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Original Research Article

Evaluation of effective anti-tumarogenic dose of ethanolic leaves extract of *Moringa oleifera* Lam. in Ehrlich ascites carcinoma bearing mice

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ABSTRACT

Background: To evaluate the optimum protective and curative antitumarogenic dose of the ethanolic leaves extract of *Moringa oleifera* Lam (ELMO). variety against Ehrlich ascetic carcinoma (EAC) cells.
Methods: The protective and curative antitumor activity of ELMO was evaluated against the EAC tumor model. The activity was assessed using survival time and increase in life span.
Results: Oral administration of all the doses of treated ELMO shown significantly increased in mean survival time and percent of life span, in that 500 mg/kg body wt. shown highest significance.
Conclusions: ELMO treated 500 mg/kg body wt. possesses showed antitumor activity.

Keywords: ELMO, Protective, Curative dose, MST, %ILS, EAC

INTRODUCTION

Medicinal plants have been used by all civilizations as a source of medicines since ancient times. In the recent times, there has been growing interest in exploiting the biological activities of different ayurvedic medicinal herbs; due to their natural origin, cost-effectiveness and lesser side effects.[1] *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.[2] Leaves of this plant are traditionally known for or reported to have various biological activities, including hypo-cholesterolemic agent, regulation of thyroid hormone status, antidiabetic, gastric ulcers, antitumor, and hypotensive agent.[3-8] 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.[9] Ehrlich ascitic carcinoma is a convenient model for the investigation of antitumor drugs side effects and the investigation of the anti-tumor immune response.[10] It is also significant in showing plasma biochemical changes; including antioxidant systems.[11] The present study aims to evaluate the effective optimum protective and curative antitumorogenic dose of the leaves extract of *Moringa oleifera* Lam. variety against EAC cells in mice.

METHODS

Plant material

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.

Preparation of the extract

The 150 gm 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 gm of the concentrated extract (17.80 w/w %).

Animals used

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^{0}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 labidum. The animals were allowed to acclimatize to laboratory conditions 48 h 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

The inoculum of EAC cells for inducing EAC was kindly provided by the Amala cancer research institute, Thrissur Kerala, India. [12]

Optimum dose studies

Various doses were employed as follows; 125 mg ELMO/kg body weight of mice, 250 mg ELMO/kg body weight of mice, 500 mg ELMO/kg body weight of mice and 1000 mg ELMO/kg body weight of mice.

Group I: 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. 6 mice received 5.0 ml of normal saline/kg body weight orally by gastric intubation daily for 6 weeks. A well-grown tumor was observed within 7-10 days.

Group II: Protective-ELMO treated-EAC induced hepatoma bearing mice

This group consists of 24 Swiss albino mice; each dose of the ELMO (125, 250, 500,1000 mg/ 5 ml/kg body wt.) was mixed in a warm aqueous solution, given orally by gastric intubation once a day for 4 days. On the 5thday, 3×10^6 EAC tumor cells were injected intraperitoneally. Later each dose of ELMO (125, 250, 500, 1000 mg/5 ml/kg body wt.) was again given orally further for 6 weeks. We noted survival period in overall experiment. In each group, we noted 1st death and last death of the animal, based on the death day we calculated the mean survival time (MST) and % of the increase in life span (%ILS) was calculated.

Group III: Curative-EAC induced hepatoma bearing mice-ELMO treated mice

This group consists of 24 Swiss albino male mice. On the 1st day, all the healthy mice were injected with 3×10^6 EAC tumor cells intraperitoneally. Then each mouse received normal saline in a dose of 5.0 ml/kg body weight orally by gastric intubation daily for 4 days. From the 5th day onwards each group of the animal takes a respected dose of ELMO (125, 250, 500,1000 mg/5 ml/kg body wt.) in to warm aqueous solution orally further for 4 weeks duration. Later we noted all the animal's survival periods in the overall experiment. In each group, we noted 1st death and last death of the animal, based on death day we calculated mean survival time (MST) and percent of increasing life span (%ILS)

Percentage increase life span (% ILS)

The effect of ELMO on tumor growth was monitored by recording the mortality daily for 6 weeks and a percentage increase in life span (%ILS) was calculated.

$$\%\ ILS = \frac{\text{Mean survival of the treated group}}{\text{Mean survival of control group}} - 1 \times 100$$

$$\text{Mean survival time} = \frac{\text{Day of 1}^{st}\text{ death + day of last death}}{2}$$

Statistical analysis

The data entry was carried out using an MS office excel worksheet and statistically evaluated. All values are expressed in mean±SEM. P value was calculated using the student's t test. P<0.05 was taken as significant and <0.001 was taken as highly significant. Whereas the group treated with 500 mg/kg/body weight showed highly significant (p<0.0001) increase in life span of tumor bearing mice treated with ELMO.

