ORIGINAL ARTICLE

Evaluation of anticancer activity of ethanol extract of *Leucas aspera* flower

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Abstract

Background: Cancer has received a major attention globally due to its high mortality rates. Yet, few successful therapies are available. Plant-based products are gaining momentum in the treatment of most diseases including cancer. **Aim:** The present study aims to assess the antitumor potential of one of the popularly known weed plants *Leucas aspera* flower (LAE) extract against Ehrlich ascetic tumor *in vitro* and *in vivo* models. **Materials and Methods:** A preliminary antioxidant assay was carried out to assess the extract showing optimal activity. Based on this, ethanol extract was used for studying the *in vitro* cytotoxicity by Trypan blue assay. Further, *in vivo* studies were performed on Ehrlich ascetic carcinoma-induced mice. The parameters assessed were mean survival time (MST) and increase in the lifespan along with hematological and enzyme profiles. **Results:** The study revealed that optimal activity was observed in the ethanol extract of LAE. Further, antioxidant studies using ferric-reducing antioxidant power and diphenylpicrylhydrazy assays revealed that best radical scavenging potential was exerted by 100 µg/ml of LAE in both the assays. Further, *in vitro* cytotoxicity showed a concentration-dependent increase in the cytotoxicity up to 200 µg/ml with an IC_{50} value of 102.14 µg/ml. Further, *in vivo* studies showed that LAE treatment enhanced the MST of tumor-bearing mice in a dose-dependent manner and this enhancement was better in 400 mg/kg body weight. The percent increase in lifespan was 119.65% at same concentration. The reduced blood cells and enzyme levels also reached normal in the treated mice groups with better results in the group treated with 400 mg/kg body weight. **Conclusion:** The study forms the basis for establishing *L. aspera* as a plant with potent anticancer activity. Further studies on these lines will pave avenues for preparing an optimal formulation from the plant for therapy against cancer.

Key words: Anticancer, Ehrlich ascetic carcinoma, Ethanol extract, *Leucas aspera*, Mean survival time

INTRODUCTION

Cancer is collectively used to describe about 200 different illnesses all of which exhibit a uncontrolled proliferation of normal cells as the common characteristic. It also triggers distant cells to undergo uncontrolled proliferation induced by a host of factors including internal (genetic) and external (physical, chemical, and biological).[1] Cancer mainly manifests because of the capacity of cell itself to proliferate uncontrollably because of the induction of a series of genes such as tumor suppressors, oncogenes, or repair genes. These transform the normal cells to cancerous cells, which may remain benign or sometimes spread through to the adjacent cells and organs of the body.[2] This type of cancers is known as malignant tumors, which are the major cause of death of cancer patients.

Epidemiology suggests that with 18.1 million cancer cases in 2018, 9.6 million cases lead to death globally according to a report given by GLOBOCAN – a WHO statistical tool. Close to 71% of cancer related deaths are in the age group between 30 and 69 years.[3] By 2050, close to 24 million deaths are predicted with an increase by 25% every year. Out of all the cancer types, lung and breast cancers were most common (close to 11.6% each) which was followed by colorectal cancer (10.2%). The organs most affected were prostrate>colorectal>stomach>liver.[4,5] Although

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most cancer cases were reported in females, most mortality due to cancer was reported in males.[6] Among these patients, cancer gets detected at an early stage only in 12.5% of the patients, whereas the rest go unnoticed until the later stages.[7-9]

