

Invitro and In Vivo Evaluation of Anticancer Activity of Boerhaavia Diffusa Linn in Experimental Animals

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ABSTRACT

The antitumor activity of the ethanolic extract of leaves of boerhaavia diffusa L. (EBD) has been evaluated against Dalton's ascitic lymphoma (DAL) in Swiss albino mice at the dose of 250 & 500 mg/kg, body weight. The experimental parameters used were tumour volume, tumour cell count, viable tumour cell count, mean survival time and increase in life span to assess antitumour activity. The extract administered orally for 14 consecutive days to tumor bearing group of animals. The extract increase the life span of DAL treated mice and restore the hematological parameters as compared with the DAL bearing mice in dose dependant manner. The study revealed that the EBD showed significant antitumour activity in tested animal models.

Keywords: Boerhaavia diffusa linn, Antitumor activity, Dalton's Ascetic Lymphoma Cell line

INTRODUCTION

Boerhaavia diffusa L. (Nyctaginaceae), commonly known as 'Punarnava' in the Indian system of medicine, is a perennial creeping herb found throughout the waste land of India. The roots are reputed to be diuretic and laxative and are given for the treatment of anasarca, ascites and jaundice¹. The Boerhaavia sp. has ancient medicinal use in different societies from the times of the B.C. The herbal medicine has evolved and changed through the years. A number of plant products have been identified through phyto-chemistry and the extract of their different plant parts are useful in various diseases without side effects.²

PHARMACOLOGY: Scientific Name: Boerhaavia diffusa Linn. Syn. B. repens; B. repens Family: Nyctaginaceae, Family Name:

Hog weed, Horse Purslane, Common Indian Names

Gujarati: Dholia-saturdo, Moto-satoda. Hindi: Snathikari Kannada: Kommegida Marathi: Tambadivasu Sanskrit: Punarnava, Raktakanda, Shothaghni, Varshabhu Bengali: Punurnava Tamil: Mukaratee-Kirei Telugu: Punernava, Habitat: Grows as common weed., Useful Parts: Root, leaves and seeds.³

PHYTOCHEMISTRY: The Boerhaavia diffusa plant contains a large number of such compounds as flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins, and glycoproteins. Punarnavine C₁₇H₂₂N₂O m.p. 236–237°C. boeravinone A-F, hypoxanthine 9-Larabinofuranoside, ursolic acid, punarnavoside, lirodendrin, and a glycoprotein having a molecular weight of 16–20 kDa have been isolated and studied in detail

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for their biological activity²⁰. Punarnava also contains arachidic acid, β -Sitosterol, α -2-sitosterol, palmitic acid, ester of β -sitosterol, tetracosanoic, hexacosanoic, stearic, urosilic acid, Hentriacontane, β -Ecdysone, triacontanol etc. Phytochemical screening of the roots from garden-grown in vivo plants of *B. diffusa* of different ages revealed that the maximum alkaloid content (2%) accumulated in the roots of 3-yearold mature plants. The aim of the present study was to evaluate antitumour activity of ethanolic extract of leaves of *boerhaavia diffusa* L. (EBD) on Dalton's ascitic lymphoma (DAL) in Swiss Albino Mice.^[3,4,5]

MATERIAL AND METHODS^[6,7,8,9,10,11]

Plant material and extraction

The leaves of *boerhaavia diffusa* L, were collected from th district of Jalna Maharashtra, India in the month of September 2010 and identified and authenticated at local Plant Anatomy and Biology Department, Jalna, India. A voucher specimen (SGSPSIOP/10/46) has been kept in our laboratory for future reference. The air-dried and coarsely powdered leaves (400g) extracted successively with 1.5 L each of petroleum ether (60–800 C) and ethanol in a Soxhlet extractor for 72 h. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40–500 C). The petroleum ether extract yielded a yellowish green stickysemisolid, weighing 3 g. The ethanol extracts yielded brown and semi-solid residues, weighing 7.0 g.

Animals

The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of the SGSPS Institute of Pharmacy, Maharashtra, India. Mature male Swiss albino mice weighing 20-25g were housed in standard isolation cages (45×35×25 cm) under environmentally controlled conditions with 12-h light/12-h dark cycle. They were allowed free access to water, standard laboratory chow (Hindustan

Liver Pvt. Ltd, Mumbai) given food and water *ad libitum*. After sufficient period of acclimatization, they were used to evaluate antitumour activity.

Tumour cell lines

Dalton's ascitic lymphoma (DAL) cells were obtained through the courtesy of the National Institute of Virology, Pune, India. DAL cells were maintained by weekly intraperitoneal (i.p.) inoculation of 1×10^6 cells/mouse.⁸

Antitumour activity in mice

After acclimatization, mature male Swiss albino mice divided into four groups (n=10) and given food and water *ad libitum*. All the groups (Table 1) except group I were injected with DAL Cells (1×10^6 cells/mouse.i.p.). This was taken as day 0. Group I served as normal saline control (5 ml/kg, p.o.) and Group II served as DAL control. On day 1, the EBD at a dose of 250 and 500 mg/kg body weight (Gr-III & IV) were administered orally and continued for 14 consecutive days. The dose of EBD was selected based on previous study on hepatoprotective activity.¹⁰ On day 15, five mice of each group were sacrificed 24 h after the last dose and the rest were kept with food and water *ad libitum* to check the increase in the life span of the tumor hosts. The effect of ethanol extract on tumor growth and host's survival time were examined by studying the parameters like tumor volume, tumor cell count, viable tumor cell count, nonviable tumor cell count, mean survival time and increase in life span.^[8,11]

Determination of tumor 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 and packed cell volume determined by centrifuging at 1000 g for 5 min.

