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Research Article

Evaluation of Antitumor Potential of *Luffa acutangula* on Ehrlich's Ascites Carcinoma Treated Mice

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ABSTRACT

The objective of the present study is to explore the anticancer activity of the ethanolic and aqueous extracts of the *Luffa acutangula* in Swiss albino mice against Ehrlich Ascites Carcinoma (EAC) cell line. Anticancer activity of ethanolic and aqueous extracts of *Luffa acutangula* was evaluated in EAC Swiss albino mice at the doses of 200 and 400 mg/kg body weight orally. Both extracts at both doses were administered for 13 consecutive days. After 24 h of the last dose and then eighteen hours of fasting, the mice were sacrificed and antitumor effect of ethanolic and aqueous extracts was assessed by evaluating tumor volume, viable and nonviable tumor cell count, tumor weight and hematological parameters of EAC bearing host. Ethanolic and aqueous extracts showed significant decrease in ($p<0.0001$) tumor volume, viable cell count, tumor weight and elevated the life span of EAC tumor bearing mice. Haematological profiles such as red blood cell (RBC), haemoglobin, and white blood cell (WBC) count reverted to normal level in treated mice. The results demonstrated that the extract has potent dose dependent anticancer activity comparable to that of cisplatin. Aqueous extract at both doses (200 and 400 mg/kg) and ethanolic extract at 400 mg/kg dose showed potent anticancer activity.

Keywords: *Luffa acutangula*, EAC cell line, anticancer activity, cisplatin.

INTRODUCTION

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. They constitute the second cause of mortality behind cardiovascular diseases in developed countries and the third after infectious and cardiovascular diseases in developing countries[1,2]. Natural products have been the mainstay of cancer chemotherapy for the past 30 years[3]. However, most of the currently used anticancer drugs cause undesirable side effects due to lack of tumor specificity and multidrug resistance. Therefore the search for potent, safe and selective anticancer compounds is crucial for new drug development in cancer research. Natural products, due to their structural diversity, provide excellent templates for the construction of novel compounds[3,4]. It is well established that plants have been a useful source of clinically relevant antitumor compounds[5]. Several studies have been conducted on herbs under a multitude of ethnobotanical grounds. Plant secondary metabolites and their semi-synthetic derivatives continue to play an important role in anticancer drug therapy[6]. These include vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide, derived from epipodophyllotoxin and paclitaxel (taxol). Sixty percent of currently used anticancer agents are derived in one way or another from natural sources[7]. One such plant, *Luffa acutangula*, (Family: Cucurbitaceae), commonly known as Ridge gourd and tiroi, is a large monoecious, annual climber, found wild and also cultivated throughout the greater parts of India. It contains crystalline bitter principle similar to cucurbitacin B, luffin, and colocynthin[8]. Seeds show presence of saturated and unsaturated fatty acid palmatic, stearic, oleic, linoleic and traces of lignoceric acid while fruits contain cucurbitacin B, E and oleanalic acid. The ancient literature also revealed that the plant is significantly used as abortifacient and antifungal agent[9]. Antioxidant activity of *Luffa acutangula* has been reported[10] and leaf extracts of *Luffa acutangula* exhibits high antiproliferative activity against various cell line as determined with MTT assay[11]. In context with the important phytochemical and therapeutic findings, it was considered worthwhile to assess the anticancer properties of fruit extract of *Luffa acutangula* against Ehlrich Ascites Carcinoma in Swiss albino mice.

MATERIAL AND METHODS

Collection of plant and preparation of extracts

Fruit of *Luffa acutangula* was purchased from market of Udaipur, authenticated and a voucher specimen has been deposited in the Department of Pharmacognosy, B. N. Institute of Pharmaceutical Sciences (Udaipur, India).

Ethanolic extract

The fruits were shade dried, powdered and about 100 g of powder was extracted with ethanol by hot extraction process (soxhlet) for 72 h. After completion of the extraction the solvent was recovered by distillation and concentrated *in vacuo*.

Aqueous extract (Chloroform: water-1:99)

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Table 1: Effect of different extracts on Mean survival time (MST) and percent increase in life span (%ILS) in EAC inoculated mice

Treatment	MST(Days)	%ILS
Control	16.16 ± 0.744	-
Standard (3.5 mg/kg)	40.66 ± 1.012***	151.60 ± 0.744***
Ethanolic (200 mg/kg)	20.83 ± 0.112**	28.89 ± 0.124**
Ethanolic (400 mg/kg)	22.70 ± 0.880**	40.47 ± 1.14**
Aqueous (200 mg/kg)	25.80 ± 0.762***	59.65 ± 0.318***
Aqueous (400 mg/kg)	28.92 ± 0.298***	78.96 ± 0.526***

*All the values are mean ± SEM of 10 animals, ***P < 0.0001 compared to control

Table 2: Effect of various extracts on tumor volume

Treatment	Tumor volume (ml)
Control	5.66 ± 1.203
Standard (3.5 mg/kg)	0.75 ± 0.144***
Ethanolic (200 mg/kg)	3.71 ± 0.320**
Ethanolic (400 mg/kg)	2.90 ± 0.142***
Aqueous (200 mg/kg)	2.28±0.124***
Aqueous (400 mg/kg)	1.87±0.214***

*All the values are mean ± SEM of 10 animals, **P < 0.01, ***P < 0.0001 compared to control.

