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## Evaluation of Anticancer Activity of *Lagenaria siceraria* Aerial Parts

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### ABSTRACT

Cancer is one of the leading causes of mortality worldwide. Many of the cucurbitaceae plants possess antitumor activity. On the basis of traditional use, the present study was carried out to evaluate the anti-cancer activity of methanol extract of *Lagenaria siceraria* (Mol.) Standley [Cucurbitaceae] aerial parts (MELS) on Ehrlich's Ascites Carcinoma (EAC) model in mice. After inoculation of EAC cells into mice, treatment with MELS (200 and 400 mg kg<sup>-1</sup>) and standard drug, 5-Fluorouracil (20 mg kg<sup>-1</sup>) were continued for 9 days. Evaluation of the effect of drug response was made by the study of tumor growth response including increase in life span, study of hematological parameters, biochemical estimations and antioxidant assay of liver tissue. Experimental results revealed that *L. siceraria* possesses significant anticancer activity which may be due to its cytotoxicity and antioxidant properties. Further research is ongoing to find out the bioactive principle(s) of MELS for its anticancer activity.

**Key words:** Anticancer, antioxidant, EAC, *L. siceraria*, Cucurbitaceae

### INTRODUCTION

Cancer is one of the most fatal diseases in human population and one of the most frequent causes of death worldwide. An extremely promising strategy for cancer prevention today is chemoprevention which is defined as the use of synthetic or natural agents (alone or combination) to block the development of cancer in humans. Plants, vegetables and herbs used in the folk and traditional medicine have been accepted currently as one of the main sources of cancer chemoprevention drug discovery and development. Plant derived natural products such as flavonoids, terpenoids and steroids have received considerable attention due to their diverse pharmacological properties which include cytotoxic and chemopreventive effects as well (Abdullaev, 2001; Uddin *et al.*, 2003; Koduru *et al.*, 2006; Zahan *et al.*, 2011; Sodde *et al.*, 2011; KunduSen *et al.*, 2011).

The plant, *Lagenaria siceraria* (Mol.) Standley from Cucurbitaceae family, popularly known as bottle gourd (English), has wide occurrence throughout India as an edible vegetable. It is a pubescent or trailing herb, with bottle or dumb-bell shaped fruits. Both of its aerial parts and fruits are commonly consumed as vegetable. Traditionally it is used as medicine in India, China, European countries, Brazil, Hawaiian island etc. for its cardiotonic, general tonic, diuretic,

antiproliferative properties (Kirtikar and Basu, 2003). Further, antihepatotoxic, analgesic and anti-inflammatory, hypolipidemic, antihyperglycemic and antioxidant activities of its fruit extract have been evaluated (Deshpande *et al.*, 2008; Deshpande *et al.*, 2007; Ghule *et al.*, 2006a, b; Shirwaikar and Sreenivasan, 1996). *Lagenaria siceraria* fruits are good source of Vitamin B complex, ascorbic acid, fibers, proteins, cucurbitacins, saponins, fucosterols and campesterols, polyphenolics, flavones-C-glycoside (Ghule *et al.*, 2006b; Shirwaikar and Sreenivasan, 1996; Krauze-Baranowska and Cisowski, 1994; Duke, 1999; Sturm and Stuppner, 2000). Methanol extract of its leaves showed the presence of sterols, polyphenolics, flavonoids, saponin, protein and carbohydrates (Shah and Seth, 2010). A novel protein, Lagenin has also been isolated from its seeds and it possesses antitumor, immunoprotective and antiproliferative properties (Wang and Ng, 2000). In spite of extensive studies on its fruits and seeds, pharmacology of the aerial parts of *L. siceraria* has remained unexplored. The present investigation was therefore, carried out to evaluate anticancer activity of methanol extract of *L. siceraria* aerial parts (MELS) against Ehrlich Ascites Carcinoma (EAC) tumor model in mice.

## MATERIALS AND METHODS

**Plant material:** The aerial parts of *L. Siceraria* were collected in November 2008, from Madanpur, West Bengal, India and identified by the Botanical Survey of India, Howrah, India. A voucher specimen (P/LS/1/08) was retained in our laboratory for further reference.