Therapies presently available are specific for the type of cancer and not universal. Yet, for most types of cancer, chemotherapy is administered which is a systemic treatment method. Others include immunotherapy, surgery, radiation, angiosuppressive, and hormonal therapies.[10] Although medical science has scaled great heights, treatment for cancer still remains a challenge.[5,11] Hence, a specific treatment regimen is required for every cancer type.[12] Pharmaceutical companies provide several chemotherapeutic drugs, which come along with a large amount of side effects including edema (24%), diarrhea/constipation, headache (10%), impaired renal and hepatic functions (16% and 6%, respectively), neutropenia (30%), skin issues (4%), inflammation and itching (20–25%), thrombocytopenia (10%), and several others.[13,14] These anticancer drugs augment more sufferings to the present condition and therefore better alternatives are always on the lookout. Literature shows enormous information on the use of medicinal plants with traditional knowledge for synthesis or formulation of anticancer agents with minimal adverse effects.[15-17] Plant-derived anticancer therapies have advantages of being less harmful as well as cost effective and therefore are widely accepted by all population.[18-20] *Leucas aspera* is one such weed plant available in Assam and other eastern parts of India. Literature review revealed that this plant was used traditionally for the treatment of several diseases.[21,22] Studies also indicated potential anticancer activity of the roots, bark, and leaves.[23,24] However, anticancer activity of the flower extracts has not been studied till date. Therefore, the present study aims to explore the anticancer potential of the selected plant against Ehrlich ascetic tumor.

MATERIALS AND METHODS

Plant Materials

The plant material was collected from Chikkahole village, Chamarajanagar district, Karnataka. The flowers of *L. aspera* plant were subjected to a thorough cleaning under tap water to remove impurities, shade dried, and powdered coarsely. Further, the powder was weighed and stored in airtight containers. Around 200 g of this powder was added to ethanol for the preparation of ethanol extract by Soxhlet extraction method for 24 h. After the extraction, *L. aspera* ethanol extract (*L. aspera* flower [LAE]) was filtered and concentrated using flash rotary evaporator and dried under vacuum.

Cytotoxicity Studies

In vitro cytotoxicity test was performed using Trypan blue dye exclusion method for the extract. Ehrlich ascetic carcinoma (EAC)-inoculated mouse peritoneum was used for the extraction of ascitic fluid. The fluid was washed using phosphate buffer saline (PBS) and the cell load was adjusted (using PBS) to 1×10^6 cells by observing under hemocytometer. These cells were subjected to LAE at different concentrations in a volume of 1 ml and incubated at 37°C for 3 h. Cisplatin was taken as positive control. Further, the samples were treated with 0.1 ml of Trypan blue, mixed thoroughly and the number of dead and living cells counted using a hemocytometer and the percentage viability/cytotoxicity was calculated.[25]

Antioxidant Activity

The antioxidant potential of the extract was evaluated using ferric-reducing antioxidant power (FRAP) and diphenylpicrylhydrazy (DPPH) assays. The FRAP assay was carried out as per the procedure given by Vijayalakshmi and Ruckmani.[26] Briefly, the freshly prepared FRAP reagent was warmed at 37°C before its use. Further, LAE at different concentration was mixed with the reagent, incubated for 10 min at 37°C and the absorbance was measured at 593 nm. The values obtained for absorbance at different concentrations were represented in terms of FRAP equivalent to that of 1 mM $FeSO_4$. In addition, DPPH assay was carried out as per the method given by Silva *et al*.[27]

In vivo Anticancer Studies

Animals

Healthy albino mice (8–10 weeks old) of either sex were considered for the experiment with an average weight of 25 ± 5 g, obtained from a registered breeder and housed in spacious, sterile polypropylene cages with paddy husk as bedding material. Each cage had four animals, and the animals were subjected to controlled temperature (23 ± 3°C) and humidity (50 ± 5%). The mice were fed on standard rat feed and water *ad libitum*. The study was performed as per the guidelines of IAEC, JSS Medical College, Mysuru, India.

