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Research Article

ANTITUMOR ACTIVITY OF *CLEOME VISCOSA* AGAINST EHRLICH ASCITES CARCINOMA (EAC) IN SWISS ALBINO MICE

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Abstract
The methanol extract of *Cleome viscosa* (Capparaceae) (MECV) were evaluated for antitumor activity against Ehrlich ascites carcinoma (EAC)-bearing Swiss albino mice. The extract was administered at the doses of 200, and 400 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 MECV on the growth of transplantable murine tumor, life span of EAC-bearing hosts and hematological profile MECV 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. The results indicate that MECV exhibited significant antitumor activity in EAC-bearing mice.
Keywords: *Cleome viscosa, Ehrlich ascites* Carcinoma, Antitumor

1. Introduction:

Man since the ancient times has been depended on medicinal plants for treating and developing immunity against various kinds of disorders. Enormous numbers of people from worldwide were using plant products in amelioration of common diseases. Cancer is a class of diseases in which a cell or a group of cells display uncontrolled growth, invasion and sometimes metastasis. It is largest non-communicable disease and it has a sizable contribution in the total number of deaths. The World cancer report documents that cancer rates are set to increase at an alarming rate globally. Cancer rates could increase by 50% new cases for the year 2020. [1]

Now a days in the global scenario is supporting the development of modern drugs from less toxic plant products with proven medicinal properties. Diverse kind of traditional system of medicines like ayurveda, sidha, unani are supporting to use medicinal plants to combat diseases. Plants contain a broad range of bioactive compounds such as lipids, Phytochemical, pharmaceutics, flavors, fragrances and pigments. Plant extracts are widely used in the food, pharmaceutical and cosmetics industries. There are various types of phytoconstituents such as bufadenolides, alkaloids, triterpenes, flavonoids, isothiocyanates etc. [2] have been used in the past and are currently employing in treating ailments including cytotoxic and cancer chemopreventive effects , this inspired many scientists to take up independent investigations on a number of medicinal plants.

Cleome viscosa (Family: Capparaceae) is a widely distributed herb with yellow flowers and long slender pods containing seeds. The whole plant is used as drugs by the traditional medical practitioners in India with beneficial action for the treatment of diarrhoea, fever, inflammation, liver diseases, bronchitis, skin diseases, and malarial fever. The plant contains lignans, flavonoids, saponins, ascorbic acid, and polyunsaturated fatty acid. Coumarino lignin glycosides cleomiscosins has isolated from seeds of *C.viscosa.*[3]. Some other chemical constituents isolated from *C. viscosa* are glucosinolates[4], cleomeolide, Stigmasta-5,24(28)-diene-3β-*O*-α-L-rhamnoside[5], kaempferide-3-glucuronide[6], and naringenin glycoside [7].

Traditionally described medicinal uses of *C.viscosa* are laxative, anti-helminthic, stomachic, and diuretic . It can be also used in treatment of malarial fevers, skin diseases, leprosy and fever due to indigestion, blood disorders and uterine complications [8]. Earlier pharmacological reports of *C.viscosa* were indicating that it has proved to be act as hepatoprotective[9], anthelminthic[10], analgesic[11], anti-inflammatory[12], antimalarial[13], immunomodulatory[14,15], mutagenic[16]. Since it has a number of medicinal properties including free radical scavenging activity. Hence, in the present study the methanolic extract of *C.viscosa* has been evaluated for antitumor activity in EAC bearing mice.

2. Experimental details

2.1. Plant material: The plant *C .viscosa* (Family: Capparaceae) was collected in the month of October 2009 from the Talakona forest, Chittor district. The plant material was taxonomically identified by the taxonomist, S.V University, Tirupathi.

2.2. Preparation of methanolic extract: The dried powder material of the bark of the *C. viscosa* was extracted with methanol (yield 14.65 %) in a soxhlet apparatus. The methanol extract was then distilled, evaporated, and dried in vacuum. Preliminary qualitative analysis of the methanol extract showed the presence of steroids, triterpenoids, flavonoids and tannins. The methanol extract of *C. viscosa* (MECV) was used for the present study.

2.3. Animals: The study was carried out after obtaining permission from Institutional animal ethics committee (No. 160/SPIPS/Wgl/IAEC/2010) and CPCSEA regulations were adhered to during the study. Male swiss albino mice (20- 25 g) were selected for this study. The animals were maintained under standard environmental conditions and fed with standard pellet feed and water *ad libitum*.

2.4. Tumor cells: EAC cells were obtained from Centre for Cellular and Molecular Biology (CCMB) (Hyderabad, India). The EAC cells were maintained by intraperitoneal inoculation of 2×10^6 cells /mouse.

2.5. Antitumor activity[17]: Male swiss albino mice weighing 20 ± 2 g. were than divided into 5 groups (n =12). All the groups were injected with EAC cells (0.2 ml of 2×10^6 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). Normal Saline, 5 ml/kg per day, was administered in group 2 (EAC control). MECV at different doses (200 and 400 mg/kg per day) and the standard drug 5-fluorouracil (20 mg/kg) were administered in groups 3, 4 and 5 respectively for 14 days orally. After the last dose and 18-h fasting, six mice from each group were sacrificed for the study of antitumor activity, hematological parameters. The rest of the animal groups were kept to check the survival time of EAC-tumor bearing hosts.