**Preparation of plant extract:** The aerial parts were dried and powdered in a mechanical grinder. The powdered material was extracted with methanol using soxhlet apparatus. This extract was filtered and concentrated *in vacuo* and kept in a vacuum desiccator for complete removal of solvent. The yield was 18.13% w/w with respect to dried powder. Aqueous suspension of MELS was prepared using 2% (v/v) Tween-80 and used for the treatment.

**Animals:** Healthy Swiss albino mice (20±2 g) were used for the study. The animals were kept in polypropylene cages with sawdust bedding and maintained under standard laboratory conditions. Standard pellet diet (Hindustan Lever, Kolkata, India) and water were given *ad libitum*. The mice were acclimatized to laboratory condition for one week before commencement of experiment. The experiments were performed based on animal ethics guidelines of University Animals Ethics Committee.

**Phytochemical analysis:** Preliminary phytochemical screening of the extract was carried out using standard methods (Kokate, 1994).

**Acute toxicity study:** Healthy Swiss albino mice (20±2 g) of either sex, starved overnight, were divided into five groups (n = 4). Group I-IV animals were orally fed with MELS in increasing dose levels of 0.5, 1.0, 1.5 and 2.0 g kg<sup>-1</sup> b.wt while group V (untreated) served as control. The animals were observed continuously for first 2 h for any gross change in behavioral, neurological and autonomic profiles or any other symptoms of toxicity and mortality if any and intermittently for the next 6 h and then again after 24, 48 and 72 h for any lethality or death. One-tenth and one-fifth of the maximum safe dose of the extract tested for acute toxicity, were selected for the experiment (Ghosh, 1984).

**Tumor cells:** Ehrlich Ascites Carcinoma (EAC) cells were obtained from Chittaranjan National Cancer Institute (CNCI), Kolkata, India. The EAC cells were maintained in Swiss albino mice, by intraperitoneal (i.p.) transplantation on every 9th days (Dagli *et al.*, 1992). The ascitic fluid was collected by syringe and the tumor cell count was performed in a Neubauer hemocytometer and  $2 \times 10^7$  cells/mL were obtained by dilution with normal saline (Orsolice *et al.*, 2005). Tumor cell suspension showing more than 90% viability (checked by trypan blue dye (0.4%) exclusion assay) was used for transplantation.

**Treatment schedule:** Healthy Swiss albino male mice were weighed and divided into five groups ( $n = 12$ ). EAC cells ( $2 \times 10^6$  cells/mouse) were injected i.p. to each mouse of each group except normal saline group. This was taken as Day 0. Extract and reference drug treatment were continued for subsequent 9 days starting from Day 1. On 10th day, 24 h after the last dose six mice were sacrificed from each group and the rest were kept for the life span study of the tumor hosts. After sacrificing the animals, blood was collected to evaluate the hematological and biochemical parameters. Liver tissue was collected from the animals for the evaluation of *in vivo* antioxidant status.

The groups and the design of the experiment were as follows (Mazumder *et al.*, 1997):

**Group I** : 2% Tween-80 (5 mL (0.9% w/v)/kg b.wt, i.p.)

**Group II** : EAC ( $2 \times 10^6$  cells/mouse) + 2% Tween-80 (5 mL  $\text{kg}^{-1}$  b.wt, i.p.)

**Group III** : EAC ( $2 \times 10^6$  cells/mouse) + MELS (200 mg  $\text{kg}^{-1}$  b.wt, i.p.)

**Group IV** : EAC ( $2 \times 10^6$  cells/mouse) + MELS (400 mg  $\text{kg}^{-1}$  b.wt, i.p.)

**Group V** : EAC ( $2 \times 10^6$  cells/mouse) + 5-fluorouracil (20 mg  $\text{kg}^{-1}$  b.wt, i.p.)

**Tumor growth response:** The effect of MELS on tumor growth were examined by studying the following parameters such as tumor volume, packed cell volume, tumor cell count, viable and nonviable tumor cell count.

