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Research Article

Effect of Methanol Extracts of *Glinus oppositifolius* and *Trianthema decandra* in Mouse against Ehrlich Ascites Carcinoma Cell Line *in vivo*

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Abstract

The plants, *Trianthema decandra* and *Glinus oppositifolius* are commonly used by tribal people in India for the treatment of cancer. The anticancer activity of methanol extracts of *Trianthema decandra* (METD) and *Glinus oppositifolius* (MEGO) was evaluated in Ehrlich Ascites Carcinoma (EAC) cell line injected intraperitoneally (i.p.) to adult Swiss albino mice at the rate of 2 x106 cells/mouse. The mean survival time, tumor volume, life span, tumor cell count, hemoglobin content and RBC count and WBC count were measured to determine the anticarcinogenic effect of METD and MEGO at the doses of 100, 200 and 400 mg\kg body weight of mice administered i.p. The life span of the tumor bearing mice, total number of RBC and haemoglobin content were significantly increased. In differential count of WBC, percentage of lymphocytes was also increased with decreased level of neutrophils in METD and MEGO treated mice. The tumor volume and the percentage of viable cells in ascitic fluid were significantly reduced in METD and MEGO treated mice. From the result of the above-mentioned parameters, it was concluded that METD and MEGO significantly elicited a potent anticancer activity as compared with that of the standard anticancer drug, 5- fluorouracil (5-FU), intraperitoneally at a dose of 2 mg/kg body weight.

***Keywords*:** Anticarcinogenic, *Trianthema decandra*, *Glinus oppositifolius*, Ehrlich ascites carcinoma (EAC), 5- fluorouracil

Introduction

The plant, *Trianthema decandra* (Family: Ficoidaceae) is commonly known as 'Gadabani', a roadside weed, which is found on dry-soil, especially in Deccan peninsula. In traditional medicinal system, the plant is widely used for the treatment of various ailments. Its decoction is used for the treatment of asthma, hepatitis, cancer and suppression of menses. Tribes normally consume root powder with milk in orchitis.[1] Another plant, *Glinus oppositifolius* (Family: Ficoidaceae) is commonly known as 'gima'. It is present in the greater part of India, especially in Assam, West Bengal and Deccan peninsula. The plant is used as anticancer, stomachic, aperients, antiseptic and suppressive agent of the lochia, bitter tonic for liver disorders.[2-3] The present study was carried out to evaluate the anticancer activity of methanol extract of *Glinus oppositifolius* (MEGO) and methanol extract of *Trianthema decandra* (METD). The mean survival time, tumor volume, life span, tumor cell count, hemoglobin content and RBC count and WBC count were measured to determine the anticancer activity of METD and MEGO at the doses of 100, 200 and 400 mg/kg intraperitoneally (i.p.) against Ehrlich's Ascites Carcinoma (EAC) cell line. 5-Fluorouracil which was synthesized by Duschinsky et al[4] (1957) has been used extensively in the treatment of certain type cancer. [5-6]

Materials and methods

Preparation of plant extracts

The whole plant material of *Glinus oppositifolius* was collected from Midnapore, West Bengal, during the month of June-August when the plant was in full leaf and another plant, Trianthema decandra were collected from Kolli Hills, Tamilnadu. The plant materials were taxonomically identified by the Botanical Survey of India, Shibpur, Howrah and the voucher specimen (GMC-1 and GMC-2) were retained in our laboratory for future reference. The collected plant material were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve # 40 and stored in an airtight container for further use. The dried powered material of *Glinus oppositifolius* was defatted by extracting with petroleum ether in Soxhlet extraction apparatus. The defatted plant material was then extracted with methanol (80%). The solvent was completely removed under reduced pressure to obtain a dry mass and stored in a vacuum desiccator. The yield of petroleum ether extract and methanol extracts were found to be 4.6% and 14.8 % w/w respectively. The dried powered plant material of *Trianthema decandra* was also extracted with petroleum ether and methanol (80%) successively in Soxhlet apparatus. The solvent was also completely removed

under reduced pressure and stored in a vacuum desiccator. The yield of petroleum ether extract and methanol extracts were found to be 7.4 % and 13.8 % w/w respectively.