Induction of Liquid Tumor

The peritoneal cavity of EAC-bearing mice was aspirated to obtain EAC cells 15 days after tumor induction. Microbial contaminations, if any, were evaluated by drawing the ascitic fluid into a sterile syringe using an 18-gauge needle. The viable cells/ml were evaluated from the ascetic fluid using Trypan blue and the concentration of cells per ml was adjusted at 10^7 cells per ml using PBS. Induction of ascitic tumor was carried out by injecting 2×10^6 EAC cells (0.25 ml of stock suspension) intraperitoneally to each mice. Post-24 h of tumor inoculation, treatment was initiated and continued for a period of 9 days.[28]

Study Design

The study was divided into five groups of having six animals in each group. Normal group was treated with only sodium

carboxymethyl cellulose (CMC) (0.5%), whereas control was treated with sodium CMC and standard cisplatin was administered to the standard group. LAE was administered at two doses, namely, 200 mg/kg body weight and 400 mg/kg body weight, based on the results of toxicity studies, as shown in Table 1.

Clinical Parameters

Hematological parameters such as red blood cell (RBC), white blood cell (WBC), platelets, neutrophils, lymphocytes, and hemoglobin contents were measured by collecting blood samples of the experimental mice in heparinized tubes intracardially on the 10th day after treatment.[29]

In addition, some of the biochemical parameters such as serum glutamate pyruvate transaminase (SGPT), serum glutamate oxalate transaminase (SGOT), alkaline phosphatase (ALP), serum creatinine, urea, and total protein content were also evaluated.[30,31]

Other parameters that were evaluated were as follows:

- % increase in body weight, which was taken by recording the body weight on day 1 and once in 3 days after tumor induction and calculated by the formula
- Mean survival time (MST) (MSL) and percent increase in lifespan (%ILS). MSL was obtained by taking the ratio of the total number of days survived by all animals in the group and number of animals in the group. Further, % ILS can be calculated by the formula:

$$\text{MST of ILS (\%)} = (\text{MST}_{\text{of treated group}} - \text{MST}_{\text{of control group}}) / \text{MST}_{\text{of control group}} \times 100$$

Overall, the lifespan increase by 25% or more was considered as an effective antitumor response.

Statistical Analysis

All the tests were performed in triplicates. The statistical tests used for comparisons between treatment and normal were evaluated by one-way ANOVA and Tukey's *post hoc* test. $P < 0.05$ was considered statistically significant.

RESULTS

Antioxidant Activity

The free radical scavenging ability of LAE was evaluated using FRAP and DDPH assays. The results obtained from one-way ANOVA and *post hoc* test suggested a significant increase with increase in the concentration of LAE. Highest free radical scavenging activity was observed at an extract concentration of 100 µg/ml (IC_{50} value of 1559.4 ± 0.9 and 68.52 ± 0.4 for FRAP and DPPH assay, respectively) [Table 2], which was only slightly lower than the standard ascorbic acid.

In vitro Antitumor Activity

LAE-treated EAC cells showed a concentration-dependent cytotoxic effect with an IC_{50} value of 102.142 µg/ml as against the standard cisplatin showing IC_{50} at 57.963 µg/ml [Table 3]. Overall, the activity was relatively lower than that of the standard drug cisplatin. However, it was observed that at 50 µg/ml, the activity of LAE was on par with that of cisplatin.

In vivo Anticancer Activity

Effect of LAE on body weight gain

EAC inoculated control animals demonstrated a substantial increase in body weight with a maximum gain of 67.19 ± 6.18% on day 15 compared as against that of day 0. Tumor development was remarkably noted on day 6th and continued till the end of study. The standard cisplatin (3.5 mg/kg) treatment significantly reduced body weight (−29.02 ± 2.22%) compared to the control. LAE at doses of 200 mg/kg and 400 mg/kg treatment significantly reduced the tumor-induced % increase in the body weight 13.80 ± 4.75% and 8.84 ± 1.988%, respectively, when compared to that of the control and efficacy was comparable to standard. On the 12th and 15th days, all treated groups including the standard cisplatin group significantly inhibited the percentage rise in body weight as compared to control [Table 4].