RESULTS

The effect of ELMO on the survival of tumor-bearing mice is shown in Table 1. The MST for the EAC control group was 14 days, where as it was 17, 20, 25, 22 days in the protective and 22, 22, 24, 23 in the curative group treated by respective doses of ELMO (125, 250, 500, 1000 mg/ 5 ml/kg body wt.). The increase in the life span of tumor-bearing mice treated with ELMO was high as compared to the EAC control group.

Table 1: Effects of ELMO treated groups on mean survival time (MST) and % of the increase in life span (%ILS) in EAC mice.

ELMO doses (mg/kg body wt.)	Protective			Curative			EAC control
	MST	% ILS	MST of treated mice/MST of control mice (T/C %)	MST	% ILS	MST of treated mice/MST of control mice (T/C %)	MST
125	17±0.80*	17.24	117.25	22±0.92*	33.33	157.14	14±0.91
250	20±1.24**	42.85	142.85	22±1.36**	57.14	157.14	
500	25±1.65***	78.57	178.57	24±1.82***	71.42	25±1.65***	
1000	22±1.12**	51.14	157.14	23±1.80**	64.28	164.28	

Values are expressed as mean ± SEM, (n=6) animals in each group, *p<0.01, **p<0.001, ***p<0.0001 vs EAC control.

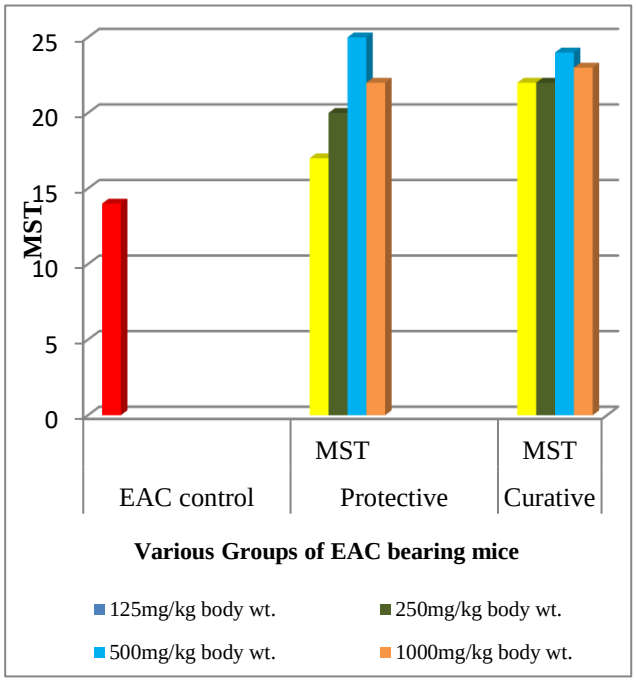

Figure 1: Anti tumorigenic effect of ELMO.

DISCUSSION

It is generally accepted that the reliable criteria for assessing the potential use of any anticancer agent are the prolongation of the life span of animals that are exposed to that anticancer agent. [13,14] Andreani et al have suggested that an increase in the lifespan of ascites bearing animals by 25% is considered indicative of significant drug activity.[15] When we compared with the control group all doses of ELMO treated protective and curative groups of animals showed a significant (p<0.01) increase form of mean survival time and % of life span. That 500 mg/kg body weight dose exhibited a highly significant (p<0.001) increase in the MST 25±1.65, %ILS 78.57 in the protective group and MST 24±1.82, %ILS 71.42 in the curative group.

The present study suggests that ELMO has significant protective as well as curative effects in EAC induced hepatoma bearing mice this may be due to antiproliferative effects because of having phytochemicals like isothiocyanate, niazimicin, niaziminin, and quercetin in this plant leaves.[16]

CONCLUSION

The present study ELMO possessed anti-cancer activity in EAC cells inoculated Swiss albino mice; with 500 mg/kg body wt. dose has shown significant prolongation of life span. It has concluded that ELMO 500 mg/kg body wt. was having maximum protective and curative action on EAC induced cancer.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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