>
<tr>
<td>Treated two consecutive days</td>
<td>8.9 ± 0.1</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (Number of
animals used = 6 in each groups).

P < 0.001 as compared to control group

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**Table 3. Effect of EEEH on tumour growth and tumor cell count induced by EAC**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumor volume (ml)</th>
<th>Viable tumor cell count (x 10⁷ cells/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAC control</td>
<td>4.0 ± 0.09</td>
<td>8.4 ± 0.13</td>
</tr>
<tr>
<td>EEEH (50 mg/kg/day i.p)</td>
<td>1.8 ± 0.14</td>
<td>5.7 ± 0.13</td>
</tr>
<tr>
<td>EEEH (100 mg/kg/day i.p)</td>
<td>1.6 ± 0.1</td>
<td>3.5 ± 0.22</td>
</tr>
<tr>
<td>5FU (20 mg/kg/day i.p)</td>
<td>1.4 ± 1.12</td>
<td>2.5 ± 0.28</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM. Days of treatment = 7; P < 0.001 as compared to control group.
Table 4. Effect of EEEH (100 mg/kg/day, i.p.) on hematological parameters

<table>
<thead>
<tr>
<th>Hematological Parameters</th>
<th>Normal</th>
<th>Tumor bearing mice (14 days)</th>
<th>EEEH Treated Tumor bearing mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB (%)</td>
<td>14.6 ± 0.3</td>
<td>8.9 ± 0.1</td>
<td>12.8 ± 0.3</td>
</tr>
<tr>
<td>RBC Cells/ml × 10¹⁰</td>
<td>3.9 ± 0.3</td>
<td>7.7 ± 0.2</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>WBC Cells/ml × 10¹⁰</td>
<td>7.5 ± 0.2</td>
<td>16.7 ± 0.2</td>
<td>10.8 ± 0.2</td>
</tr>
<tr>
<td>Protein (g%)</td>
<td>8.4 ± 0.3</td>
<td>13.5 ± 0.3</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>PCV (mm)</td>
<td>16.9 ± 0.3</td>
<td>25.8 ± 0.3</td>
<td>18.9 ± 0.2</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>69.7 ± 0.3</td>
<td>25.8 ± 0.2</td>
<td>56.2 ± 0.2</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>30.1 ± 0.3</td>
<td>72.2 ± 0.2</td>
<td>42.7 ± 0.3</td>
</tr>
<tr>
<td>Monocytes</td>
<td>2.33 ± 0.42</td>
<td>1.33 ± 0.33</td>
<td>1.17 ± 0.31b</td>
</tr>
</tbody>
</table>

Days of drug treatment = 14, n = 6 animals in each group. *P < 0.001 as compared to normal mice; **P < 0.01, ***P < 0.001 as compared to tumor bearing mice. *Non significant as compared to normal group; bNon significant as compared to control group.

Differential counts were significantly reduced by EEEH treatment and monocytes counts were not significant when compared with the control group (tumor treated groups). At the same time interval, EEEH (100 mg/kg/day i.p) treatment could change altered hematological parameters to near normal.

**DISCUSSION**

The present study was carried out to evaluate the anti-tumor effect of *Euphorbia hirta* on Ehrlich’s ascites carcinoma bearing mice in *in vivo*. Various reports on the mechanism behind the anti-tumor activity of various plant extracts indicate that different plant extracts exhibited their anti-tumor activities through different mechanisms in the host (Sakagami et al., 1987; Silchenmyer et al., 1991; Tanaka et al., 1996; Chaudhuri et al., 1998; Rosangkima et al., 2002; Das et al., 2004). In the anti-tumor effect of tea plant (*Camellia sinensis* var assamica, Theaceae) root extract, the activity of superoxide dismutase, a free radical scavenger, was found to be increased in the serum of tumor bearing mice, suggesting the involvement of the tea root extract in the enhancement of the defense mechanism (Chaudhuri et al., 1998).

Ascites fluid is the direct nutritional source to tumor cells and the faster increase in ascites fluid with tumor growth would be means to meet the nutritional requirement of tumor cell (Prasad et al., 1994). The reliable criteria for judging the value of any anticancer drug are the prolongation of life span of animals and disappearance of leukemia cells from blood (Oberling et al., 1994; Mazumdar et al., 1997). The results of the present study demonstrate that EEEH treatment suppresses the EAC cells growth, by reducing the tumor volume and viable tumor cell count (Table 2). EEEH- treated animals at the dose of 200 mg/kg significantly inhibited the tumor volume, packed cell volume, tumor cell count and brought the hematological parameters near to normal values.

The cytotoxicity of oxygen free radicals can cause several diseases (Emerit et al., 1994). Body cells and tissues are continuously threatened by free radicals and reactive oxygen species, which are produced during normal oxygen metabolism or are induced by exogenous damage (De Groot, 1994; Grace, 1994). Reactive oxygen species (ROS) can damage DNA and division of cells with unpaired or mispaired damage leads to mutation. If these changes appear in critical genes, initiation or progression may result. ROS can interfere directly with cell signaling and growth. The cellular damage caused by ROS can induce mitosis, increasing risk that damaged DNA will lead to mutations, and can increase the exposure of DNA to mutagens. Free radicals can attract various inflammatory mediators; contribute to a great inflammatory response and tissue damage.

All these data point to the possibility of developing EEEH as a potential agent in the area of cancer.
chemotherapy. Preliminary phytochemical screening indicated that presence of alkaloids and flavonoids in *Euphorbia hirta* (Baslas et al., 1980; Galvez et al., 1993). Flavonoids have been shown to possess free radical scavenging antimutagenic and anti-malignant effects (Brown et al., 1980; Husain et al., 1987; Rebak et al., 1988; Hirano et al., 1989). More over flavonoids have chemopreventive role in cancer through effects on signal transduction in cell proliferation and angiogenesis (Weber et al., 1996; Fotis et al., 1997). Flavonoids can also prevent injury caused by free radicals in various ways. One way is the direct scavenging of free radicals. Flavonoids are oxidized by radicals, resulting in a more stable, less reactive radical. In other words, flavonoids stabilize the reactive oxygen species by reacting with the reactive compound of the radical. Because of the high reactivity of the hydroxyl group of the flavonoids, radicals are made inactive, according to the following equation: flavonoid (OH) + R > flavonoid (O) + RH, where R is a free radical and O' is an oxygen free radical (Korkina et al., 1997) It has been stated that flavonoids, as antioxidants, can inhibit carcinogenesis (Stefani et al., 1999). Specific flavonoids are known to chelate iron thereby removing a casual factor for the development of free radicals.

The cytotoxicity and anti-tumor properties of the extract of *Euphorbia hirta* may be due to its quercetin nature (Khandiya et al., 2002). Quercetin in particular is known for its iron chelating and iron stabilizing properties. Direct inhibition of lipid peroxidation is another protective measure. Quercetin and apigenin also inhibited melanoma growth and influenced the invasive and metastatic potential in mice (Caltagirone et al., 2000). Quercetin, in particular, inhibits both cyclo-oxygenase and lipo-oxygenase activities, thus diminishing the formation of these inflammatory metabolites (Robak et al., 1996; Kim et al., 1998).

The exact mode of action of EEEH is not known. As far as present findings are concerned, the exact mechanism of action the plant extracts against Ehrlich’s ascites carcinoma may not be elucidated in detail at present. However, the increase of lifespan and reduced tumor volume, viable tumor cell count by EEEH treatments seems to be playing a significant role in mediating cytotoxic and anti-tumor activity against Ehrlich’s ascites carcinoma.

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