Table 1: The grouping of animals and their respective treatment with *Leucas aspera* flower extract

Groups	Grouping of animals	Treatment
Group I	Control	Sodium CMC of 0.5% (p.o.) – 15 days
Group II	EAC cells + control	Sodium CMC of 0.5% (p.o.) – 15 days 24 h post-induction of EAC
Group III	EAC cells + standard (cisplatin)	3.5 mg/kg of standard was prepared in 0.5% sodium CMC i.p (administered for 2 alternate days)
Group IV	Dose 1: 200 mg/kg of LAE+EAC cells	LAE was prepared in 0.5% sodium CMC (p.o.) – 15 days
Group V	Dose 2: 400 mg/kg of LAE+EAC cells	

CMC: Carboxymethyl cellulose, EAC: Ehrlich ascetic carcinoma, LAE: *Leucas aspera* flower extract

Effect of LAE on Body Weight Gain on MST and % Increase in Lifespan

MST of EAC inoculated analyzed mice was 21.17 ± 0.749 days. Standard cisplatin treatment at 3.5 mg/kg also significantly enhanced MST to 54.50 ± 5.94 days when compared to the control. Likewise, LAE at 200 mg/kg and 400 mg/kg significantly increased MST to 38.5 ± 1.97 and 46.5 ± 3.71, respectively, when compared to the control, which is therefore on par with the standard.

The percent % ILS of animal treated with LAE 200 mg/kg and 400 mg/kg were 81.86% and 119.65%, respectively. The efficacy of 400 mg/kg treatment was potential in enhancing lifespan of tumor-bearing animal when compared to that of the standard (cisplatin 3.5 mg/kg) which was 158% [Table 5 and Figure 1].

Table 2: Antioxidant assay of *Leucas aspera* flower extract by FRAP and DPPH methods

S. No.	Concentration in µg/ml	IC_{50} for FRAP assay	IC_{50} for DPPH assay
Ascorbic acid	-	-	24.63±0.11
LAE5	5	95.0±0.15	15.83±0.40
LAE25	25	183.10±0.30	37.10±0.50
LAE50	50	865.69±2.50	25.30±0.28
LAE100	100	1559.40±0.90	65.32±0.68

Values are expressed as Mean±SEM, *n*=3, LAE: *Leucas aspera* flower extract, FRAP: Ferric-reducing antioxidant power, DPPH: Diphenylpicrylhydrazy

Table 3: Effect of *Leucas aspera* flower extract on EAC cell line

Concentration in µg/ml	LAE	Cisplatin
10	17.70±0.61	19.50±0.18
25	28.61±1.10	34.60±0.70
50	42.40±0.13	44.50±0.38
100	58.43±1.05	75.40±0.47
200	74.21±0.48	84.30±1.76
IC_{50}	102.14±0.10	57.96±0.05

Values are expressed as Mean±SEM, *n*=3, LAE: *Leucas aspera* flower extract, EAC: Ehrlich ascetic carcinoma

Effect of LAE on Hematological Parameters

On the 10th day after tumor induction, the hematological parameters such as the total RBC, WBC, and hemoglobin content were assessed to check the effects of LAE treatment. It was noted that the total RBC count in EAC inoculated control mice (2.58 ± 0.14) was remarkably reduced in comparison with that of the normal mice (4.91 ± 0.06) as a result of the treatment. Conversely, this effect was reversed by the treatment with cisplatin 3.5 mg/kg (4.06 ± 0.09) in comparison to that of the control. Treatment with LAE at both doses elevated the total RBC count to near normal and the efficacy was comparable with standard cisplatin [Table 6]. Likewise, the extract elevated the total WBC count in EAC inoculated control mice (24.77 ± 0.729) as against that of the normal mice (9.06 ± 0.12). However, treatment with standard cisplatin at 3.5 mg/kg dose significantly reversed the elevated WBC count in the tumor-induced mice (11.23 ± 0.26) when compared with that of the control [Table 6]. Treatment with the extracts at both the concentrations significantly reduced (16.93 ± 0.2 and 15.14 ± 0.2) the WBC count showing similar results as that of the standard. In addition, a significant reduction in hemoglobin level was also observed in EAC inoculated control (8.30 ± 0.15) mice as compared to that of the normal mice (14.02 ± 0.14). Standard cisplatin treatment at a dose of 3.5 mg/kg significantly reversed the tumor-induced reduction in hemoglobin level to 13.28 ± 0.15