Table 3: Effect of various extracts on tumor weight

Treatment	Tumor weight (g)
Control	6.79 ± 0.320
Standard (3.5 mg/kg)	0.90 ± 0.172**
Ethanolic (200 mg/kg)	4.10 ± 1.145*
Ethanolic (400 mg/kg)	3.52 ± 0.630**
Aqueous (200 mg/kg)	3.19 ± 0.214**
Aqueous (400 mg/kg)	2.12 ± 0.820***

*All the values are mean ± SEM of 10 animals, *P < 0.05, **P < 0.01, ***P < 0.0001 compared to control

The fruits were shade dried, powdered and macerated with chloroform water for seven days.

Chemicals

Sodium chloride, propylene glycol, tryphan blue, methyl violet, sodium sulphate, methylene blue (Merck Limited, Mumbai, India), cisplatin (Sigma Aldrich, USA). All other chemicals and reagents used were of pure analytical grade.

Animals

Male Swiss albino mice weighing 22-28 g were used in the experiment. They were obtained from animal house of B. N. Institute of Pharmaceutical Sciences, Udaipur and were acclimatized to the experimental room having temperature 23±2 °C, controlled humidity conditions and 12-h light - dark cycle. Animals were caged in poly acrylic cages (38 x 23 x 10 cm) with maximum of four animals per cage. The mice were fed with standard food pellets and water ad libitum. Before commencement of the experiment the mice were acclimatized to laboratory conditions for 7 days. All procedures described were reviewed and approved by the Animal Ethical Committee and study was conducted after obtaining ethical committee clearance (Clearance certificate No. 100/LSC/BNCP-12/IAEC).

Acute Toxicity Study

Acute toxicity study was carried out for ethanolic and aqueous extracts of *Luffa acutangula* according to the method described by Litchfield and Wilcoxon, 1949 using male Swiss albino mice orally[12]. The LD_{50} values were found to be 4 g/kg body weight respectively.

Transplantation of Tumor

The EAC induced mice were originally obtained from the animal house of B. N. Institute of Pharmaceutical Sciences, Udaipur. The EAC cells propagated for 12- 14 days were used in experiment. The ascitic fluid from mice was drawn using an 18 gauge needle into sterile syringe and was tested for microbial contamination. Tumor viability was determined by Tryphan blue exclusion test and cells were counted using Haemocytometer. The ascitic fluid was suitably diluted in normal saline to get a concentration of 10 x 10^6 cells/mL of tumor cell suspension. From this stock suspension 0.25 mL (2.5 x 10^6 cells/mice) was injected intraperitonially (i.p.) to obtain ascitic tumor.

Treatment Schedule

140 Swiss albino mice were used in the experiment, which were divided into seven groups (n=20), they were fed with food and water ad libitum. All the animals in each groups received EAC Cells (2.5 x 10^6 cells/mouse i.p.) except Group-I. This was taken as day '0'. Group-I animals served as normal saline control (5 mL/kg i.p.) and group-II animals served as EAC control without any drugs. 24 h after EAC transplantation, groups-III and IV animals received ethanolic extract of *Luffa acutangula* at a dose of 200 and 400 mg/kg orally, groups-V and VI animals received aqueous extract at a dose of 200 and 400 mg/kg orally for 13 alternative days, respectively. Group-VII animals received reference drug cisplatin (3.5 mg/kg i. p) on the first day[13]. After 24 h of the last dose and then 18 h of fasting, ten animals of each group were sacrificed by cervical dislocation to measure tumor volume, tumor weight, cell viability and haematological parameters and the rest of the animals were kept with food and water ad libitum to check percentage increase in life span (% ILS) of the tumor host.

Evaluation of the Antitumor Activity

The antitumor activity of the ethanolic and aqueous extracts of *Luffa acutangula* was measured in EAC animals using the following parameters:

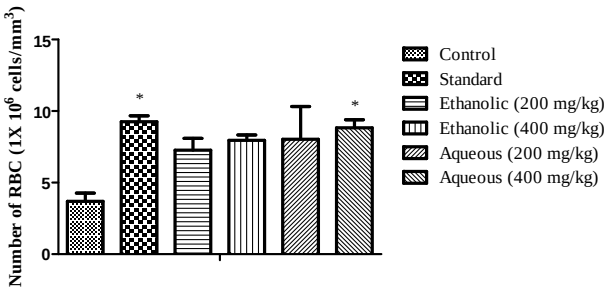

Fig. 1A: Effect of various extracts on RBC count

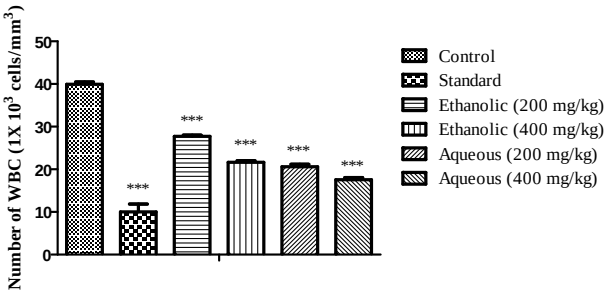

Fig. 1B: Effect of various extracts on WBC count

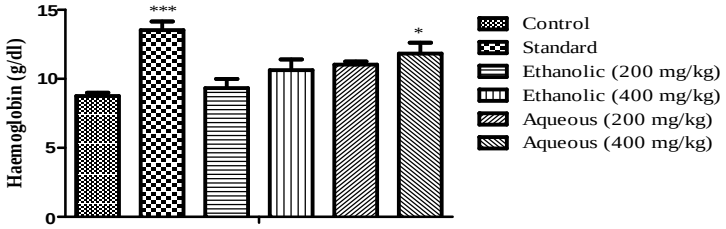

Fig. 1C Effect of various extracts on Haemoglobin count
*All the values are mean ± SEM of three animals, *P < 0.05, **P < 0.01, ***P < 0.0001 compared to control.

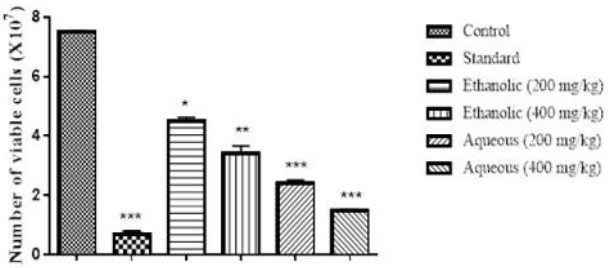

Fig. 2A: Effect of various extracts on viability of cells*All the values are mean ± SEM of 10 animals, ***P < 0.0001 compared to control

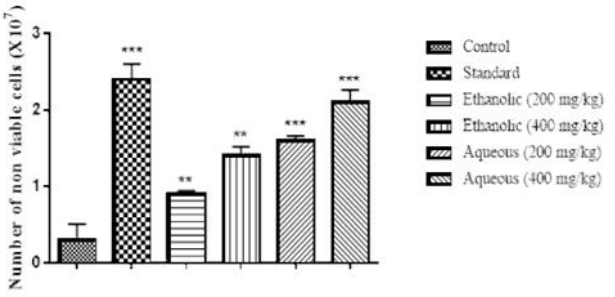

Fig. 2B: Effect of various extracts on non-viable cells *All the values are mean ± SEM of 10 animals, **P < 0.01, ***P < 0.0001 compared to control.

(1) Tumor volume: The ascitic fluid was collected from the peritoneal cavity of Swiss albino mice and the volume was measured by using a graduated centrifuge tube.
(2) Tumor weight: The tumor weight was measured by taking the weight of the mice before and after the collection of the ascitic fluid from peritoneal cavity.
(3) Percentage increase in life span: The effect of ethanolic and aqueous extracts of *Luffa acutangula* on percentage increases in life span (% ILS) of the animals was calculated on the basis of mortality of the experimental mice.
%ILS = Mean survival time (MST) of treated group- MST of control group / MST of control group x 100,
MST= Mean survival time
Mean survival time* = [First Death + Last Death]/2,
*Time denoted by days.
(4) Tumor cell count: The ascitic fluid withdrawn from the peritoneal cavity of the mice was taken with a WBC pipette and diluted 100 times with normal saline. A drop of the diluted cell suspension was placed on the Neubauer's counting chamber and the numbers of cells in the 64 squares were counted.
(5) Viable/ nonviable tumor cell count by tryphan blue assay: The viability and nonviability of the cells were checked by tryphan blue assay. The cells were stained with tryphan blue (0.4 % in normal saline) dye. Upon staining, the viable cells did not take the stain while the non viable cells were stained blue.
Cell count = Number of cells x dilution factor / Area x thickness of liquid film.
(6) Blood parameters: At the end of the experimental period, the next day after an overnight fasting, blood was withdrawn from the retro-orbital plexus and used for the estimation of haemoglobin (Hb) content, red blood cell (RBC) count and white blood cell (WBC) count by using an automatic analyzer (ERMA INC Tokyo, - PEC-21 OVET).

Statistical Analysis

Statistical significance (p) was calculated by one-way ANOVA between the treated groups and the EAC control group followed by Dunnett's post hoc test of significance

where, $p<0.05$, $p<0.01$ and $p<0.0001$ considered being significant, very significant and highly significant, respectively. All datas are expessed as mean ± S.E.M (n=10 mice per group).

RESULTS

The ethanolic and aqueous extracts of *Luffa acutangula* at the doses of 200 and 400 mg/kg body weight, when administered orally, elevated the MST and life span of EAC tumor bearing mice (ILS) (Table 1). The haematological profile such as RBC count and haemoglobin content increased (Fig. 1A and 1C) but the WBC count was decreased as compared to that of EAC control (Fig. 1B). The extracts showed increased nonviable cell count (Fig. 2B) and decreased viable cell count (Fig. 2A), tumor volume (Table 2) and tumor weight (Table 3) when compared to that of EAC control mice.

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