Determination of tumor cell count

The ascitic fluid was taken in a RBC pipette and diluted 1000 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the number of cells in 64 small squares was counted.

Estimation of viable 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.

Cell count = (No. of cells x Dilution) / (Area x Thickness of liquid film)

Percentage increase life span

Recording the mortality monitored the effect of the EBD on tumor growth and percentage increase in life span (ILS %) were calculated.¹²

ILS (%) = [(Mean survival of treated group/ Mean survival of control group)-1]x100

Mean survival time = [1st Death + Last Death] / 2

Hematological studies

The effect of EBD on peripheral blood was investigated. RBC, WBC counts and estimation of hemoglobin were done by standard procedures from freely flowing tail vein blood. Serum protein conc. was estimated by Lowry's method and packed cell volume (PCV) was determined by the method described by Docie et al.¹³.

Statistical analysis

The experimental results were expressed as the mean \pm S.E.M. Data were assessed by the method of One way ANOVA followed by Dunnett post hoc test. *P* value of <0.05 was considered as statistically significant.

RESULTS

Results of the preliminary phytochemical analysis carried out on the crude ethanol

extract indicated the presence of alkaloids, glycosides, lignin, flavonoids and saponins. The effect of EBD on the survival of tumour bearing mice showed MST (Table 1) for the tumour control group (DAL treated) to be 18.40 \pm 1.41 days, while it was 31.12 \pm 1.0 days (41%) and 33.12 \pm 0.9 days (46.23%) for the group treated with EBD at the dose of 250 & 500 mg/kg respectively. The average number of tumour volume (Table 1) in DAL treated animals was found to be 2.68 \pm 0.11. EBD treatment at both dose level significantly (*P*<0.05) reduced tumour volume which was found to be 1.36 \pm 0.10 and 0.98 \pm 0.04 respectively. Viable cell count of the tumor bearing mice was significantly decreased while non-viable cell count were increased in EBD treated groups in dose dependant fashion when compared with DAL treated group. Moreover, hematological parameters of (Table 2) tumour bearing mice on day 15 were found to be significantly altered from normal group. The total WBC count, protein and PCV were found to be increased with a reduction of the haemoglobin and RBC. In a differential count of WBC, the percent of neutrophils increased while the lymphocyte count decreased. At the same time interval, EBD treatment could change those altered parameters to near normal.

DISCUSSION

In cancer chemotherapy, the major problems that are being encountered are of myelosuppression and anaemia^{17,18} but the results have clearly shown that EBD has not only brought back hemoglobin content to normal but also the RBC count to normal. Analysis of the other hematological parameters showed minimum toxic effect in the mice which were treated with EBD. After 14 days of transplantation, EBD treated groups were able to reverse the changes in the haematological parameters consequent to tumour inoculation. All these data point to the possibility of developing an ethanolic extract of leaves of

boerhaavia diffusa as a novel, potential agent in the area of cancer chemotherapy. Flavonoids have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation²¹ and angiogenesis. Thus, antitumour effect produced by the EBD may be due to its flavonoids as well as its antioxidant

potential. Thus our present study suggests that EBD possess potent anticancer activity and increase life span. Further studies to characterize the active principle and elucidate the mechanism of action of EBD are in progress using different cell lines.

Table 1. Effect of ethanolic extract of *Boerhaavia Diffusa* on survival time, life span, tumor volume, viable and non-viable cell count in DAL bearing mice.

Treatment group	Survival time (Days)	Increase of life span	Tumour volume	Viable cell count X 10 ⁶ cells/ml	Non-Viable cell count X 10 ⁶ cells/ml
Normal Saline(5ml/kg p.o)	--	--	--	--	--
DAL control (1 x 10 ⁶ cells)	18.40±1.41	--	2.68±0.11	8.41±0.12	2.41±0.20
DAL (1 x 10 ⁶ cells) + EBD (250 mg/kg p.o)	31.12±1.0*	41.00	1.36±0.10*	2.89±0.02*	0.91±0.05*
DAL (1 x 10 ⁶ cells) + EBD (250 mg/kg p.o)	33.12±0.9*	46.23	0.98±0.04*	2.01±0.02*	1.05±0.06*

Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's test.

*p < 0.01 calculated by comparing treated groups with DAL control group.

Table 2. Effect of ethanolic extract of *Boerhaavia Diffusa* on hematological parameters in dal bearing mice.

Treatment group	Hb (g%)	RBC (million/mm ³)	WBC(103 cells/mm ³)	Proteins (g%)	PCV (mm)
Normal Saline(5ml/kg p.o)	12.2±0.1	4.5±0.2	7.1±0.1	7.4±0.1	15.6±0.7
DAL control (1 x 10 ⁶ cells)	6.8±0.1a**	2.8±0.1 ^{a**}	14.2±1.0 ^{a**}	13.6±1.2 ^{a**}	24.6±0.2 ^{a**}
DAL (1 x 10 ⁶ cells) + EBD (250 mg/kg p.o)	10.1±0.2 ^{b**}	4.6±0.5 ^{b**}	10.2±0.7 ^{b*}	9.98±0.01 ^{b*}	20.2±0.12 ^{b**}
DAL (1 x 10 ⁶ cells) + EBD (250 mg/kg p.o)	12.4±0.4 ^{b**}	5.3±0.23 ^{b**}	7.6±0.5 ^{b**}	8.6±0.11 ^{b**}	16.4±0.1 ^{b**}

Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's test.

*P < 0.05, **P < 0.01, nsP > 0.05; a vs. Normal group, b vs. DAL control. n = 5.

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