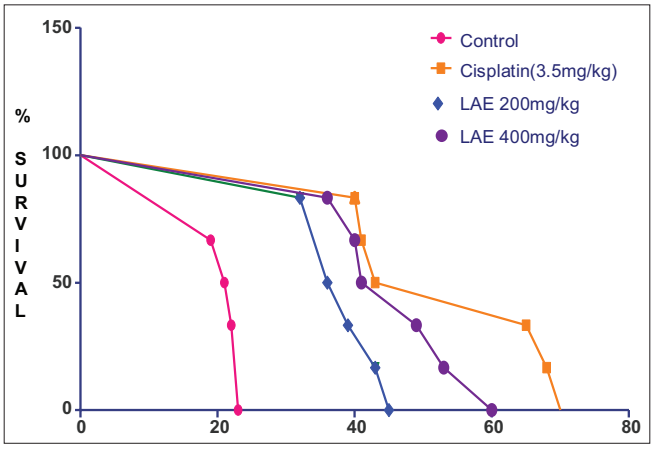

Figure 1: Kaplan–Meier estimate of survival of Ehrlich ascetic carcinoma inoculated mice after treatment with *Leucas aspera* flower extract and standard cisplatin. Values are expressed as Mean±SEM, *n*=6

Table 4: Effect of *Leucas aspera* flower extract on body weight changes in EAC inoculated mice

Days	3	6	9	12	15
Control	7.05±2.35	26.38±1.58	34.70±1.36	46.33± 2.72	68.90 ±5.18
Standard cisplatin (3.5 mg/kg)	5.70±2.45	1.15±2.77[a]	1.33±3.74[a]	-11.74±2.66[a]	-29.02±2.22[a]
LAE 200 mg	6.80±2.17	23.57±2.376[b]	29.41±2.017[b]	19.22±2.31[ab]	13.80±4.08[ab]
LAE 400 mg	4.26±1.30	10.79±1.677[a]	23.75±2.14[b]	17.11±1.99[ab]	8.84±1.98[ab]

*Values are expressed as MEAN±SEM, *n*=6. The mean % of body weight was compared to day 0. The data were analyzed by one-way ANOVA and *post hoc* Tukey's multiple comparison tests ([a]*P*<0.05: Compared with control; [b]*P*<0.05: Compared with standard). LAE: *Leucas aspera* flower extract, EAC: Ehrlich ascetic carcinoma

when compared to control. LAE treatment at both the doses also caused a significant increase in hemoglobin content compared to control [Table 6].

