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Anti Cancer Activity of Methanolic Extract of Aerial parts of *Momordica Cymbalaria* Hook F. Against Ehrlich Ascites Carcinoma in Mice

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Abstract
Cancer is a malignant disease that is characterized by rapid and uncontrolled formation of abnormal cells which may mass together to form a growth or tumor or proliferate throughout the body. Next to heart disease cancer is a major killer of mankind. Present study aims at a preliminary phytochemical screening and anti cancer evaluation of methanolic extract of aerial parts of *Momordica Cymbalaria* Hook F. against Ehrlich Ascites Carcinoma in animal model. *Momordica Cymbalaria* Hook F. is a wild crop, well known as Athalakkai. The collected plant was identified and dried under shade and coarsely powdered and passed through 40 mesh sieve. The powdered material was extracted with Methanol. The methanolic extract of its aerial parts given orally to mice at the dose of 100 and 200 mg/kg body weight for 14 days caused significant($p< 0.001$) reduction in body weight, packed volume and viable tumor cell count when compared to the mice of the EAC control group. All these results suggest the potent antitumor properties of the MEMC. However, the standard cyclophaspomide treatment at 50 mg/kg body weight produced better results than the extract treatment in all these parameter. Restoration of hematological parameters towards normal was also observed. Histological observations of liver indicated repair of tissue damage caused by tumor inoculation.

Key words: (EAC) Ehrlich ascites carcinoma, cyclophosphamide, Carboxy methyl cellulose (CMC), *Momordica Cymbalaria* Hook F,Methanol.

INTRODUCTION

The use of plant preparations and extracts of their anti fertility properties has a long-standing history among Indian physicians. In this regard, one such plant which has number of traditional uses is *Momordica Cymbalaria* Hook F (Family-Cucurbitaceae). It is perennial herb distributed over tropical parts of Tamil nadu, India and well known as Athalakai.It is also found in this states of Karnataka, Andhra Pradesh in India. It is traditionally used as abortifacient. Ethanol extract is reported to have anti-ovulatory, abortifacient and anti implantation activity[1-3]. The extracts and the dried form of fruit and leaves were shown to have anti diabetic, hypolipidemic, anti hyperglycemic activities[4-5] and recently shown anti microbial activity[6]. Hence, we have undertaken this study to evaluate the anti cancer activity of methanol extract of *Momordica Cymbalaria* against Ehrlich Ascites Carcinoma in Mice.

MATERIALS AND METHODS

Plant material:
The plant material of the (*Momordica Cymbalaria* Hook F (Family-Cucurbitaceae)Aerial parts were collected in the month of July 2010 from the village sivarakottai of Madurai district and authenticated by Dr.G.V.S.Murthy, Scientist & Head of office Government of India, Botanical Survey of India, Southern Circle, T.N.A.U. Campus, Lawley Road, Coimbatore -641 003. A voucher specimen No. BSI / SRC/ 23 / 10 / 10-11/Tech.-575.

Treatment of Aerial Parts
The dried aerial parts were powdered by means of mixer grinder and were sieved through sieve no.40 to get the coarse powder and it was extracted with methanol and obtained extract is subjected to qualitative phytochemical analysis.

Chemicals:
cyclophosphamide were obtained from Cadila Healthcare Ltd.,Kundaim Industrial Estate,Goa, India. Carboxy methyl cellulose (CMC), were obtained from S.D. Fine Chemicals, Mumbai, India. All other chemicals used in the study were of analytical grade.

Animals and Animal Care:
Male Wistar albino mice, weighing 25 - 30 g were used in this study. Protocols were in accordance with and approved by the institutional animal ethical committee (JKKMMRFCP/IAEC/2010/016). These animals were kept in an environment with controlled temperature (25°C), humidity (45–57%), and photoperiod (12: 12-h light-dark cycle). The animals were fed with rat pellet feed supplied by Hindustan Ltd, Bangalore in India and free access to water ad libitum. The mice were acclimatized and laboratory conditions for 10 days before commencement of experiment.

Tumor Cells:
Ehrlich ascites carcinoma (EAC) cell were supplied by Amala Cancer Research Centre, Trissur, Kerala,

India. The cells were maintained in vivo in Swiss albino mice by intraperitonial transplantation .EAC cells aspirated from the peritonial cavity of mice were washed with salaine and given intraperitoneally to develop ascetic tumor.

