ANTITUMOR ACTIVITY OF *CAPPARIS SEPIARIA* ON EHRLICH ASCITES CARCINOMA IN MICE

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ABSTRACT

The methanol extract of *Capparis Sepiaria* (Capparaceae) bark (MECS) 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 MECS on the growth of transplantable murine tumor, life span of EAC-bearing hosts and hematological profile MECS 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 MECS exhibited significant antitumor activity in EAC-bearing mice.

KEY WORDS: *Capparis sepiaria*, Ehrlich ascites carcinoma, Antitumor

INTRODUCTION

The use of medicinal plants to treat diseases is as old as human civilization. Human beings of all ages in both developing and undeveloped countries use plants in seek to treat numerous diseases and to get relief from physical ailments. 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].

India is a rich source of medicinal plants and a number of plant extracts are used against diseases in various systems of medicine such as Ayurveda, Unani, and Siddha. Only a few of them have been scientifically explored. There were good number of plant products such as flavonoids, terpenes; alkaloids [2, 3, 4] and these can are used as remedies to treat various diseases and disorders. Because of their distinct pharmacological qualities including cytotoxic and cancer chemopreventive effects, it is inspired many scientists to take up independent investigations on a number of medicinal plants[5].

Many species of *Capparis* are reported from India and different parts of the world. *Capparis sepiaria* is widely distributed in India, Srilanka, Mayanmar, and Pakisthan.

It is a climbing shrub with curved thorns, elliptical leaves and simple flowers, fruits are in clusters, pisiform and black. It is distributed throughout salty ranges of Andhra Pradesh, Maharashtra, and Karnataka. ethnobotanical studies are stating that *C.sepiaria* used as blood purifier, stomachic, tonic and appetizer[6, 7]. It's flowers, leaves and roots are used in cough and toxemia and root powder is also used as a cure for the snake bite. It is also possesses febrifuge properties and also used to treat skin diseases, tumors and diseases of muscles. Various bioactive compounds can be seen in *Capparis sepiaria* like alkaloids, phenols, sterols or glycosides. ß-sitosterol, present in whole plant and leaf extract of *C. sepiaria*. Betulin is also identified from whole plant and leaf extracts. Leaf contains a, ß-amyrin, taraxasterol, erythrodiol, whole plant n-octacosanol[8].

Extracts of different parts of *capparis* show biological activities against various diseases. *C. sepiaria* seed have been considered as antidote for snake bite. Root can be used in earache and mumps. Stem root bark having therapeutic value in curing dropsy gout, aphthae. This further suggests that *Capparis species* roots might have chemical compound with anticancer properties as well, which requires standardization.

MATERIALS AND METHODS

Plant material and toxicity studies

The plant C*apparis sepiaria* (Family: Capparaceae) was collected in the month of August 2010 from the Talakona forest, Chittor district. The plant material was taxonomically identified by the taxonomist, S.V University, Tirupathi. The dried powder material of the whole plant of the C*apparis sepiaria* were extracted with methanol in a soxhlet apparatus. The methanol extract was then distilled, evaporated, and dried in vacuum. The resulting methanolic extract yield was observed to be 10.5 %. Preliminary qualitative analysis of the methanol extract showed the presence of saponins, tannins, flavonoids, alkaloids. The methanol extract of *Capparis sepiaria* (MECS) was used for the present study. The LD_{50} value of *C.sepiaria* was found to be 894.43 mg/kg (i.p) according to method described by Lorke[9].

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*.

Tumor cells and Drugs

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. Standard drug 5-fluorouracil procured as a gift sample from Cadila Pharmaceuticals Ltd, Ahmedabad.

Antitumor activity [10]

The activity was carried out according to the method described by[10]. Male Swiss albino mice weighing 20 ± 2 g. were randomly divided into 5 groups (n =12). All the groups were injected with EAC cells (0.2 ml of 2×10^6 cells/mouse) intraperitoneally (i.p) except the normal group. This was taken as day zero. On the first day, 5 ml /kg of normal saline was

administered orally (p.o) in group 1 (Normal). Normal Saline, 5 ml/kg per day, was administered in group 2 (EAC control). MECS at different doses (200 and 400 mg/kg per day) and the standard drug 5-fluorouracil [11] (20 mg/kg) were administered in groups 3, 4 and 5 respectively for 14 days orally (p.o). 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.

Effect of MECS on Tumor growth response

The antitumor effect of MECS 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 [12, 13]

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

Effect of MECS 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 [14,15]. Differential leukocyte count of WBC was carried out from Leishman stained blood smears [16] of normal, EAC control, and MECS treated groups, respectively.

Effect of MECS 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 MECS 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 trypan blue exclusion method[17].

STATISTICAL ANALYSIS

The experimental results were expressed as the mean ± S.E.M. Data were assessed by Student's t-test; P value of < 0.05 was considered as statistically significant.