Effect of LAE Extract on Biochemical Parameters in EAC Inoculated Mice

The effects of the extract on biochemical parameters such as SGOT, SGPT, ALP, creatinine, serum urea, and total protein content were evaluated by assessing their levels in all the treatment groups on the 10th day of tumor induction. The serum SGOT level was remarkably elevated in EAC inoculated control mice (82.00 ± 3.055) in comparison with that of the normal animal (42.00 ± 1.36). Cisplatin at 3.5 mg/kg body weight remarkably nullified the tumor-induced increase in the SGOT level (52.67 ± 1.606). LAE at both doses significantly decreased the elevated SGOT level compared to control. A significant increase in serum SGPT level was also observed in EAC inoculated control mice (95.17 ± 2.040) when compared to normal animal (64.33 ± 2.076), which was significantly reduced by cisplatin at a dose of 3.5 mg/kg (70.33 ± 1.25) as well as both the doses of LAE when compared with that of the control. Similarly, elevated levels of ALP (189.3 ± 1.282) when compared to normal animal (126.2 ± 2.197) were also decreased by the treatment with standard cisplatin treatment at a dose of 3.5 mg/kg and LAE at both doses. Standard cisplatin 3.5 mg/kg induced toxicity was manifested by significant increase in serum creatinine (2.55 ± 0.17) when compared to normal (0.717 ± 0.079). LAE dose at 200 mg/kg significantly increased (1.72 ± 0.16[a]) when compared to normal. LAE dose at 400 mg/kg significantly decreased (1.37 ± 0.13) when compared to that of the standard cisplatin. Likewise, the increased level of urea (58.01 ± 1.99) was seen in the cisplatin-treated EAC-induced mice when compared to that of the normal (22.85 ± 1.07). LAE both the doses (36.13 ± 2.61 and 32.57 ± 1.20) also significantly decreased when compared to standard cisplatin [Table 7]. Furthermore, the total protein content was also significantly decreased by LAE treatment.

Table 5: Effect of *Leucas aspera* flower extract (LAE) on mean survival time and % increase in life span

Treatment	MST	%ILS
Control	21.17±0.75	-
Cisplatin (3.5 mg/kg)	54.50±5.94[a]	157.94%
LAE 200 mg/kg	38.50±1.97	81.86%
LAE 400 mg/kg	46.50±3.71	119.65%

*All the values are expressed as Mean±SEM, *n*=6. The data were analyzed by one-way ANOVA and *post hoc* Tukey's multiple comparison tests ([a]*P* < 0.05: Compared with control; [b]*P*<0.05: Compared with standard). LAE: *Leucas aspera* flower extract, MST: Mean survival time, %ILS: Percent increase in lifespan

DISCUSSION

Several plants have been screened as rich antitumor agents and their anticancer abilities are well documented in many scientific literatures.[32] It is well established that oxidative free radicals are carcinogenesis promoting agents and several studies have proved that plants with potential antioxidant principles also possess antitumor activity.[33] Therefore, *L. aspera*, a folklore plant, with reported antioxidant properties was taken in our study.[34,35] In the present study, the *in vitro* and *in vivo* anticancer efficacy of LAE was exploited. The use of cell lines and implanted tumor models in animals is among the few sophisticated techniques in screening of prospective cancer agents. However, all the extracts cannot be subjected to such expensive techniques, and therefore, the use of some of the *in vitro* screening models such as brine shrimp lethality bioassay, Trypan blue exclusion assay, sulforhodamine B assay, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay is still widely used in the preliminary cytotoxicity screening. Several chemical agents including plant extracts are still tested on appropriate transplantable animal models such as mice for the preliminary studies on drug discovery. Therefore, in our study, the *in vitro* and *in vivo* effects of LAE were evaluated using transplantable tumor model Ehrlich's ascites carcinoma in mice.

The extract was subjected to a preliminary phytochemical analysis to ascertain the previously obtained results of phytochemical screening. In this present study, LAE showed a relatively high percentage phenolic acid (5.4 mg/ml) content. The preliminary phytochemical screening revealed that the n-hexane, ethyl acetate, and ethanol extracts

Table 6: Effect of *Leucas aspera* flower extract on total RBC, WBC, and HB% in EAC inoculated mice

Treatment	RBC (1×10^6 cells/mm^3)	WBC (1×10^3 cells/mm^3)	Hb (g %)
Normal	4.91±0.07	9.03±0.12	14.02±0.14
Control	2.52 0.15[a]	24.71±0.73[ac]	8.31±0.15[ac]
Cisplatin (3.5 mg/kg)	4.04±0.09[ab]	11.24±0.26[ab]	13.21±0.15[ab]
LAE (200 mg/kg)	3.36±0.16[abc]	16.93±0.27[abc]	11.45±0.20[abc]
LAE (400 mg/kg)	3.82±0.12[ab]	15.14±0.26[abc]	12.17±0.17[ab]

