Anticancer screening of *Gomphrena globosa* against ehrlich ascites carcinoma in swiss albino mice

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**ABSTRACT**

Based on the ethno pharmacological evidence, the present study was performed to investigate the anticancer activity of chloroform extract of aerial parts of *Gomphrena globosa* against Ehrlich Ascites Carcinoma (EAC) induced solid tumor. Acute toxicity study shows that, the extract was non-toxic up to 2000 mg / kg body wt. Administration of 200 or 400 mg / kg p.o. in mice, caused significant reduction in body weight, packed cell volume and viable cell count compared to the EAC control group, and restored the hematological and biochemical parameters towards normal value. Histological study indicated that the damaged tissue was recovered by extract and solid tumor volume was reduced significantly. Thus, the findings reveal a significant anti-cancer activity of the extract.

**Key words:** *Gomphrena globosa*, Anticancer, Hematological, Biochemical, Histological, Tumor.

**INTRODUCTION**

Cancer is a second leading cause of death in developing countries, with uncontrolled division of cells in the part of the body [1]. The cancer burden in developing countries is raising as a result of increasing population ageing and growth, and adaptation of cancer-associated lifestyle choices including smoking, physical inactivity and ‘Westernized diet’ [2]. Recently it has been reported that there were more than five lakh people died of cancer in India in the year 2010, and in female most deaths were due to breast cancer. The standard methods used currently to cure or control the cancer, exhibit severe toxicity on normal tissues. Therefore, worldwide research is going on to investigate the best effective antitumor agents from different sources. Among the different sources, herbal source remains important in identifying lead molecule in the plants with proven anticancer property that ultimately occupies the platform for clinical use. More than 60% of the commercially available anticancer drugs are related to natural origin. The numbers of ethno medicinal plants with anticancer properties reported in India are around 62, with the commonest phytochemical constituents like alkaloids, glycosides, terpenoids, stearic, oleic, palmitic acid, flavonoid, β-sitosterol, amino acids, saponins etc.

Different species of the same genus have been reported to possess similar pharmacological activity [3, 4]. Literature search on this line, indicates that *G. martiana* [5] and *G.macrocephala* [6] have been reported for anti-tumor activity; however, the anticancer property of *Gomphrena globosa*, yet another plant belonging to the same genus has not been investigated though the plant has been reported for antihypertensive property and containing saponins,
alkaloids, reducing sugars and coumarins [7]. Therefore, the present study was undertaken to investigate the anticancer property of *Gomphrena globosa* using chloroform extract of aerial parts of the plant.

**EXPERIMENTAL SECTION**

**Plant material**

The aerial parts of *Gomphrena globosa* (Amaranthaceae) were collected freshly in the month of June, in and around Tiruchengode, Namakkal district, Tamilnadu, India. The plant was authenticated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Coimbatore (Specimen Number: 1368). The aerial parts of *Gomphrena globosa* were dried under shade, coarse powdered, and extracted (500 gm) with chloroform by continuous hot extraction method for 20 h by using soxhlet apparatus [8]. The extract was concentrated to a dry mass by vacuum distillation and controlled temperature (40°C-50°C). The crude chloroform extract was 36 g by wt. (Yield-7.2%). The extract was preserved in a refrigerator at 4°C until further use.

**Chemicals**

5-Flurouracil (5-FU) was obtained from Ranbaxy, Ltd., Gurgaon, India. Tryphan blue was obtained from Hi-Media Laboratories, Ltd., Mumbai, India. All other chemicals used were of analytical grade.

**Tumor cells**

Ehrlich Ascites Carcinoma (EAC) cells were supplied by Amala Cancer Research Centre, Trissur, Kerela, India. The cells were maintained in vivo in Swiss Albino Mice by intraperitoneal transplantation. EAC cells aspirated from the peritoneal cavity of mice were washed with phosphate buffer solution (pH 7.4) and used for inducing ascitic tumor in mice under study.

**Dose suspension and solution**

For the short term cytotoxicity assay against the EAC cell lines, chloroform extract of *Gomphrena globosa* (CEGG) was dissolved in phosphate buffer solution (pH 7.4) and the volume made up to 10 ml to obtain a 1000 µg/ml stock solution, which was stored at -20°C until further use. The CEGG and standard 5-FU were suspended using sodium carboxy methyl cellulose (CMC, 0.2%) and administered orally to the animals to evaluate the potency of anticancer activity.