Treatment schedule

Mature male Swiss albino mice were weighed (20-22 g) and divided into 9 groups (n=10) and given food and water ad libitum. EAC cells (2 x10^6 cells/mouse) obtained from Chittaranjan National Cancer Research Centre, Kolkata were injected to the mice of all groups except normal control group. This was taken as Day 0. On Day 1, the drug was administered at the doses mentioned below and continued for 9 consecutive days. On Day 10, six mice from each group were sacrificed after 24 h of the last dose and 18 h fasting condition. The rest mice were kept with food and water ad libitum to check the lifespan.[7] 5-Fluorouracil was used as standard anticancer drug to compare the anticarcinogenic effect. The treatment protocol is given below:

Group	Treatment	Dose
Group I	Control	[5 ml/kg body weight of normal saline i.p.]
Group II	EAC	[2 $\times$ 10^6 cells/mouse i.p.]
Group III	EAC+5FU	[2 X 10^6 cells/mouse i.p.] [2 mg/kg body weight i.p.]
Group IV	EAC+ METD	[2X10^6 cells/mouse i.p.] [100 mg/kg body weight i.p.]
Group V	EAC+ METD	[2 X 10^6 cells/mouse i.p.] [200 mg/kg body weight i.p.]
Group VI	EAC+ METD	[2 X 10^6 cells/mouse i.p.] [400 mg/kg body weight i.p.]
Group VII	EAC+ MEGO	[2 X 10^6 cells/mouse i.p.] [100 mg/kg body weight i.p.]
Group VIII	EAC+ MEGO	[2 X 10^6 cells/mouse i.p.] [200mg/kg body weight i.p.]
Group IX	EAC+ MEGO	[2 X 10^6 cells/mouse i.p.] [400 mg/kg body weight i.p.]

Tumor growth response

The effect of METD and MEGO on tumor growth and host's survival time were examined by studying the following parameters-tumor volume, tumor cell count, viable tumor cell count, nonviable tumor cell count, mean survival time and increase in lifespan.

Tumor volume

The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and packed cell volume determined by centrifuging at 1000 g for 5 min.

Tumor cell count

The ascitic fluid was taken in a RBC pipette and diluted 1000 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the number of cells in the 64 small squares was counted.

Viable tumor cell count

The cells were then 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 stain were non viable. These viable and nonviable cells were counted. Cell count = (No. of cells x Dilution) / (Area x Thickness of liquid film).

Percentage increase in life span

The effect of METD and MEGO on tumor growth was monitored by recording the mortality everyday for 6 weeks and percentage increase in life span (% ILS) was calculated.[8]

ILS (%) = [(Mean survival of treated group / Mean survival of control group)-1] x 100

Mean survival time = [1st Death + Last Death]/ 2

Haematological studies

The effect of METD and MEGO on peripheral blood was investigated. RBC, WBC counts and estimation of hemoglobin was done by standard procedures from freely flowing tail vein blood.[9-11]

Statistical analysis

Results are expressed as mean $\pm$ S.E.M. and the test of significance of the results were evaluated by ANOVA analysis followed Dunnet's *t*-test.

Table 1. Effect of methanol extracts of *Glinus oppositifolius* and *Trianthema decandra* on life span, mean survival time in EAC treated mice

