



ANTICANCER STUDIES ON ETHANOL EXTRACT OF *IMPATIENS BALSAMINA*

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ABSTRACT

Ethanol Extract of *Impatiens balsamina* was investigated for anticancer and *in-vitro* cytotoxic activities against transplantable tumors and human cell line. *In vitro* cytotoxicity was evaluated in Hela and NIH3T3 cells by MTT assay and *in-vivo* antitumor activity with Dalton's ascites lymphoma (DLA) tumor bearing mice. Activity was measured by monitoring the Cancer Cell number, packed cell volume and increase in life span. Hematological parameters were evaluated and compared with the respective controls. The extract exhibited strong *in vitro* cytotoxicity against Hela cell line, it was found to be safe with normal cell. EEIB at the doses 200 and 400 mg/kg significantly increase the life span and decrease in the cancer cell number and body weight ($P<0.01$) and exert a protective effect on the hemopoietic system. The results showed significant antitumor and cytotoxic effects against DLA and human Cancer Cell line and support the ethno-medical use of *Impatiens balsamina* in cancer therapy

Key words: *Impatiens balsamina*, Dalton's Lymphoma, Anticancer activity, 5-Fluorouracil.

INTRODUCTION

Over the past few years, cancer has remained a major cause of death and the number of individuals affected with cancer is continuing. Hence, a major portion of the current pharmacological research is devoted to anticancer drug design customized to fit new molecular targets¹. Due to enormous propensity of plants, which synthesize a variety of structurally diverse bioactive compounds, the plant kingdom is a potential source of chemical constituents with antitumor and cytotoxic activities². The rich and diverse plant sources of India are likely to provide effective anticancer agents. One of the best approaches in the search of anticancer agents from plant sources is the selection of plants based on ethnomedical leads³.

Impatiens balsamina (Balsaminaceae) is distributed in the tropical and sub-tropical part of India. The Annual herb of *Impatiens balsamina* is used as emetic, cathartic, diuretic, in Hawaii island. It is used as anticancer. Flower is used as cooling, tonic and antiseptic⁴. Many compounds have been reported from the genus *Impatiens*. Previous phytochemical investigations with *I. balsamina* revealed the occurrences of Apigenin, flavonoids, naphthaquinone, glycosides, kaempferol 3 – rhamnosyl glycoside, kumarins.^{5,6}

MATERIALS AND METHODS

Extraction

The whole plant of *Impatiens balsamina* were collected from the surrounding areas of trichy in the month of July 2010. The plant was authenticated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Coimbatore, Tamilnadu, India. A voucher specimen is preserved in our laboratory for future reference (Voucher No.bsi/sc/5/23/10-11). The plant material was shade dried, pulverized and extracted (500 g) with 80% ethanol at room temperature for 72 hour. The extract was filtered and concentrated to dryness under reduced pressure and

controlled temperature (40°C to 50°C) in a rotary evaporator. The extract was dark, yellowish brown and solid, preserved in vacuum desiccators until further use.

Preliminary Phytochemical Screening

The extract was screened for the presence of various phytochemical constituents⁷. The presence of steroids, alkaloids, tannins, flavonoids, glycosides confirmed using standard tests.

Tumor Cells and Inoculation

Normal Mouse Embryonic Fibroblast (NIH 3T3), Human Cervical Cancer Cells (HeLA), were obtained from National Centre of Cell Sciences (Pune, India). The cultures were maintained in Dulbecco's modified eagles medium (DMEM) containing 10% inactivated calf serum and were grown in 25cm² tissue culture flasks (Tarson Products Ltd, Kolkata, India) until confluent and used for cytotoxic assays. DLA cells were supplied by Amala Cancer Research Centre (Trissur, Kerala, India). The cells were maintained *in vivo* in Swiss albino mice by intraperitoneal transplantation. Tumor cells aspirated from the peritoneal cavity of mice were washed with normal saline and were used for further studies.

Preparation of Suspension and Solutions

For cytotoxicity assays, the extract was dissolved in dimethyl sulfoxide (DMSO) and the volume made up to 10ml to obtain a 1000µg/ml stock solution. Serial two-fold dilutions were made using DMSO to get lower concentration. EEIB was suspended in distilled water using sodium carboxy methyl cellulose (CMC, 0.3%) and administered orally to the animals with the help of an intragastric catheter to study *in vivo* antitumor activity.

In vitro Cytotoxicity Studies on Human Cancer Cell Lines

Stock cells of Normal Mouse Embryonic Fibroblast (NIH 3T3), Human Cervical Cancer Cells (HeLA), were cultured in RPMI-1640 and DMEM supplemented with 10% fetal bovine serum, penicillin (100IU/ml) and streptomycin (100µg/ml) in a humidified atmosphere of

5% CO₂ at 37°C until confluent. The cells were dissociated with 0.2 % trypsin and 0.02% EDTA. The cytotoxic assay was carried out by adding 0.1 ml of cell suspension containing 10,000 cells to each well of a 96-well microtitre plate (Tarson, Kolkatta, India) and fresh medium containing various concentrations of extract was added at 24 h after seeding. Control cells were incubated without the extract and with DMSO. The microtitre plates were incubated at 37°C in a humidified atmosphere with 5 % CO₂ for a period of 72 h. The percentage cytotoxicity and IC₅₀ were determined by the standard MTT assay method⁸.

Animals

Healthy male Swiss albino mice weighing 25±2g were obtained from Venkateshwara Enterprises, Bangalore, India. The mice were grouped and housed in polypropylene cages and maintained under standard conditions (25±2°C) with 12 h dark/light cycle. The animals were fed with standard animal pellet diet and water ad libitum. The experiment protocols received clearance from the Institutional Animal Ethical Committee (IAEC) and CPCSEA, Chennai, India. (Proposal NO P.col/62/2011/IAEC/VMCP).