*All the values are expressed as Mean±SEM, *n*=6. The data were analyzed by one-way ANOVA and post hoc Tukey's multiple comparison tests ([a]*P*<0.05: Compared with normal; [b]*P*<0.05: Compared with control; [c]*P*<0.05: Compared with standard). LAE: *Leucas aspera* flower extract, RBC: Red blood cell, WBC: White blood cell

Table 7: Effect of *Leucas aspera* flower extract on SGOT, SGPT, ALP, creatinine, serum urea, and TPC in EAC inoculated mice

Treatment	SGOT	SGPT	ALP	Creatinine	TPC	Urea
Normal	42.00 ±1.36	64.33±2.07	126.20±2.19	0.72±0.08	6.80±0.29	22.85±1.07
Control	82.00±3.05^{ac}	95.15±2.04^{ac}	189.30±1.28^{ac}	1.94±0.13a	12.89±0.42^{a,c}	48.13±2.34^{ac}
Cisplatin (3.5 mg/kg)	52.67±1.60^{ab}	70.41±1.25^{b}	130.00±2.34^{b}	2.55±0.17a	7.45±0.25^{b}	58.01±1.99^{ab}
LAE 200 mg/kg	75.13±1.84^{ac}	82.15±1.94^{abc}	141.10±1.38^{abc}	1.72±0.16^{a,c}	10.16±0.38^{a,b,c}	36.13±2.61^{abc}
LAE 400 mg/kg	64.98±2.08^{abc}	76.97±1.34^{ab}	135.98±2.36^{b}	1.37±0.13^{a,c}	9.37±0.43^{a,b,c}	32.57±1.20^{abc}

*All the values are expressed as Mean±SEM, *n*=6. The data were analyzed by one-way ANOVA and *post hoc* Tukey's multiple comparison tests (^{a}P<0.05: Compared with normal; ^{b}P<0.05: Compared with control; ^{c}P<0.05: Compared with standard). LAE: *Leucas aspera* flower extract, SGPT: Serum glutamate pyruvate transaminase, SGOT: Serum glutamate oxalate transaminase, ALP: Alkaline phosphatase, TPC: Total protein content

demonstrated a wide variety of phytochemicals which further ascertained that these extracts were rich in antioxidant properties. Among these extracts, ethanol extract was proved to be the best in terms of antioxidant activity. In addition, the previous studies also reported that the extract contained profound phytochemicals including flavonoids and alkaloids that are responsible for the aforementioned activity.[36,37] The results from this study as well as previous findings led to the bioactivity guided isolation and characterization of leading compounds in due course.

Our study also demonstrated a remarkable antioxidant activity identified by the DPPH assay, which showed potential similar to that of the standard ascorbic acid. A study done by Ramu *et al*.[38] demonstrated that the inhibition exhibited by the ethanol extract on DPPH was potential than other solvent extracts. In our study also, the crude ethanolic extract exhibited highest DPPH radical scavenging activities (70.6%) inhibition at highest concentration. Thus, it could be suggested that LAE altered the intracellular redox state, thereby contributing to enhance the antitumor activity. The previous studies have reported that extracts obtained from plant sources with potential antioxidant capacity proved beneficiary for antitumor activity in experimental animals.[32]