**Tumor volume and packed cell 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. Packed cell volume was determined by centrifuging at 1000 rpm for 5 min.

**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 and nonviable 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.

**Percentage increase in life span:** The effect of MELS on tumor growth was monitored by recording the mortality daily for 6 weeks and percentage increase in life span (%IMST) was calculated. An enhancement of life span by 25% or more was considered as effective antitumour response (Mazumder *et al.*, 1997; Gupta *et al.*, 2000):

$$\text{IMST (\%)} = \left( \frac{\text{Median survival of treated group}}{\text{Median survival of control group}} - 1 \right) \times 100$$

$$\text{Median Survival Time (MST)} = \frac{\text{1st death} + \text{Last death}}{2}$$

**Hematological studies:** RBC, WBC counts and estimation of hemoglobin was done by standard procedures from the blood obtained intracardially (D'Amour *et al.*, 1965; Wintrobe *et al.*, 1961).

**Hemoglobin estimation:** The 0.1 mL of heparinized blood was taken in Sahli's Hemoglobinometer and diluted with 0.1 N HCl until the color matched with standard. The reading was then taken from the graduated cylinder and expressed as g/100 mL of blood.

**Counting of erythrocytes:** The blood sample was diluted (1:200) with the diluting fluid using Thoma pipette. After vigorous mixing, a drop of resultant mixture was discharged under the cover glass of Neubauer hemocytometer and the corpuscles were allowed to settle for 3 min. The number of erythrocytes in 80 small squares was counted under light microscope. The number of cells in 1 cumm of undiluted blood was calculated.

**Total count of leukocytes:** Blood was diluted 1:20 with a diluting fluid. The Neubauer hemocytometers were filled with the mixture and the number of cells in four corner blocks (each block subdivided into 16 squares) was determined and the total leukocyte count per cumm of blood was calculated.

**Biochemical estimation:** The changes in the biochemical parameters of EAC bearing mice due to the treatment of MELS were evaluated by the estimation of serum biochemical enzymes such as Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) activities by the method of Reitman and Frankel (1957) and Alkaline Phosphatase (ALP) activities by Kind and King (1954) method.

**In vivo antioxidant assay:** The antioxidant assay was performed with the liver tissues of the mice from different groups and evaluation was carried out by measuring the level of lipid peroxidation (Ohkawa *et al.*, 1979) and the amount of enzymatic (CAT) and nonenzymatic antioxidant system (GSH) by the methods of Luck (1963) and Ellman (1959), respectively.

**Statistical analysis:** Values were presented as Mean $\pm$ SEM Data were statistically evaluated by one-way Analysis of Variance (ANOVA) followed by post hoc Dunnett's test using SPSS software.  $p < 0.05$  was considered as statistically significant.

## RESULTS

Preliminary phytochemical screening of MELS revealed the presence of polyphenolics, flavonoids, glycosides, triterpenoids, saponin and carbohydrates.

In acute toxicity study, MELS did not show any toxic effect upto the dose of 2 g kg<sup>-1</sup> b.wt., accordingly 200 and 400 mg kg<sup>-1</sup> b.wt were taken as low and high dose of MELS for the experiment.

Antitumor activity of MELS against EAC tumor bearing mice was assessed by the parameters such as tumor volume, packed cell volume, viable and non-viable cell count, median survival time and % increase in life span. In case of tumor growth response study, MELS treatment significantly ( $p < 0.01$ ) reduced tumor volume (from 4.5 to 1.03 mL), packed cell volume (upto 0.52 from 2.57 mL) and viable cell count (from 97.80 to 68.01%) compared to those of EAC control mice while nonviable cell count was found to be increased significantly (from 2.20 to 31.99%) in the treated groups (Table 1). Table 2 depicts the effects of MELS on prolongation of life span. In life span study, the median survival time for the control group was 19.0 days, whereas it was 26.5, 31.5 and 34.0 days for low and high dose of MELS and the standard drug, 5-FU treated mice, respectively. The extract was found to be able to increase the MST upto 61.54% (Table 2) and were found to be significant ( $p < 0.01$ ) with respect to the EAC control mice and it reflects the antitumor property of the extract MELS.

