ORIGINAL RESEARCH PAPER

Pharma

ANTITUMOR ACTIVITY OF ETHANOLIC LEAVES EXTRACT OF MORINGA OLEIFERA LAM. AGAINST EHRLICH ASCITES CARCINOMA IN SWISS ALBINO MICE

KEY WORDS: ELMO, Protective, Curative dose, Bodyweight, Tumor cell count, EAC.

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ABSTRACT

Aim &Objective: To evaluate the antitumor activity of ethanolic leaves extract of Moringa oleifera Lam. (ELMO) against Ehrlich Ascetic Carcinoma in Swiss albino mice. **Material and Methods:** The effect of ethanolic extract of Moringa oleifera Lam. (ELMO) on tumor growth was studied by the following parameters: body weight, Abdominal circumference, tumor volume, viable and non-viable cell count. ELMO was administered at 500mg/kg body. once a day for 15 days, after 24 h of tumor inoculation. Decreases in tumor volume and viable cell count were observed in ELMO treated animals when compared to EAC treated animals. The extract also decreased the body weight of the EAC tumor bearing mice. Hematological studies reveal that the Hb content was decreased in EAC treated mice; whereas restoration to near normal levels was observed in ELMO treated protective group animals. **Results:** Oral administration of 500mg/kg body wt. of treated ELMO has shown a significantly decreased in both protective and curative group of body weight, tumor volume, abdominal circumference, and increased all blood cells when compared to the EAC tumor group. **Conclusion:** ELMO treated 500mg /kg body wt. possesses showed antitumor activity

INTRODUCTION

Despite substantial progress in the understanding of the molecular basis, diagnosis, and treatment, cancer is still a major health concern. It is the second leading cause of death in the world after cardiac diseases. According to the recently available information, globally in the year 2002, excluding the non-melanoma skin cancers, there were more than 10 million new cases of cancer recorded, with nearly 7 million cancer deaths[1]. Projections are that by the year 2020, these figures will increase to over 16 million new cases, with 10 million deaths, and that in 2030 there may be more than 20 million new cases of cancer, with 70% of cancer deaths in the low-income countries, which have minimal resources to treat[1].

Irrespective of the cause, localized malignant tumors are best managed by surgical removal or radiotherapy, or both, while the treatment options for advanced and metastasized tumors are mostly chemotherapy [2]. However, most of the clinically used synthetic chemotherapeutic agents exhibit severe normal tissue toxicity and cause undesirable side effects in the patients receiving them [3]. Therefore, there is a need is felt to find alternative drugs, which at non-toxic doses are highly effective, inexpensive, and affordable to the common man. One of the best approaches in searching for novel anticancer agents from plant resources is identifying time-tested ethnomedical practices and evaluating their actual efficacy and safety through established methods of modern science [4,5]. The use of ethnomedical knowledge has contributed to health care and systematic studies have shown that the evaluation of traditionally used medicines may lead to effective drug discovery [5]. Since time immemorial, vast ethnobotanical knowledge exists in India and most people use traditional medicine as an alternative treatment for diseases, including cancer, due to its lower toxicity [6]. These herbal systems are still in place today because of their organizational strengths, based on the principles of Ayurveda, the traditional system of Indian Medicine. The herbal drugs used by traditional healers primarily focus on multicomponent mixtures and are mostly prepared by boiling the plants in water [6].

Moringa oleifera is an important food commodity that has had enormous attention as the 'natural nutrition of the tropics. The leaves, fruit, flowers of this tree are used as a highly nutritive vegetable in many countries, particularly in India, Pakistan, Philippines, Hawaii, and many parts of Africa [7] Leaves of this plant are traditionally known for or reported to have various biological activities, including hypocholesterolemic agent [8], regulation of thyroid hormone status[9], antidiabetic[10], gastric ulcers [11], antitumor [12], and hypotensive agent [13]. Active oxygen species and free radicals play an important role in the pathogenesis of several human diseases, such as rheumatoid arthritis, cardiovascular diseases, and cancer. Any natural compound with antioxidant properties may help in maintaining health when continuously taken as components of dietary foods, spices, or drugs[14]. Ehrlich ascitic carcinoma is a convenient model for the investigation of antitumor drugs side effects and the investigation of the anti-tumor immune response [15]. It is also significant in showing plasma biochemical changes; including antioxidant systems [16]. The present study aimed to evaluate the antitumor activity of the ethanolic leaves extracts of *Moringa oleifera* Lam. (ELMO) against Ehrlich ascites carcinoma (EAC) in albino mice.

MATERIALS AND METHODS

Collection and extraction

The leaves of *Moringa oleifera* Lam. were collected in January 2019 from Anantapur district, Andhrapradesh, India. The leaves were dried under a shade with occasional shifting and then powdered with a mechanical grinder and stored in an air-tight container. 150g of the dried leaves powder of *Moringa oleifera* Lam were soaked in 90% ethanol, and 10% distilled water for 24 hours in a percolator. After 24 hours, it was allowed to percolate slowly and the extract was collected in Petri dishes. The extract was concentrated in a vacuum using a rotary flash evaporator (40°C). There was a net yield of 23.00 g of the concentrated extract (17.80 w/w %).

