



Ethnopharmacological communication

Evaluation of anticancer activity of *Cleome gynandra* on Ehrlich's Ascites Carcinoma treated mice

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ABSTRACT

Ethnopharmacological relevance: The plant *Cleome gynandra* L. (Capparidaceae), is commonly known as 'Hurhur' and 'Karaila' in India and 'Cat's whiskers' in English. Traditionally the whole plant is used in the treatment of tumor, anti-inflammatory and lysosomal stability actions.

Aim of study: The objective of present study is to explore the anticancer activity of the methanol extract of the *Cleome gynandra* in Swiss albino mice against Ehrlich Ascites Carcinoma (EAC) cell line.

Materials and methods: Anticancer activity of methanol extract of *Cleome gynandra* (MECG) was evaluated in Swiss albino mice against Ehrlich Ascites Carcinoma (EAC) cell line at the doses of 200 and 400 mg/kg body weight intraperitoneally. MECG was administered for nine consecutive days. Twenty-four hours of last dose and 18 h of fasting, the mice were sacrificed and antitumor effect of MECG assessed by evaluating tumor volume, viable and nonviable tumor cell count, tumor weight and hematological parameters of EAC bearing host.

Results: MECG showed significant decrease in ($p < 0.01$) tumor volume, viable cell count, tumor weight and elevated the life span of EAC tumor bearing mice. Hematological profile such as RBC, hemoglobin, WBC and lymphocyte count reverted to normal level in MECG treated mice.

Conclusion: From the result it was showed that the extract has potent dose dependent anticancer activity and that is comparable to that of 5-fluorouracil.

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Plant: The whole plant of *Cleome gynandra* L. (Capparidaceae) was collected from Jalpaiguri, West Bengal, India, in January 2009 and identified by the Botanical Survey of India, Botanic garden, Howrah, West Bengal, India. A voucher specimen (No.-CNH/I-I/(293)/2009/Tech.II/335) has been preserved at our laboratory for future reference. Air-dried whole plant (215 g) material except roots were powdered in a mechanical grinder and the plant material were successively extracted by chloroform and methanol by using Soxhlet extraction apparatus. Then solvent was completely removed under reduced pressure and stored in a vacuum dessicator. The methanol extract of *Cleome gynandra* (MECG) was used during the whole study. The yields of the chloroform and methanol fraction were about 7.25% and 9.30%, respectively. Preliminary phytochemical study of the methanol extract indicated the presence of flavonoid, alkaloids and tannins.

Uses in traditional medicine and reported activities: The plant *Cleome gynandra* L. (Capparidaceae), is commonly known as

'Hurhur' and 'Karaila' in India and 'Cat's whiskers' in English (Borgio et al., 2008). It is an herb indigenous to the tropical and subtropical regions. The leaves and seeds of cat's whiskers are used in indigenous medicine in many countries. Cat's whiskers grow as a weed in most tropical countries. It has been used for several years in Indian traditional medicine as an anthelmintic and antimicrobial agent (Ajaiyeoba, 2000). Leaves are applied externally over the wounds to prevent the sepsis. The decoction of the root is used to treat fevers. Leaves with a high percentage of vitamin C is taken as a pot herb in soups, fresh or dried. The leaves are used as disinfectants. Inhalation of the leaves also relieves headaches; leaf juice and oil, for earache and eye wash. Seeds have been reported to have anti-helmintic properties and oil is used as fish poison. Stems are used as analgesic and anti-inflammatory agent (Gupta and Chakravarty, 1957).

The whole plant is also used in the treatment of malaria, piles, rheumatism and in tumor (Mule et al., 2008). The methanol extract of *Cleome gynandra* possess very good antioxidant property (Muchuweti et al., 2007). The plant *Cleome gynandra* also possess anti-inflammatory and lysosomal stability actions in adjuvant induced arthritic rats (Narendhirakannan et al., 2007). In the present study it was evaluated that the anticancer effect of *Cleome gynandra* against Ehrlich's Ascites Carcinoma (EAC) in Swiss albino mice.

Abbreviations: MECG, methanol extract of *Cleome gynandra*; EAC, Ehrlich Ascites Carcinoma; %ILS, percentage increases life span; MST, mean survival time; B.W., body weight; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin.

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Previously isolated classes of constituents: Cleogynol, a novel dammarane triterpenoid isolated from *Cleome gynandra* (Das et al., 1999).