Administration of MELS significantly reduced WBC count in both the low and high dose of extract treated groups (upto 8.92 thousand/cumm) with respect to that of EAC control group (15.50 thousand/cumm). RBC count and hemoglobin content which were decreased after EAC inoculation (3.85 million/cumm and 10.20 g%, respectively), were found to be significantly ( $p < 0.01$ ) restored to the normal levels in the animals treated with MELS of both 200 and 400 mg kg<sup>-1</sup> b.wt, as well as standard drug 5-FU. The results (Fig. 1) imply the protective role of MELS on the hematological profile of EAC bearing mice.

Biochemical estimation as shown in Fig. 2 indicates the significantly elevated level of liver functional enzymes, such as, SGOT, SGPT, ALP, in serum of EAC inoculated mice with respect to normal ones. However, these were significantly reduced to near normal value in the extract treated groups, indicating the protection of the tumor cell induced hepatotoxicity by MELS.

Antioxidant studies (Fig. 3) in the present investigation indicate that, as compared to normal mice, the level of lipid peroxide in liver tissue of the EAC bearing mice was significantly ( $p < 0.01$ )

Table 1: Effect of Methanol extract of *L. siceraria* (MELS) on Tumor growth response of EAC bearing mice

Groups	Ascitic tumor volume (mL)	Packed cell volume (mL)	Tumor cell count ( $\times 10^7$ mL <sup>-1</sup> )	
			Viable (% cell count)	Non viable (% cell count)
EAC Control	4.50±0.58	2.57±0.25	13.99±0.85 (97.80%)	0.25±0.19 (2.20%)
MELS (200 mg kg <sup>-1</sup> )	2.23±0.23 <sup>b</sup>	1.30±0.19 <sup>b</sup>	9.04±0.85 <sup>b</sup> (87.21%)	1.33±0.50 <sup>b</sup> (12.79%)
MELS (400 mg kg <sup>-1</sup> )	1.03±0.17 <sup>b</sup>	0.52±0.08 <sup>b</sup>	6.05±0.45 <sup>b</sup> (68.01%)	2.85±0.68 <sup>b</sup> (31.99%)
5 Fluorouracil (20 mg kg <sup>-1</sup> )	0.80±0.10 <sup>b</sup>	0.40±0.05 <sup>b</sup>	4.50±0.46 <sup>b</sup> (60.85%)	2.75±0.32 <sup>b</sup> (39.15%)

Values are Mean±SEM; n = 6 in each group. Drug treatment was done for 9 days. <sup>b</sup> $p < 0.01$  for treated groups vs. EAC control group; where the significance was performed by One way ANOVA followed by post hoc Dunnett's test

Table 2: Effect of Methanol extract of *L. siceraria* (MELS) on prolongation of life span of EAC bearing mice

Groups	MST (days)	IMST (%)
EAC control	19.0	-
MELS (200 mg kg <sup>-1</sup> )	26.5	35.90
MELS (400 mg kg <sup>-1</sup> )	31.5	61.54
5 Fluorouracil (20 mg kg <sup>-1</sup> )	34.0	74.35

MST: Median survival time; % IMST: % Increase in MST =  $[(T/C)-1] \times 100$  where T is median survival time of treated group and C that of control group

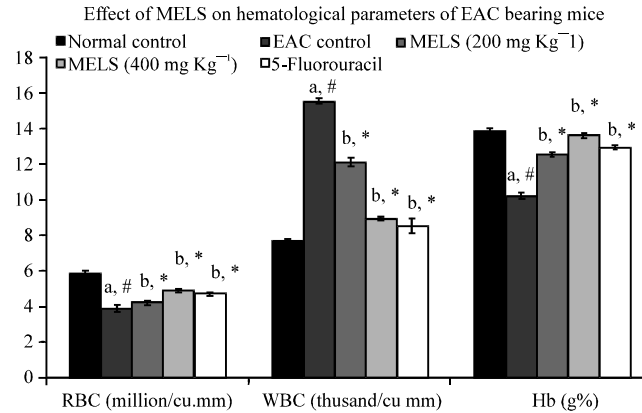


Fig. 1: Effect of menthol extract of *L. siceraria* (MELS) on hematological parameters of EAC bearing mice: Values are Mean±SEM; n = 6 in each group. Drug treatment was done for 9 days. <sup>a</sup>EAC control group vs. Normal group, <sup>#</sup>p<0.01; <sup>b</sup>Treated group vs. EAC control group, \*p<0.01; where the significance was performed by one way ANOVA followed by post hoc Dunnett's test