**Animals**

Adult female albino mice of Swiss strain were used for the studies. The animal ethical clearance for animal experiments was obtained from the Institution Animal Ethical Committee, Swamy Vivekanandha College of Pharmacy (889/ac/05/CPCSEA dated 29th April 2005). Female mice which were nulliparous and non-pregnant and weighing 25 to 30 g were used for the study. Each animal, at the commencement of its dosing, was 8 weeks old and weight variation was within ± 20% of the mean weight of any previously dosed animals. The temperature in the experimental animal room was 22°C (± 3°C) and the relative humidity was between 50-60%. These animals were fed with pellet diet supplied by Amrut Laboratory, Animal Feed Company, Sangli, Maharashtra, India and drinking water *ad libitum*. They were kept in 12h/12h light/dark cycle and maintained for at least 5 d prior to dosing for acclimatization to the laboratory conditions.

**Preliminary short term in-vitro cytotoxic study**

Preliminary investigation of *in-vitro* cytotoxic study was performed on chloroform, ethanol and aqueous extract of *Gomphrena globosa* using EAC cell line. The viability of the cells was determined using Tryphan blue dye exclusion method [9]. EAC cell line was aspirated from the peritoneal cavity and washed three times with phosphate buffer solution (pH 7.4). One million cells were incubated with various concentrations (61.25-1000 µg/ml) of the extract in a total volume of 1 ml for 3 h at 37°C. After incubation the percentage of dead cells was calculated from which the IC₅₀ was determined.

**Acute oral toxicity study**

The acute toxicity study was carried out as per the guidelines of OECD-423 [10]. Three albino mice were fasted over night and the test sample CEGG was given orally at a starting dose of 5 mg/kg. p.o. Animals were observed for a period of 2 h, then occasionally for 4 h for severity of any toxic signs and mortality. Since no mortality was observed, same dose was repeated with another group of animals. The procedure was repeated for doses of 50, 300 and 2000 mg/kg, p.o. in separate group of animals. The maximum dose of 2000 mg/kg did not produce any mortality and toxic symptoms. So, for further studies 1/10⁶ and 1/5⁶ of the maximum dose (2000 mg/kg. p.o) values were taken as treatment dose [11, 12]. Behavior as well as other toxic symptoms if any was observed for 24, 48 and 72 h [13]. The animals were kept under observation up to 14 d after drug administration to find out delayed mortality if any [14].
In-vivo anticancer activity

Adult female Swiss albino mice were divided into 5 groups each group consisting of 12 animals for studying two parameters (mean survival time, n=6 and hematological parameter, n=6). All the animals were inoculated with 1×10^6 EAC cells / intraperitoneally / mouse except for the normal control groups. This was considered as day '0' and group I and group II were normal control and tumor control respectively. These two groups were treated with the equal volume of 0.9% sodium chloride solution p.o. Group III, which served as the positive control, was treated with the suspension of 5-FU at 20 mg/kg p.o. Groups IV and V were treated with the CEGG at 200 and 400 mg/kg, p.o, respectively. All these treatments were given 24 h after the tumor inoculation, once daily for 14 days. After the last dose and 24 h fasting, 6 mice from each group were selected randomly. The blood was collected from these animals by retro-orbital puncture under mild anesthesia condition; and the hematological parameters such as red blood cells (RBC), white blood cells (WBC) and hemoglobin content (Hb) were studied using cell diluting fluids and hemocytometer. Differential cell count (DC) was carried out from Leishman stained blood smears [15]. The ascitic fluid was collected from the peritoneal cavity of the animals once on 10th and 20th d from each animal and observed for appearance, color and cell count. The rest of the animals were noted for the Mean Survival Time (MST) and changes in the body weight were recorded up to 45 days. The anticancer efficacy of CEGG was compared with that of 5-fluorouracil [16]. The MST of the treated group was compared with that of the control group using the following formula.

\[ \text{MST} = \frac{(T-C)}{C} \times 100 \]

T = Treated group; C =Control group

Solid tumor volume

Mice were divided into three groups, each group consisting of six animals, and tumor cells 1×10^6 cells per mouse were injected into the right hind limb (thigh) of all the animals intramuscularly. Group I was tumor control, group II and III received CEGG 200 and 400 mg/kg, p.o respectively and given for 10 d continuously. Tumor mass was measured from 7th d of tumor inoculation. The measurement was carried out every 6th d for a period of 48 d [17].