Parameters	Survival time (days)	Increase Life span (%)	Tumor volume (ml)	Viable cells in ascitic fluid (%)
Nomal Saline (5ml/kg b.w)		—	—	—
EAC (2×10^6 cells/mouse)	20.12±1.93	—	3.21±0.04	94.68±3.53
EAC (2×10^6 cells/mouse) +5-FU (2mg/kg)	40.53±3.45	101.44	1.43±0.03 *	21.87± 2.15 *
EAC (2×10^6 cells/mouse) + METD (100mg/kg)	24.05±1.41	19.53	2.72±0.05	75.34±2.81
EAC (2×10^6 cells/mouse)+ METD (200mg/kg)	27.94±2.07	38.87	2.45±0.03 *	54.67±2.34*
EAC (2×10^6 cells/mouse) + METD (400mg/kg)	32.18±2.18	59.94	2.06±0.02 *	41.32±1.52*
EAC (2×10^6 cells/mouse) + MEGO (100mg/kg)	23.67±1.09	17.64	2.67±0.08	72.75±2.86
EAC (2×10^6 cells/mouse) + MEGO (200mg/kg)	28.83±2.84	43.24	2.28±0.04	51.28±2.13*
EAC (2×10^6 cells/mouse) + MEGO (400mg/kg)	34.54±2.35	71.66	1.81±0.03 *	35.56±1.45*

p-values calculated by ANOVA analysis followed by Dunnet's 't' test of significance with five animals in comparison with EAC treated group. $p<0.05$. All values represent mean ±SEM

Table 2. Effect of methanol extracts of *Glinus oppositifolius* and *Trianthema decandra* on hematological profile in EAC treated mice

Parameters	Hemoglobin (g/dl)	RBC (count ×106 /mm^3)	WBC(count × 103 /mm3)	Neutrophyl %	Lymphocytes %	Monocytes %
Nomal Saline (5ml/kg bw)	13.5±0.4	5.4±0.8	6.5±1.2	68.26±2.42	28.35±1.15	1.78 0.09
EAC (2×106 cells/mouse)	8.8±0.6	4.1±0.7	13.5±2.5	78.68±2.26	18.56±0.98	1.56 0.07
EAC +5-FU (2mg/kg)	11.5±0.4 *	5.2±1.6 *	7.9±1.6*	70.28±3.09	26.81±1.31	1.74 0.08
EAC + METD (100mg/kg)	9.3±0.3	4.3±0.7	12.4±0.5*	75.18±2.82*	22.38±1.16	1.62 0.09
EAC + METD (200mg/kg)	10.1±0.3	4.8±0.6	10.2±1.8	74.47±2.75	24.10±1.42	1.65 0.06
EAC + METD (400mg/kg)	11.6±0.4*	5.0±0.8 *	8.8±0.9	72.81±2.96	25.67±1.23	1.71 0.08
EAC + MEGO (100mg/kg)	9.4±0.4	4.4±0.8	12.8±2.0*	75.88±3.62	22.48±1.37	1.57 0.04
EAC+MEGO (200mg/kg)	10.3±0.3	4.8±0.7	9.8±1.2	74.32±2.92	23.82±1.47	1.68 0.05
EAC + MEGO (400mg/kg)	11.5±0.3 *	5.1±0.9 *	8.7±0.9*	71.56±2.35	26.63±1.38	1.65 0.08

p-values calculated by ANOVA analysis followed by Dunnet's't' test of significance in comparison (n=6) with EAC treated group. $p<0.05$. All values represent mean±SEM