Animals

Swiss albino male mice, weighing 20-25g, male, were procured from the animal house of the Basaveshwara Medical College and Hospital, Chitradurga, Karnataka, India. All the animals were kept in standard polypropylene cages under standard conditions: temperature (24±1°C), relative humidity (40-45%), and a12:12 light: dark cycle. The animals were fed a standard rodent diet(Amruth Rat Feed, manufactured and supplied by Pranav Agro Industries, Pune, India), and water was given *ad labitum*. The animals were allowed to acclimatize to laboratory conditions 48h before the start of the experiment. The experimental protocol is duly approved by the institutional animal ethical committee (Reg. no.1284/ac/09/CPCSEA).

Tumor Cell Line and Their Maintenance

EAC cells were obtained through the courtesy of Amala Cancer Research Center, Thrissur. They were maintained by weekly intraperitoneal inoculation of $3x10^6$ cells/mouse[17].

Experimental design:

In this study total of 24 albino male mice were taken, divided into four groups. Each group contains 6 animals. These groups are as follows:

Group I: Normal Control (Non-EAC induced hepatoma bearing mice):

This group consists of 6 Swiss albino mice; all mice received 5.0 ml of normal saline/kg body weight orally by gastric intubation daily for 15 days.

Group II: EAC Control (EAC induced hepatoma bearing mice):

This group consists of 6 Swiss albino mice with experimentally induced hepatoma. About $3x\ 10^6$ EAC tumor cells were injected intraperitoneally into healthy mice. All mice received 5.0 ml of normal saline/kg body weight orally by gastric intubation daily for 15 days.

Group III: Protective group (ELMO treated-EAC induced hepatoma bearing mice):

This group consists of 6 Swiss albino mice; each animal treated ELMO (500 mg / 5ml / kg body wt.) was mixed in 5.0ml of a warm aqueous solution, given orally into gastric intubation once a day for 4 days. On the 5^{th}day, $3x10^6$ EAC tumor cells were injected intraperitoneally. Later ELMO (500, mg / 5ml /kg body wt.) was again given orally to each animal further for 11 days.

Group IV: Curative group (EAC induced hepatoma bearing-ELMO treated mice):

This group consists of 6 Swiss albino male mice. About $3x\ 10^6$ EAC tumor cells were injected intraperitoneally into healthy mice. These mice received 5.0 ml of normal saline/kg body weight orally by gastric intubation daily for 4 days. From the 5^{th} day, 5.0 ml warm aqueous solution of ELMO (500 mg /kg body weight) was given orally for 15 days.

On the 15^{th} day, all control protective and curative groups of animals' body weight and abdominal circumferences were recorded. Then the mice were anesthetized and sacrificed. The ascitic fluids were immediately collected in clean dry graduated tubes by puncturing the abdomen. The fluid volumes were noted. The ascitic fluids were assayed for total cell count, and viable and non-viable tumor cell count was assessed microscopically by using a Neubauer chamber. Blood samples from each mouse were collected from the eyes by sino orbital puncture of mice using micro-capillary tubes [18]. Blood samples were withdrawn in clean and dry test tubes containing ethylene diamine tetraacetic acid (EDTA) and were then centrifuged at 3000 rpm for 15 minutes. The supernatant plasma was separated and stored deep-frozen at -20°C until assayed. The remnants (the packed RBCs) were stored in a deep freezer at -20°C until assayed.

The anti-tumor activity of the ethanolic leaves extract of *Moringa oleifera* (ELMO) was measured in EAC animals concerning the following parameters:

Bodyweight: Body weights of the experimental mice were recorded both in the treated and control group at the beginning of the experiment (day 0) and sequentially on every 5th day during the treatment period [19].

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 was determined by centrifuging at 1000 rpm for 5 min[20].

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 counting chamber and the numbers of cells in the 64 small squares were counted.

Viable / non-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 nonviable. These viable and nonviable cells were counted.

$$\text{Cell count} = \frac{\text{No. of cells X Dilution}}{\text{\% Area X Thickness of liquid film}}$$

Hematological Parameters: At the end of the experimental period, all mice were killed the next day after an overnight fast by decapitation. Blood was collected from a freely flowing tail vein and used for the estimation of Hemoglobin (Hb) content, red blood cell count (RBC), and white blood cell count (WBC) [21]. WBC differential count was carried out from Leishman stained blood smears[22].

Statistical Analysis

The experimental results were expressed as mean ± S.E.M. Data was assessed by ANOVA followed by the Students *t*-test, *p*-value < 0.05 was considered statistically significant.