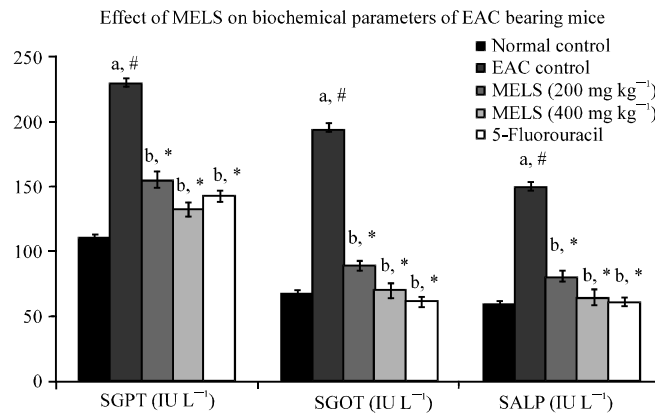


Fig. 2: Effect of Methanol extract of *L. siceraria* (MELS) on biochemical parameters of EAC treated mice. Values are Mean±SEM; n = 6 in each group. Drug treatment was done for 9 days. <sup>a</sup>EAC control group vs. normal control group, <sup>#</sup>p<0.01 <sup>b</sup>Treated groups vs. EacS control group, \*p<0.01 where the significance was performed by one-way ANOVA followed by post hoc Dunnett's test

elevated. It was however reduced to near normal level by the treatment of MELS, both at low and high dose. Depletion of GSH was observed in EAC control group animals indicating high oxidative stress which was however, significantly improved (p<0.01) in case of the extract treated animals. Inoculation of EAC drastically decreased the catalase activity in EAC control group mice when compared to normal group. Administration of MELS both at low and high dose to the EAC bearing mice significantly (p<0.01) improved the catalase activity in a dose dependent manner, indicating a potent antioxidant and free radical scavenging property of the extract MELS.

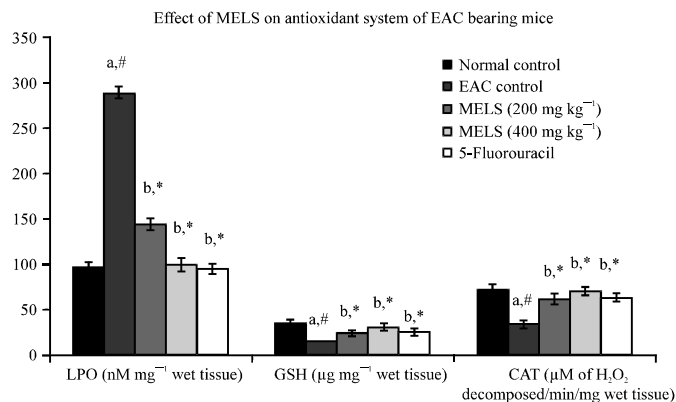


Fig. 3: Effect of methanol extract of *L. siceraria* (MELS) on antioxidant status of EAC bearing mice. LPO: Lipidperoxide; GSH: Reduced Gluathione; CAT: Catalase. Values are Mean±SEM; n = 6 in each group. Drug treatment was done for 9 days. <sup>a</sup> EAC control group vs. normal control group, <sup>#</sup>p<0.01; <sup>b</sup>Treatment groups vs. EAC control group, <sup>\*</sup>p<0.01 where the significance was performed by one-way ANOVA followed by post hoc Dunett's test

## DISCUSSION

The present investigation was carried out to evaluate the antitumor activity of methanol extract of *L. siceraria* (MELS) in EAC tumor bearing mice. The MELS treated animals at the doses of 200 and 400 mg kg<sup>-1</sup> significantly inhibited the tumor volume, packed cell volume, tumor (viable) cell count. 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 the means to meet the nutritional requirement of tumor cells (Prasad and Giri, 1994). Treatment with MELS inhibited the tumor volume, packed cell volume and viable tumor cell count, increasing the non viable cell count.