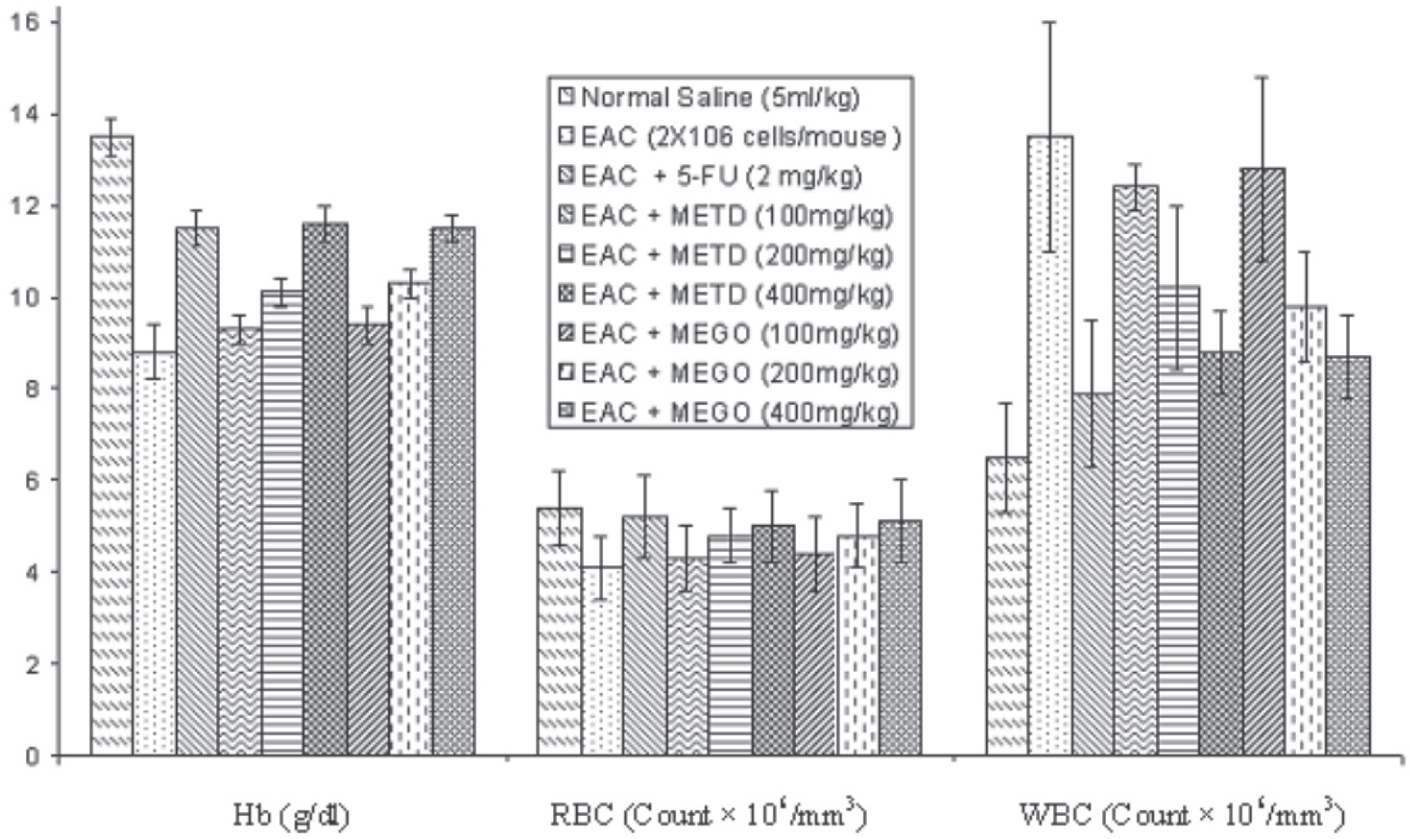

Fig. 1: Effect of methanol extracts of Glinus oppositifolius and Trianthema decandra on hemoglobin percentage, RBC and WBC in EAC treated mice

Results and Discussion

Oral administration of METD and MEGO at the dose of 100, 200 and 400 mg/kg exhibited the percentage of increase in life span of the tumor bearing mice 19.53, 38.87,59.97 and 17.64, 43.24, 71.66 respectively, when compared to that of EAC control mice i.e. 101.44. Both MEGO and METD also restored the hematological parameters i.e., RBC, WBC and haemoglobin content.

Total number of RBC and haemoglobin content were also increased and in differential count of WBC, percentage of Lymphocytes was increased with decreased level of neutrophils in METD and MEGO treated mice that were shown in Fig.1.

The tumor volume and the percentage of viable cells in ascitic fluid were reduced in METD and MEGO treated mice. All values were summarized in Table 1 and Table 2.