Acute Toxicity Studies

Acute toxicity study on ethanol extracts of whole plant of *Impatiens balsamina* carried out in Swiss albino mice using the OECD guidelines 423.

Antitumor Studies

Swiss Albino mice were divided in to five group of six each. All the animals in four groups were injected with DLA cells (1x10⁶ cells per mouse) intraperitoneally, and the remaining one group is normal control group. Group 1 served as the normal control. Group 2 served as the tumor control. Group 1 and 2 receives normal diet and Water. Group 3 served as the positive control, was treated with injection fluorouracil at 20 mg/kg body weight, intraperitoneally. Group 4 served as a treatment control group and was administered ethanolic Extract of *Impatiens balsamina* (EEIB) at a dose of 200mg/kg through orally. Group 5 served as a treatment control group and was administered ethanolic extract of *Impatiens balsamina* (EEIB) at a dose of 400 mg/kg orally. In this study, drug treatment was given after the 24 hrs of inoculation once daily for 14 days. On day 14, after the last dose, all mice from each group were sacrificed; the blood was withdrawn from each mouse by retro orbital plexus method and the following parameters were checked. Tumor cell count, Packed cell volume, Percentage increase of life span, Hemoglobin, RBC, WBC, platelets, Cholesterol, Aspartate amino transferase, Alanine amino transferase, Alkaline phosphatase, Tri glycerides and body weight.⁹

Table 1: Effect of EEIB and on the life span, body weight and cancer cell count of tumor induced mice

Treatment	Number of animals	% ILS Life span	Body weight grams	Cancer cell count; ml X 10 ⁶
G ₁	6	>30 days	2.22±0.50	-
G ₂	6	52%	7.85±1.10 ^{a**}	2.62±0.32 ^{a**}
G ₃	6	90%	3.70±0.55 ^{b**}	1.37±0.31 ^{b**}
G ₄	6	72%	6.55±0.80 ^{b*}	2.05±0.47 ^{b*}
G ₅	6	76%	6.25±0.72 ^{b*}	1.90±0.32 ^{b*}

All values are expressed as mean ± SEM for 6 animals in each group.

The Data was evaluated using one way ANOVA followed by Newman Keuls multiple range test.

**a – Values are significantly different from control (G₁) at P < 0.001, *b – Values are significantly different from cancer control (G₂) at P < 0.01

**b – Values are significantly different from cancer control (G₂) at P < 0.001

Table 2: Effect of EEIB on Hematological parameters

Treatment	Total Wbc Cells/Mlx10 ³	Rbc Count Mill/Cumm	Hb Gm/Dl	PCV %	Platelets Lakhs/Cumm
G1	9.98 ±1.30	4.24±0.99	12.35 ±2.30	14.60±2.70	3.20±0.74
G2	14.25 ±2.60 ^{a**}	2.40±0.45 ^{a**}	7.30 ±0.88 ^{a**}	31.50±3.60 ^{a**}	1.65±0.60 ^{a**}
G3	11.45 ±1.90 ^{b**}	3.98±0.88 ^{b**}	11.3 ±1.75 ^{b**}	19.30±2.55 ^{b**}	2.68±0.55 ^{b**}
G4	12.40±2.60 ^{b*}	3.25±0.60 ^{b*}	9.95±1.30 ^{b*}	23.45±2.90 ^{b*}	2.15±0.50 ^{b*}
G5	12.05 ±2.14 ^{b*}	3.52±0.70 ^{b*}	10.32±1.55 ^{b*}	21.32±2.23 ^{b*}	2.28±0.58 ^{b*}

RESULTS AND DISCUSSION

The preliminary phytochemical screening revealed that the extract contains glycosides, alkaloids, saponins, flavonoids, phenolic compounds and terpenoids.

The IC₅₀ of EEIB was found to be 33.7 µg/ml for HELA cells and 49.6 µg/ml for NIH 3T3 cells. In MTT assay, the percentage cytotoxicity progressively increased in a concentration dependent manner. The IC₅₀ of EEIB was found to be less than 100 µg/ml against all the human cancer cell lines used. However the IC₅₀ values against the normal mouse embryonic fibroblast (NIH3T3) were found to be very high when compared to that of cancer cell lines. This indicated that EEIB possess selective cytotoxicity against the cancerous cell lines, but is safer towards the normal cells. In acute toxicity studies,

animals treated with EEIB did not show any toxic symptoms or mortality when dosed up to 2000 mg/kg body weight by oral route. This indicated that the extract was found to be safe at the tested dose level. Hence 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for the *in vivo* studies.

In the DLA tumor control group, the average life span of animal was found to be 52% where as EEIB at the dose of 200 mg/kg and 400mg/kg body weight increase the life span to 72% and 76% respectively. These values were significant. However the average life span of 5- FU treatment was found to be 90%, indicating its potent antitumor nature. The antitumor nature of EEIB was evidenced by the significant reduction in percent increase in body weight of animal treated with EEIB at the dose of

200 mg/kg and 400mg/kg body weight when compared to DLA tumor bearing mice. It was also supported by the significant reduction in packed cell volume and viable Tumor cell count in both the extent of treatment when compared to the DLA tumor control. (Table 1)

Effect of hematological parameter as shown in (Table 2) RBC, Hgb, Platelets were decreased and WBC count was significantly increased in the DLA control group compared to the normal control group. Treatment with EEIB at the dose of 200 mg/kg and 400mg/kg significantly increases the Hgb content, RBC, Platelets and significantly decreased the WBC count to about normal level. All these results suggest the anticancer nature of the EEIB. However, the standard 5-FU at the dose of 20 mg/kg body weight produced better result in all these parameters.

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