1. Materials and methods

1.1. Acute toxicity

As per reported method (Litchfield and Wilcoxon, 1949) the LD₅₀ value of MEGC in male Swiss albino mice was determined and it was found to be 2 g/kg body weight i.p.

1.2. Chemicals

The chemicals used were sodium chloride, propylene glycol, trypan blue, methyl violet, sodium sulphate, methylene blue, 5-fluorouracil (MERCK Limited, Mumbai, India). All other chemicals and reagents used were of highest analytical grade.

1.3. Animals

Male Swiss albino mice weighing 20–22 g were taken. They were obtained from the animal house, B. N. Ghosh & Co. Kolkata, India. The mice were grouped and housed in poly acrylic cages (38 cm × 23 cm × 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 °C and 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 7 days before commencement of the experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee.

1.4. Transplantation of tumor

EAC cells were obtained from Chittaranjan National Cancer Institute (CNCI), Kolkata, India. The EAC cells were maintained *in vivo* in Swiss albino mice by intraperitoneal transplantation of 2 × 10⁶ cells per mouse after every 10 days. Ascitic fluid was drawn out from EAC tumor bearing mouse at the log phase (days 7–8 of tumor bearing) of the tumor cells. Each animal received 0.1 ml of tumor cell suspension containing 2 × 10⁶ tumor cells intraperitoneally.

1.5. Treatment schedule

100 Swiss albino mice were divided into five groups (*n* = 20) and given food and water *ad libitum*. All the animals in each groups except Group-I received EAC cells (2 × 10⁶ cells/mouse i.p.) This was taken as day '0'. Group-I served as normal saline control (5 ml/kg i.p.) and Group-II served as EAC control. 24-h after EAC transplantation, Group-III and Group-IV received methanol extract of *Cleome gynandra* (MEGC) at a dose of 200 and 400 mg/kg i.p. for nine consecutive days, respectively. Group-V received reference drug 5-FU (20 mg/kg i.p) for nine consecutive days (Mazumder et al., 1997). Twenty-four hours of last dose and 18 h of fasting, 10 animals of each group were sacrificed by cervical dislocation to measure antitumor and hematological parameters and the rest were kept with food and water *ad libitum* to check percentage increase in life span of the tumor host. The antitumor activity of the methanol extracts of *Cleome gynandra* was measured in EAC animals with respect to the following parameters.

1.6. Tumor volume

The ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube.

1.7. Tumor weight

The tumor weight was measured by taking the weight of the mice before and after the collection of the ascetic fluid from peritoneal cavity.

1.8. Percentage increase in life span

The effect of MEGC on percentage increases in life span was calculated on the basis of mortality of the experimental mice (Sur and Ganguly, 1994).

$$ILS (\%) = \left[\frac{\text{Mean survival time of the treated group}}{\text{Mean survival time of the control group}} - 1 \right] \times 100$$

$$\text{Mean survival time}^* = \frac{\text{First death} + \text{last death}}{2}$$

*time denoted by days.

1.9. Tumor cell count

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

1.10. Viable/nonviable tumor cell count

The viability and nonviability of the cell were checked by trypan blue assay. The cells were stained with trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those that took the dye were nonviable. These viable and nonviable cells were counted.

$$\text{Cell count} = \frac{\text{Number of cells} \times \text{dilution factor}}{\text{Area} \times \text{thickness of liquid film}}$$

1.11. Hematological parameters

At the end of the experimental period, the next day after an overnight fasting blood was collected from freely flowing tail vein and used for the estimation of hemoglobin (Hb) content, red blood cell (RBC) count, white blood cell (WBC) count and lymphocyte count by standard procedures.

1.12. Statistical analysis

All data are expressed as mean ± S.E.M. (*n* = 10 mice per groups). Statistical significance (*p*) calculated by one-way ANOVA between the treated groups and the EAC control followed by Dunnett's post hoc test of significance where *p* < 0.05 and *p* < 0.01 considered to be significant and highly significant, respectively.

2. Results

Intraperitoneal administration of the MEGC at the dose of 200 and 400 mg/kg body weight increased the life span (ILS) and nonviable cell count, decreased tumor volume, tumor weight and viable cell count of the tumor bearing mice, when compared to that of EAC control mice (Table 1). The MEGC also restored the hematological parameters towards the saline control. The number of RBC count

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