The methanol extract of *Glinus oppositifolius* and *Trianthema decandra* significantly increased the life span of EAC bearing mice. Increased life span is major factor in the cancer treatment, because most of the anticancer drugs having less life span and also it produced more side effects. The reliable criteria for judging the value of any anticancer drug are prolongation of life span and decrease of WBC count.[12-13]

Hematological parameters such as RBC, WBC, and hemoglobin levels are frequently affected by the cancer chemotherapy. Most of modern antineoplastic agents produce anaemia due to their cytotoxic effect. MEGO significantly enhanced the erythrocyte count and hemoglobin level and decreased WBC count in EAC bearing mice. This indicates that MEGO alters the hematological profile to more or less normal. METD also enhanced RBC count and hemoglobin level and reduced WBC count in EAC bearing mice. Most of the anticancer drugs alter the hematological profile to more or less normal.[14] MEGO shows better anticancer activity than METD.

MEGO and METD decreased viable cell count and increased non-viable cell count. These suggested that MEGO having direct relationship with tumor cells because these anticancer agents cause the lysis of the cells by direct cytotoxic mechanism.

Conclusion

METD and MEGO treated mice restore the mean survival time, tumor volume, life span, tumor cell count and increased hemoglobin content and RBC count but decreased WBC count. These findings indicate that methanol extracts of *Trianthema*

decandra and *Glinus oppositifolius* could be beneficial as anticancer agents.

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References

1. Kirtikar KR, Basu BD. Indian Medicinal Plants. Dehra Dun: M/S Bishensingh, Mahendra pal Singh, New Connaught Place, Vol. 2, 2nd ed, 1975, p. 1182.

2. Kirtikar KR, Basu BD. Indian Medicinal Plants. Dehra Dun: M/S Bishensingh, Mahendra pal Singh, New Connaught Place Vol. 2, 2nd ed, 1975, p. 1184.

3. Chatterjee A, Pakrashi SC. The Treatise on Indian Medicinal Plants. New Delhi: Publication & Information Directorate, Vol. 1, 1991, p. 78.

4. Duschinsky R, Pleven E, Heidelberger C. The synthesis of 5-fluoropyrimidines. J Ameri Chem Soc 1957; 79: 4559-60.

5. Heidelberger C, Ansfield FJ. Experimental and clinical use of fluorinated pyrimidines in cancer chemotherapy. Cancer Res 1963; 23:1226-43.

6. Seifert P, Baker HL, Recd ML. Comparison of continuously infused 5-fluorouracil with bolus injection in the treatment of patients with Colorectal adenocarcinoma. Cancer 1975; 36:123-28.

7. Mazumder UK, Gupta M, Maiti S, Mukherjee M. Antitumor activity of Gygrophila spinosa on Ehrlich ascites carcinoma and sarcoma-180 induced mice. Ind J Expt Biol 1997; 35: 473-77.

8. Fastier FN, Spenden RN, Waal H. Prolongation of chloral hydrate sleeping time by 5-hydroxytryptamine and by certain other drugs. Br J Pharmacol 1957; 12: 251-256.

9. Dacie JV, Lewis SM. Practical Hematology. London: J&A Churchill Ltd., 1958, pp. 38-48.

10. Osser LB. Hawk's Physiological Chemistry. Bombay and New Delhi: Tata Mc-Graw Hill Publishing Co. Ltd., 1954, pp.1067 -74.

11. D'Amour FE, Blood FR, Belden DA. Jr Manual for Laboratory Work in Mamalian Physiology. Chicago:The University of Chicago Press, 3 ed, 1965: 4 -6.

12. Clarkson BD, Burchenal JH. Preliminary screening of antineoplastic drugs. Prog Clin Cancer 1965; 1: 625-29.

13. Oberling C, Guerin M. The role of viruses in the production of cancer. Adv can Res 1965; 2: 353-423.

14. Gupta M, Mazumder UK, Rath N, Mukhopadhyay DK. Antitumor activity of methanol extract of Cassia fistula L. seed against Ehrlich ascites carcinoma. J Ethnopharma 2000; 72(1-2): 151-56.