In preliminary cytotoxic screening, considerable cytotoxicity of plant extracts was observed in Trypan blue assay. The LAE showed promising activity as compared to cisplatin. A dose-dependent cytotoxic activity was observed with an IC_{50} of 102 and 142 µg/ml. On the basis of this cytotoxicity study, LAE was taken forward to assess its role *in vivo*. Ascitic tumor study showed that LAE lowered the tumor volume and live cell count as compared to that of the tumor control group along with a corresponding increase in the number of dead cells. The study reveals that LAE could directly affect the tumor cells by exerting cytotoxicity or indirectly by leading to a more local effect which in turn leads to the activation of macrophages and then cell death.[39] Studies have shown that EAC implantation as such leads to a local inflammatory response, along with an elevated vascular permeability, leading to intense edema, cellular migration, and a progressive ascitic fluid formation. This fluid furnishes all the necessary nutrients to the cell and therefore essential for tumor growth.[40,41]

In this ascites tumor model, because of a rapid accumulation of the tumor cells, the body weight increased substantially in the control mice-bearing EAC. There was a remarkable reduction of body weight in the LAE-treated mice at higher dose in comparison to that of the lower doses indicating the inhibition of tumor cell progression. MST of the tumor-bearing mice was elevated by LAE treatment dose dependently and this enhancement was better in 400 mg/kg body weight. The percent increase in lifespan of tumor-bearing mice, following treatment with LAE 400 mg/kg, was 119.65%. However, the lifespan of tumor-bearing mice was least affected by the LAE at lower dose. It is well known that for assessing optimal anticancer efficacy of any therapy, prolongation of lifespan is one of the most important and reliable criteria.[41] In this regard, any enhancement of lifespan over 25% that of the control was considered as effective antitumor response. In the present study, LAE meets these criteria and thus can be considered potent anticancer formulation.

Hematological data of tumor control group showed that animals were suffering from loss of RBCs, severe anemia, and accumulation of WBCs. The reduced RBCs and/or hemoglobin is primarily responsible for anemia observed in the EAC mice in the present study. This condition is probably because of deficiency of iron or hemolytic or myelopathic conditions.[42] It was found that LAE therapy restored the RBC and Hb level in the treated mice. The remarkable elevation of WBC in EAC-induced group is probably because of the defense exerted by the animals against cancer-causing cells.[43] As the tumor development was reduced by LAE treatment, the WBC count also become normal. Ascites carcinoma often exhibits symptoms such as myelosuppression and anemia. In our study, the EAC control mice exhibited high WBC count, along with a corresponding reduction in the hemoglobin and RBC count. Anemia (reduced hemoglobin) observed in this carcinoma is a resultant of iron deficiency, either by hemolytic or myelopathic conditions. All chemotherapies target the blood cells as well because of which it leads to myelosuppression and anemia. The highest concentration

of LAE (400 mg/kg) in our study led to the reversal of hematological alterations (such as reduced WBC count that was elevated due to the cancer induction and a concomitant increase in t-RBC and hemoglobin count) were observed in our study. The serum enzyme levels SGPT, SGOT, ALP, serum creatinine, total protein, and serum urea were also restored to basal levels indicating that the extract was not toxic to the other cells. Most tumor cells are known to produce reactive oxygen species therefore resulting in causing oxidative stress. This, in turn, induces mutation in cancer cells causing it to activate the redox pathways along with the release of pro-survival factors, namely, NF-αB and AP-1.[44] A great deal of studies report that elevated phenol content in plants indicates its potential antitumor and antioxidant attributes.[45] Since the ethanolic extract of LAE showed similar anticancer activity as that of standard, the mechanism of action may be because of the potent antioxidant activity of the extracts. However, these assumptions need to be furthered by additional research.

CONCLUSION

The quest for an ideal antitumor agent along with limited side effects is an ever-demanding area. On these lines, in the present study, the possible antitumor activity of flowers of *L. aspera* in various *in vitro* and *in vivo* models was evaluated. LAE showed promising results in increasing the lifespan, which is an able indicator of anti-tumor activity. Alongside, the extract also showed improvement in the blood cells and biochemical parameters. These results thus suggest the potential efficacy of *L. aspera* against tumor development. This is the first study on the anticancer activity of the flower extract of this plant and thus requires further research to establish it as a medicine in itself.

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