The present study revealed that MELS treatment at the dose of 200 and 400 mg kg<sup>-1</sup> significantly increased the life span of the mice when compared to the EAC control. The steadfast criteria for judging the potency of any anticancer drug is prolongation of life span (Hogland, 1982). MELS reduced the EAC volume, delayed the cell division and thereby increased survival time of EAC inoculated mice which suggested the antiproliferative effect of the extract (Karthikeyan *et al.*, 2007).

Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia (reduced haemoglobin) (Price and Greenfield, 1958). Anemia encountered in ascites carcinoma mainly due to iron deficiency, either by haemolytic or myelopathic conditions which finally lead to reduced RBC number (Fenninger and Mider, 1954). In this study, elevated WBC count, reduced haemoglobin and RBC count were observed in EAC control mice and the oral administration of *L. siceraria* restored haemoglobin content and maintained normal values of RBC and WBC, thus supporting its hematopoietic protecting activity without inducing myelotoxicity, the most common side effects of cancer chemotherapy.

Significant elevation in the levels of SGOT, SGPT, SALP reflects the hepatocellular damages caused by a number of agents. Biochemical measurements of these parameters showed that to some extent hepatotoxicity was associated after 9 days of inoculation with EAC. Treatment with the MELS restored the elevated biochemical parameters more or less to normal range, indicating the protection of the tumor cell induced hepatotoxicity by MELS (Sallie *et al.*, 1991).



Epidemiological studies have suggested that high endogenous level of oxidative adducts and deficiencies in antioxidant levels are likely to be important risk factors for cancer (Preston-Martin *et al.*, 1990). Excessive generation of free radicals results in the oxidative stress which in turn may lead to the damage of the macromolecules such as lipids and can induce lipid peroxidation *in vivo* (Yagi, 1987). In EAC bearing mice the level of lipid peroxide in liver was found to be elevated significantly which was however reduced to near normal level in the MELS treated animals. This reflects the decrease in free radical production and the subsequent reduction in oxidative stress, one of the main risk factors for the disease.

Reduced glutathione (GSH) is a potent free radical scavenger and can play an important protective role in the neoplastic process by lowering the oxidative stress (Slinclair *et al.*, 1990). Depletion of GSH in EAC control group animals indicates high oxidative stress which was however significantly improved ( $p < 0.01$ ) in the liver of the extract treated animals.

Free radical scavenging enzyme catalase is present in all oxygen-metabolizing cells and its function is to provide a direct defense against the potentially damaging reactivities of superoxide and hydrogen peroxide. The inhibition of CAT activities as a result of tumor growth has also been reported (Marklund *et al.*, 1982). Similar findings were observed in the present investigation with EAC bearing mice. The administration of MELS at different doses increased the CAT levels in a dose dependent manner which along with the restoration of lipid peroxide and GSH content to near normal indicates the antioxidant and free radical scavenging property of MELS.

## CONCLUSION

The present study thus explores the potent anticancer activity of MELS which may be either because of a direct cytotoxic effect of the extract on tumor cells or due to its indirect local effect which may involve macrophage activation and vascular permeability inhibition. Along with this, the significant antioxidant property of the extract probably potentiates its anticancer activity further. This relevant pharmacological activity of the MELS may be attributed to the presence of polyphenolics, flavonoids or the protein in the extract. Flavonoids such as quercetin, kaemferol and their glycosides have shown to possess antimutagenic and antimalignant effect (Labbe *et al.*, 2009). The anticancer activity of methanolic extract is probably due to its flavonoids content. However, further research is ongoing to isolate the pure bioactive compound(s) from it.

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