From the mass of solid tumor obtained, the solid tumor volume was calculated using the following formula, \( V = \frac{4}{3}\pi r^2 \), where 'r' is the mean of the \( r_1 \) and \( r_2 \).

Statistical analysis

All values were expressed as mean ± SEM. The data were statistically analyzed by one way ANOVA followed by Turkey Kramer multiple comparison test. P value <0.05 was considered significant.

RESULTS

Preliminary short term in-vitro cytotoxic study

The results obtained from the study are given in Fig-1. It was observed that CEGG produced potent cytotoxic activity with the inhibitory concentration of (IC50) 56.87 µg/ml for EAC cell line as compared to 201.94 µg/ml and 858.96 µg/ml for ethanol and aqueous extracts respectively and therefore, chloroform extract was used for further studies.

Acute oral toxicity study

There was no significant change in the behavioral response of the animal at the different doses of CEGG tested (2000, 300, 50 and 5 mg/kg, p.o). No mortality of the animals was observed even in the maximum dose of 2000 mg/kg.p.o.

In-vivo anticancer activity

The mean survival time and percentage increase in life span of CEGG in both doses (200 and 400 mg/kg p.o) on ECA were increased significantly to 36.50±1.06 (78%), (p<0.001) and 37.83±0.67 (84.54%), (p<0.001) respectively as compared to tumor control (20.50±0.99) and the effects were almost near to that of the standard (5-Fluoro Uracil, 40.67±0.88), (90.4%), however, no significant difference in the activity was observed between 200 and 400 mg/Kg p.o (p>0.05). The results of the packed cell volume and body weight of the treated doses show the potency of the anti-cancer property is dose dependent (p<0.001). The experimental data of MST, packed cell volume and reduction of body weight are given in Fig - 2 a, b and c respectively. The CEGG in both doses (200 and 400 mg/kg p.o) reversed the changes in the hematological parameters induced by tumor to normal values in EAC significantly. The analytical data of the haematological parameters are given in Table-1.
Solid tumor
The results of solid tumor volume are given in Fig-2 d. The solid tumor mass of EAC of the CEGG in both doses (200 and 400 mg/kg p.o) was reduced significantly. The results of the solid tumor volume of the CEGG in both doses (200 and 400 mg/kg p.o) for EAC were found to be 5.01 ± 0.25 (p<0.001) and 3.22 ± 0.21 (p<0.001) respectively as compared to tumor control (12.51 ± 0.62) and the effect was found to be dose dependent (p<0.05).

Fig 1 IC₅₀ values of different extracts of *Gomphrena globosa* by Tryphan blue method

Fig 2a Mean Survival Time of CEGG on EAC bearing mice; n = 6 animals in each group; Values are expressed as Mean ± SEM.
Fig 2b Packed Cell Volume of CEGG on EAC bearing mice; n = 6 animals in each group; Values are expressed as Mean ± SEM.

Fig 2c Reduction of body weight by CEGG on EAC bearing mice; n = 6 animals in each group; Values are expressed as Mean ± SEM.
**Fig 2d Reduction of Solid Tumor Volume by CEGG in EAC bearing mice; n = 6 animals in each group; Values are expressed as Mean ± SEM.**

**Table 1 Haematological parameters of CEGG treated mice bearing EAC cell line**

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>Normal control</th>
<th>Tumor control</th>
<th>EAC + 5-FU</th>
<th>EAC + CEGG 200</th>
<th>EAC + CEGG 400</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total WBC (x 10³/mm³)</strong></td>
<td>5.73 ±0.11</td>
<td>10.18 ±0.17***</td>
<td>5.22 ±0.10***</td>
<td>4.93 ±0.09***</td>
<td>5.08 ±0.15***</td>
</tr>
<tr>
<td><strong>RBC (x 10⁶/mm³)</strong></td>
<td>9.66 ±0.21</td>
<td>5.02 ±0.11***</td>
<td>7.54 ±0.22***</td>
<td>6.21 ±0.19***</td>
<td>7.04 ±0.23***</td>
</tr>
<tr>
<td><strong>Hb (g/dl)</strong></td>
<td>11.04 ±0.18</td>
<td>4.43 ±0.11***</td>
<td>10.63 ±0.09***</td>
<td>9.62 ±0.07***</td>
<td>10.28 ±0.16***</td>
</tr>
<tr>
<td><strong>Lymphocyte</strong></td>
<td>1.76 ±0.14</td>
<td>71.77 ±0.84***</td>
<td>47.50 ±0.57***</td>
<td>60.23 ±0.79***</td>
<td>58.88 ±0.69***</td>
</tr>
<tr>
<td><strong>Neutrophils</strong></td>
<td>26.12 ±0.63</td>
<td>26.12 ±0.41***</td>
<td>51.07 ±0.41***</td>
<td>22.20 ±0.69***</td>
<td>39.55 ±0.62***</td>
</tr>
<tr>
<td><strong>Eosinophils</strong></td>
<td>1.56 ±0.11***</td>
<td>2.15 ±0.14</td>
<td>1.56 ±0.11***</td>
<td>1.99 ±0.16***</td>
<td>1.63 ±0.15***</td>
</tr>
</tbody>
</table>