RESULTS

Antitumor activity of ELMO against EAC tumor-bearing mice was assessed by the parameters such as body weight, abdominal circumference, tumor volume, and tumor cell count (viable, non-viable, and total count), The results are shown in Table 1. The tumor volume, and body weight to be significantly ($p < 0.01$) increased and the non-viable cell count was significantly ($p < 0.01$) low in EAC control animals when compared with normal control animals. Administration of ELMO at the dose of 500 mg/kg body wt. significantly ($p < 0.05$) decreased the bodyweight, abdominal circumference, tumor volume, and viable cell count. Non-viable cell count was significantly ($p < 0.05$) higher in ELMO-treated animals when compared with EAC control animals. On administration of ELMO at 500 mg/kg body weight respectively. Finally, the change in body weights of the animals suggests the tumor growth-inhibiting property of ELMO. All these results indicate that the ELMO has a remarkable capacity to inhibit the growth of tumor-induced by EAC cell line in a dose of 500mg/kg body wt. in experimental animals.

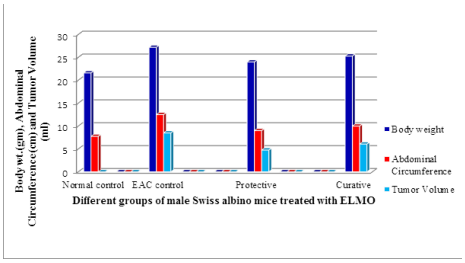
Table 1: Bodyweight, Abdominal Circumference, And Tumor Cell Count Of Different Groups Of EAC- Bearing Mice.

Group	Bodyweight (g)	Abdominal circumference (cm)	Tumor volume (ml)	Tumor cell count		
				Viable	Nonviable	Total Count
Normal control	21.60 ± 0.23	7.75±0.17	---			
EAC control	27.20 ± 0.61	12.50±0.43	8.50 ± 0.32	8.5± 02	2.17± 03	10.67± 05
Protective	24.00 ±0.63	9.00±0.26	4.76 ± 0.26 **	3.17± 12*	5.83± 03*	9.00± 15*
Curative	25.30 ± 0.46	10.00±0.26	6.06 ± 0.14 **	4.0± 10*	5.5± 05*	9.5± 15*

Data are expressed as Mean ± S.E.M., (n = 6) animals in each group, *p<0.01, **p<0.001, ***p<0.0001 Vs control. When compared to EAC control *p<0.01, **p<0.001.

Graph 1: Showing Bodyweight, Abdominal Circumference, and Tumor Volume of different groups of

EAC-bearing mice.



Graph 2: Showing Tumor cell count of different groups of EAC bearing mice

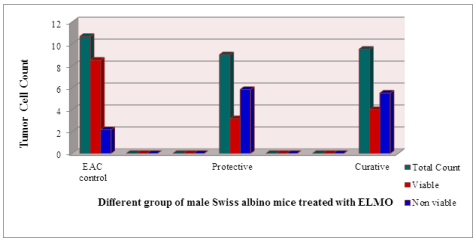


Table 2: Hematological parameters of different groups of EAC bearing mice

Group	Hb content (g. %)	RBC (cells/ml X10^6)	WBC (cells/ml X10^6)	Differential count		
				Lymphocytes (%)	Neutrophils (%)	Monocytes (%)
Normal control	13.05± 0.35	5.3 ± 0.12	7.8 ± 0.40	68 ± 1.3	27 ± 1.1	2.2 ± 1.5
EAC control	10±0.23	3.9 ± 0.80	15.4 ± 0.21	35 ± 1.5	65 ± 1.4	1.6 ± 0.5
Protective	12.6±0.50	4.0 ± 0.36	9.7 ± 0.08	54 ± 1.6	35 ± 1.0	1.7 ± 1.5
Curative	11.4±0.55	4.1 ± 0.15	12.1 ± 0.33	45 ± 1.1	52 ± 1.6	1.6 ± 1.2

Data are expressed as Mean ± S.E.M. (n = 6) animals in each group, *p<0.01, **p<0.001, ***p<0.0001 Vs control,

DISCUSSION

The present investigation was carried out to evaluate the antitumor activity and antioxidant status of ethanolic leaves extract of Moringa oleifera (ELMO) in EAC tumor bearing mice. The ELMO treated animals at the doses of 500mg/kg body wt. inhibited the tumor volume, packed cell volume, and tumor (viable) cell count and brought back the hematological parameters to more or less normal levels. In EAC tumor bearing mice, a regular rapid increase in ascitic tumor volume was observed. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells [23]. Treatment with ELMO inhibited the tumor volume, abdominal circumference, body eight, viable tumor cell count, and increased the life span of the tumor-bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the life span of animals [24]. It may be concluded that ELMO by decreasing the nutritional fluid volume and arrests the tumor growth of EAC bearing mice. Thus, ELMO has antitumor activity against EAC bearing mice.

Usually, in cancer chemotherapy, major problems that are being encountered are myelosuppression and anemia [25, 26]. The anemia encountered in tumor bearing mice is mainly due to a reduction in RBC or hemoglobin percentage, and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions [27]. Treatment with ELMO brought back the hemoglobin (Hb), RBC, and WBC count more or less to normal levels to normal levels. This indicates that ELMO possesses protective action on the hemopoietic system.

CONCLUSION

In conclusion, the present study demonstrates that the ethanolic leaves extract of Moringa oleifera (ELMO) increased the life span of EAC tumor bearing mice. All these parameters suggest that the ethanolic leaves extract of Moringa oleifera exhibits potential antitumor activity.

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