RESULTS

The present investigation indicates that the MECS showed significant antitumor activity in EAC-bearing mice. The effects of MECS 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.

Effect on mean survival time

In the EAC control group, the mean survival time was 18.54 ±0.16 days, while it increased to 28.62 ± 0.16 (200 mg/kg), and 33.87 ± 0.14 (400 mg/kg) days, respectively, in the MECS-treated groups, whereas the standard drug 5-fluorouracil (20 mg/kg)-treated group had a mean survival time of 37.58 ± 0.25 days.

Effect on tumor growth

Treatment with MECS 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 MECS were significantly ($P < 0.01$) increased in a dose-dependent manner.

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 MECS 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 MECS 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 MECS at different doses changed these altered parameters more or less to the normal values.

DISCUSSION

The present study was carried out to evaluate the antitumor effect of MECS in EAC-bearing mice. The MECS-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 [18]. Treatment with MECS inhibited the tumor volume, tumor cell count, and 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[19]. The MECS decreased the ascites fluid volume, viable cell count, and increased the percentage of life span. It may be concluded that MECS by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of EAC-bearing mice. Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia[20, 21]. 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[22]. In EAC control group, a differential count the presence of neutrophils increased, while the lymphocyte count decreased, the observed leucocytopenia indicates a common symptom of immunosuppression in many types of cancers[23, 24] and one of the causes of neutrophilia is myeloid growth factors which are produced in malignant process as part of a paraneoplastic syndrome. In addition to this another factor granulocyte colony stimulating factor produced by the malignant cells has also been attributed to be the cause of neutrophilia because of its action on bone marrow granulocytic cells in cancer. After the repeated treatment, MECS able to reverse the changes in altered neutrophils and lymphocytes count.[25, 26]. Treatment with MECS brought back the hemoglobin content, RBC, and WBC count more or less to normal levels and This indicates that MECS posses protective action on the hemopoietic system.

Flavonoids are found to have promising anticancer and antioxidant activity. MECS shows the presence of flavonoids, alkaloids and Betulin which may act as anticancer and antioxidant principles with MECS[27,28]. In our earlier studies, we found that MECS possess antidiabetic and antioxidant properties[29]. 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 MECS increased the life span of EAC-tumor bearing mice in the liver. The above parameters are responsible for the antitumor and antioxidant activities of *Capparis sepiaria*.

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Table 1: Effect of the methanol extract of MECS 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	MECS (200 mg/kg) + EAC	MECS (400 mg/kg) + EAC	Standard 5-fluorouracil (20 mg/kg)+ EAC
Body weight (g)	26.4 ± 0.13	24.84 ± 0.17**	22.3 ± 0.13**	21.2 ± 0.19**
Mean survival time (days)	18.54 ±0.16	28.62 ± 0.16**	33.87 ± 0.14**	37.58 ± 0.25**
Increase life span (%)	-----	58.23**	92.56**	119.35**
Tumor volume (ml)	4.64 ± 0.11	3.15 ± 0.06**	1.83 ± 0.04**	1.02 ± 0.03**
Packed cell volume (ml)	27.2 ± 1.36	22.8 ± 0.12**	18.5 ± 0.03**	17.4 ± 0.35**
Viable tumor cell count ($\times 10^7$ cells/ml)	11.34 ± 0.05	5.6 ± 0.07**	0.76 ± 0.04**	-----
Nonviable tumor cell count ($\times 10^7$ cells/ml)	0.32 ± 0.03	0.83 ± 0.03**	1.48 ± 0.04**	-----

Data are expressed as the mean of results in (n= 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 2. Effect of the methanol extract of MECS on hematological parameters of EAC-bearing mice.

Parameters	Normal (saline, 5 ml /kg)	EAC Control	MECS (200 mg/kg) + EAC	MECS (400 mg/kg) + EAC
Hemoglobin (g %)	14.2 ± 0.12	10.7 ±0.15 ***	11.8±0.14**	13.4 ±0.13**
RBC ($\times 10^9$/ul)	6.2 ± 0.15	3.1 ±0.08 ***	4.6 ± 0.24	5.6 ±0.45**
WBC ($\times 10^9$/ul)	5.3 ± 0.08	16.9 ±0.21 ***	9.1 ±0.05**	5.9 ±0.03
Monocyte (%)	1.6 ±0.04	1.0 ±0.04 ***	1.2 ±0.03	1.5 ±0.04**
Neutrophil (%)	18.7 ±1.08	59.4 ± 4.12 ***	41.6 ± 3.14**	29.1 ±3.15
Lymphocyte (%)	75.3 ±2.36	37.6 ±2.45 ***	54.8 ±2.35	63.1 ±2.18**

Data are expressed as the mean of results in (n= 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.