2.6. Effect of MECV on tumor growth response: The antitumor effect of MECV 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[18,19]:

MST = (Day of first death + Day of last death*)* / 2

ILS (%) = [(Mean survival time of treated group) / (Mean survival time of control group) **-** 1 ×100

2.7. Effect of MECV on hematological studies: Blood was withdrawn from each mouse by retro orbital plexus method and the hemoglobin content, red blood cell (RBC), and white blood cell (WBC) counts were measured[20,21]. Differential leukocyte count of WBC was carried out from leishman stained blood smears[22] of normal, EAC control, and MECV treated groups, respectively.

2.8. Effect of MECV on in vitro cytotoxicity: Short-term cytotoxicity was assessed by incubating 1 X 10^6 EAC cells in 1 ml phosphate buffer saline with varying concentrations of the MECV at 37^0C for 3 h in CO_2 atmosphere ensured using a McIntosh field jar. The viability of the cells was determined by the tryphan blue exclusion method. [23]

2.9. Statistical analysis: 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.

3. Results

The present investigation indicates that the MECV showed significant antitumor activity in EAC-bearing mice. The effects of MECV at the doses of 200 and 400 mg/kg on survival time, % ILS, tumor volume, packed cell volume, and tumor cell count (viable and nonviable cell) are shown in Table 1.

3.1. Effect on mean survival time: In the EAC control group, the mean survival time was 17.95 ± 0.13 days, while it increased to 27.62 ± 0.16 (200 mg/kg), and 34.67 ± 0.14 (400 mg/kg) days, respectively, in the MECV-treated groups, whereas the standard drug 5-fluorouracil (20 mg/kg)-treated group had a mean survival time of 38.68 ± 0.27 days.

3.2. Effect on tumor growth: Treatment with MECV at the doses of 200 and 400 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 MECV were significantly ($P < 0.01$) increased in a dose-dependent manner.

3.3. 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 MECV at the dose of 200 and 400 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 MECV at the dose of 200 and 400 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 MECV at different doses changed these altered parameters more or less to the normal values.

4. Discussion

The present study was carried out to evaluate the antitumor effect of MECV in EAC-bearing mice. The MECV-treated animals at the doses of 200 and 400 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. 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 ascitis fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells [24]. Treatment with MECV 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[25]. The MECV decreased the ascites fluid volume, viable cell count, and increased the percentage of life span. It may be concluded that MECV by decreasing the nutritional fluid volume and arresting the tumor growth, this could be the reason for the increase life span of EAC-bearing mice. Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia[26,27]. 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 [28]. After the repeated treatment, MECV able to reverse the changes in hematological parameters hemoglobin content, RBC, and WBC counts near to normal levels. This indicates that MECV is showing protective action on the hemopoietic system.

Some triterpinoids and flavonoids are found to have promising anticancer and antioxidant activity. MECV shows the presence of triterpenes and flavonoids which may act as anticancer and antioxidant principles with MECV [29,30]. In our earlier studies, we found that MECV possess hepatoprotective and antioxidant properties[31]. 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.

The present study demonstrates that MECV increased the life span of EAC-tumor bearing mice in the liver. The above parameters are responsible for the antitumor and antioxidant activities of *Cleome viscosa*.

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Results Tables:

Table I. Effect of the methanol extract of MECV on body weight, mean survival time, % ILS, tumor volume, packed cell volume, and viable and nonviable tumor cell count of EAC-bearing mice.

Parameters	EAC Control	MECV (200 mg/kg) + EAC	MECV (400 mg/kg) + EAC	Standard 5-fluorouracil (20 mg/kg)+ EAC
Body weight (g)	25.7 ± 0.11	23.3 ± 0.17**	21.5 ± 0.13**	21.2 ± 0.19**
Mean survival time (days)	17.95 ±0.13	27.62 ± 0.16**	34.67 ± 0.14**	38.68 ± 0.27**
Increase life span (%)	-----	62.23**	93.56**	119.35**
Tumor volume (ml)	4.58 ± 0.11	3.27 ± 0.06**	1.53 ± 0.04**	1.06 ± 0.03**
Packed cell volume (ml)	27.2 ± 1.36	23.1 ± 0.12**	18.5 ± 0.03**	17.3 ± 0.25**
Viable tumor cell count (× 10^7 cells/ml)	12.34 ± 0.05	4.6 ± 0.07**	0.96 ± 0.04**	-----
Nonviable tumor cell count (× 10^7 cells/ml)	0.35 ± 0.03	0.73 ± 0.03**	1.41 ± 0.04**	-----

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.

Table II. Effect of the methanol extract of MECV on hematological parameters of EAC-bearing mice.

Parameters	Normal (saline, 5 ml /kg)	EAC Control	MECV (200 mg/kg) + EAC	MECV (400 mg/kg) + EAC
Hemoglobin (g %)	13.5 ± 0.12	9.7 ±0.15 ***	11.7±0.13**	14.4 ±0.13**
RBC (×10^9/ul)	6.4 ± 0.15	3.5 ±0.08 ***	4.4 ± 0.24	5.9 ±0.45**
WBC (×10^9/ul)	5.9 ± 0.08	15.6 ±0.21 ***	10.1 ±0.05**	6.4 ±0.03
Monocyte (%)	1.7 ±0.04	1.2 ±0.04 ***	1.7 ±0.03	1.9 ±0.04**
Neutrophil (%)	17.9 ±1.08	56.4 ± 4.12 ***	43.6 ± 3.14**	25.1 ±2.15
Lymphocyte (%)	79.3 ±2.16	33.6 ±2.55 ***	58.2 ±2.45	69.4 ±2.58**

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.