*** p<0.001 compared to normal control; ** p<0.01 compared to tumor control; n = 6 animals in each group; Values are expressed as Mean ± SEM.

**DISCUSSION**

Cancer is one of the leading causes of mortality worldwide and the failure of conventional chemotherapy to effect major reduction in the mortality indicates that new approaches are critically needed. Recently a greater emphasis has been given towards the researches on complementary and alternative medicine that deals with cancer management. Several studies have been previously conducted on herbs under the multitude of ethno botanical ground. And several compounds isolated from plants, like terpenoids, glycosides, alkaloids and flavanoids, are reported for anticancer property. Based on this ground much number of plants with anti-tumor properties have been reported [18].

From the literature, we found that *Gomphrena martiana* and *Gomphrena macrocephala* belonging to the genus *Gomphrena* and their phytoconstituents have been earlier reported for invitro and in vivo anticancer property [5, 6]. The petroleum ether extract of *Gomphrena martiana* was found to possess a lipophilic flavanoid fraction with cytotoxic activity [5]. The in vitro cytotoxicity of the ethanol extract of the leaves of *Gomphrena globosa* belonging to the same genus was investigated; however the activity was found to be low [7]. These variable anticancer properties of Gomphrena species support the effect of solvent system on the activity of the plant. Considering the above factors the aerial parts of the *Gomphrena globosa* was extracted out with chloroform, ethanol and water and the extracts were subjected to preliminary in vitro cytotoxic activity. Study was carried out with different solvents using chloroform, ethanol and water. The IC₅₀ values obtained from the study indicated that, the chloroform extract
showed potent anticancer activity, indicating the cytotoxic responsible compounds are lipophilic in nature and the chloroform extract was used for further studies.

The acute toxicity study performed for the CEGG indicated that the extract was safe and did not produce significant changes in its behavior or mortality. The in vivo anticancer study was carried out with EAC cell line, because this can grow in almost all strains of mice rapidly with aggressive behavior [19], which induces per se a local inflammatory reaction on inoculation, which increases the vascular permeability with an intense edema formation, cellular migration and a progressive ascetic fluid formation [20]. The ascetic fluid constitutes a direct nutritional source for tumor cells, which is responsible for tumor growth with the significant increase in body weight in the EAC tumor control bearing mice [21]. The significant reduction in the values of body weight, packed cell volume and viable cell count indicate that the extract may act on either directly on tumor cells or indirect local effect in a dose dependent manner.

Tumor growth normally affects various hematological parameters like decreasing hemoglobin content, RBC and increasing WBC resulting in the myelosuppression and suppression of humoral immunity. So the anti-cancer activity is generally assessed by restoration of the changes in these parameters to normal. The acceptance criteria for determining the antitumor activity of a compound is the determination of circulating WBC [22] and the life span prolongation [23]. The CEGG significantly decreased WBC Count and increased RBC count, lymphocyte and neutrophils count as compared to tumor control. The changes in hemoglobin, neutrophil, monocyte and lymphocyte brought about by the induced cancer reversed values close to normal.

The MST was extended nearly two fold by the extract treatment as compared to tumor control, and the MST of extract treatment was almost comparable to that of 5-fluorouracil, which reveals that the plant has potential anti-cancer property. The changes in RBC, WBC and eosinophils count brought about by the CEGG was found to be dose independent; however CEGG reversed Hb, lymphocyte and neutrophilsin a dose dependent manner. Thus it can be conclusively stated that the plant *Gomphrena globosa* is a potential source for anti-cancer property. Further study on the phytoconstituents of the plant may help identify the lead molecule with potent anti-cancer property.

REFERENCES