Acute oral toxicity:

The acute oral toxicity of the Methanolic aerial parts extract of the *Momordica cymbalaria* Hook f was carried out as per OECD 423 – guidelines. The acute toxicity studies revealed that LD_{50}, Up to 5000mg/kg did not produce any toxic effect. Hence the biological dose was fixed as 100mg/kg and 200mg/kg respectively[7].

EAC-Induced Ascitic Antitumor Studies:

Ehrlich's Ascites Carcinoma cells were cultured in the peritoneal cavity of healthy albino mice weighing between 25 to 30 g by injecting a suspension of EAC cells ($1X10^6$cells/ml) intraperitoneally. The cells were aspirated aseptically from the peritoneal cavity of the mice on day 15 and washed with normal saline and centrifuged for 15 min at 1,500 rpm in a cooling centrifuge. The pellet was re-suspended with normal saline and the process was repeated three times. Finally, the cells were suspended in a known quantity of normal saline and the cell count was adjusted to ($2X10^6$cells/ml).Sample showing more than 90 % viability was used for transplantation. Each animal received 0.1 ml of tumor cell suspension containing ($2X\ 10^6$ cells /ml) intraperitoneally[8].

EXPERIMENTAL DESIGN:

The mice were divided into five groups comprising twelve animals in each group. The entire animal was injected with EAC cells ($2X10^6$cells/mouse) intraperitoneally except for the normal group as follows:

Group I:	Normal with sodium CMC Suspension (0.1%)
Group II:	Induced EAC cell ($2\ X10^6$) with sodium CMC Suspension (0.1%)
Group II:	Induced EAC cell ($2X10^6$) with MEMC 100mg/kg body weight With sodium CMC Suspension (0.1%)
Group IV:	Induced EAC cell ($2X10^6$) with MEMC 200mg/kg body weight With sodium CMC Suspension (0.1%)
Group V:	Induced EAC cell ($2X10^{6)}$ with cyclophosphamide 50mg/kg body Weight with sodium CMC Suspension (0.1%)

All this treatments were given 24 h after the tumor inoculation, once daily for 14 days after the last dose and 24 h fasting, six mice from each group were sacrifice. The blood was collected from the animals by retro-orbital puncher under slight anesthesia (di ethyl ether) conditions; and the hematological parameter such as red blood cells (RBC), white blood cells (WBC), differential count (DC), and hemoglobin(Hgb) were estimated by cell analyzer. The differential count of WBC was carried out in the blood smear. The ascitic fluid was collected from the peritoneal cavity of the animals and divided into two parts. One part was centrifuged in a graduate centrifuge tube at 1,000 rpm for 10 min and the packed cell volume was measured. The cells in the other part of the aseptic fluid were separated by centrifugation and stained with trypan blue (0.4% in normal saline). The number of viable cells was counted .the rest of the animals were kept to check average life span and change in body weight for 6 weeks. Percent increase in life span (ILS) was calculated by the following formula[9].

% ILS = {(life span of treated group/ life span of controlled group)-1} X 100.

Statistical Analysis:

Results of all the estimations done were indicated in terms of Mean ± SEM. Statistical significance of data were assessed by analysis of variance (one way ANOVA), followed by multiple comparison test and student's 't'test . The significance was at the level of $p<0.05$. The group II was compared with the group I and all other groups were compared with the group II.

RESULT AND DISCUSSION:

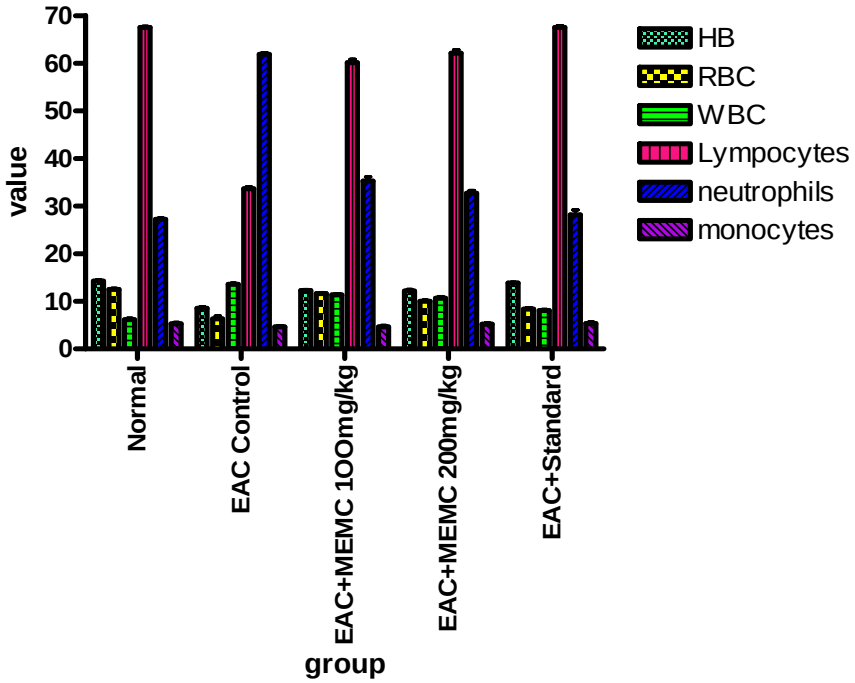
The animals of the tumor control group inoculated with EAC survived for a period 14.38 ± 1.242 days. The treatment with MEMC at 100 and 200 mg/kg body weight increased the average life span of animals by 23.73 ± 1.639, and 22.54 ± 0.783 days, respectively (fig.9a). The increases in life span at 100 and 200 mg/kg body weight were found to be significant. The MEMC at the 100 mg/kg body weight dose was found to be more potent in inhibiting the proliferation of EAC with the percentage increases in life span of 43.01%. The percent increase in body weight of the EAC tumor control group was found to be 22.41 ±1.422%. The MEMC treatment at 100 and 200 mg/kg doses significantly inhibited the percent increase in body weight when compared to the tumor control ($p<o.oo1$). The packed cell volume (ml), viable tumor cell count ($x10^7$celles/ml) and total WBC ($x10^3/mm^3$) were found to decreases significantly in animal treated with the MEMC at almost all the

Table: 1-Effect of MEMC on hematological parameters of EAC-bearing mice on day 15 of the Experiment

Treatment		*Hb (g %)*	*RBC (million /mm^3)*	*WBC (103cells / mm^3)*	*WBC Differential Count %*		
					Lymphocytes	**Neutrophils**	**Monocytes**
Group I	Normal	14.26 ± 0.48	12.54 ±0.43	6.16 ±0.64	67.52 ±0.49	27.23 ±0.57	5.25 ± 0.71
Group II	EAC control	8.54 ± 0.61[a]	6.34 ±1.32[a]	13.54 ±0.57[a]	33.63 ±1.56[a]	61.85 ±0.87[a]	4.62 ± 0.54
Group III	EAC + MEMC 100(mg/kg)	12.26 ± 0.47[b]	11.62 ±0.39[b]	11.34 ±0.56[d]	60.11 ±1.56[b]	35.25 ± 1.98[b]	4.64 ± 0.63
Group IV	EAC + MEMC 200(mg/kg)	12.14 ± 0.78[c]	10.03 ±0.48[c]	10.65 ±0.76[e]	62.12 ±1.47[c]	32.69 ±1.34 [c]	5.21 ± 0.54
Group V	EAC + Cyclophosphamide 50(mg/kg)	13.76 ± 0.45	8.36 ±0.62	8.09 ±0.47	67.53 ±0.82	28.15 ±2.31	5.32 ± 0.71

[a]P < 0.001 Vs Normal mice; [b, c] P < 0.001; [d] P< 0.01; [e]P< 0.05 Vs Tumor mice; n = 6

Figure: 1- Effect of MEMC on hematological parameters of EAC-bearing mice on day 15 of the Experiment



doses tested when compared to EAC tumor control, which indicating the antitumor nature of the extract. Similarly, RBC count, hemoglobin content, and lymphocytes count, which were decreased after EAC inoculation, were found to be significantly return to the normal levels in the animals treated with the MEMC at all the two doses (Table 1) The neutrophile count, which was increased in EAC tumor control animals, was found to be decreased towards the normal by the MEMC significantly ($p< 0.001$) at all the doses. All these results suggest the potent antitumor properties of the MEMC. However, the standard cyclophaspomide treatment at 50 mg/kg body weight produced better results than the extract treatment in all these parameter.

CONCLUSION

In the present pharmacological evaluation the methanolic extract of aerial parts of *Momordica Cymbalaria* Hook F (Family-Cucurbitaceae) was extensively investigated for its anticancer activity against EAC induced cancer in mice. The Methanolic extract of aerial parts of *Momordica cymbalaria* Hook f (100,200mg/kg) has been showed significant anticancer activity as compared to standard cyclophosphamide in EAC induced cancer model.

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