

## Research Article



## Evaluation of Anticancer Potential of *Bacolepsis nervosa* decne. Ex. Moq. Against Ehrlich Ascites Carcinoma Induced Cancer in Swiss Albino Mice

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### ABSTRACT

The present investigation was designed to determine the *in vitro* anticancer potential of the ethanol extract of *Bacolepsis nervosa* stem and leaves against Ehrlich Ascites Carcinoma (EAC) induced cancer in Swiss albino mice. The anticancer activity was evaluated using mean survival time, tumor volume and hematological studies. The ethanol extract of stem and leaves given orally to mice at the dose of 150 and 300 mg/kg body weight for 14 days. The high dose of *B. nervosa* stem and leaf extract (300 mg/kg) significantly reduced the tumor growth, increased life span of the mice and restoration of hematological parameters. The results suggest that the ethanol extract of *B. nervosa* exhibit significant antitumor effects in EAC bearing mice.

**Keywords:** Anticancer, *Bacolepsis nervosa*, Ehrlich ascites carcinoma, Solid tumor.

### INTRODUCTION

The body is made up of hundreds of millions of living cells. Normal body cells grow, divide into new cells and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace the worn-out or dying cells or to repair injuries. Cancer begins when cells in a part of the body start to grow out of control. There are many kinds of cancer, but they all start because of out of control growth of abnormal cells. Cancer cell growth is different from normal cell growth. Instead of dying, cancer cells continue to grow and form new, abnormal cells. Cancer cells can also invade other tissues, something that normal cells cannot do. Cells become cancerous because of damage done to DNA. In a normal cell, when DNA gets damaged, the cell either repairs the damage or the cell dies. In cancer cells, the damaged DNA is not repaired but the cell does not die. Instead, this cell goes on making new cells that the body does not need. These new cells will all have the same damaged DNA as the first cell.<sup>1</sup>

Cancer is one of the most dreaded diseases of the 20<sup>th</sup> century and spreading continuously with increasing incidence in 21<sup>st</sup> century. It is world's second killer after cardiovascular disease. Cancer is a major public health burden in both developed and developing countries. World Health Organization (WHO) reported that there are 7.6 million deaths in 2008 and it is estimated up to 13.1 million deaths in 2030.<sup>2</sup> New cancer patients in India are estimated between 7 to 9 lakhs.<sup>3</sup> Cancer is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions and mutations that occur from metabolism). The failure of conventional treatment modules including chemotherapy and radiation therapy poses a magnitude of severe side effects and in

this regard, phototherapy is emerging as a promising field in cancer therapy.

The rich and diverse plant sources of India are likely to provide effective anticancer agent. The National Cancer Institute collected about 35000 plant samples from 20 countries and has screened about 1, 14,000 extracts for anticancer activity.<sup>4</sup> In any cancer drug discovery program, a paradigm based on ethanobotanical and ethnopharmacological data would be more economical and beneficial in identifying potential anticancer modules than mass screening of plant species.<sup>5</sup> India is a rich source of medicinal plants and a number of plant extracts are used against diseases in various systems of medicines. Only few of them have been scientifically explored.<sup>6</sup>

The plant *Bacolepsis nervosa* is an endemic plant in Nilgiri Biosphere Reserve. This plant contains rich source of bioactive compounds such as phenolic compounds, flavonoids, steroids and alkaloids. The impact of this plant in cancer treatment should be considered to discover new drug molecule or its derived compounds for cancer research. But no such literatures are revealed for its activity against treatment for cancer. Therefore, the present study was planned to explore the possible *in vitro* anticancer activity of stem and leaves of *B. nervosa* against EAC in Swiss albino mice.

### MATERIALS AND METHODS

#### Collection of plant sample

The stem and leaves of *Bacolepsis nervosa* Decne. ex. Moq. were freshly collected from Kothagiri, Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu. The plant specimen was identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethno



pharmacology unit, Research department of Botany, V. O. Chidambaram College, Tuticorin, Tamil Nadu.

### Preparation of plant extract for anticancer activity

The stem and leaves of *B. nervosa* were cut into small pieces, washed, dried at room temperature; the dried stem and leaves were powdered in a Wiley mill. Hundred grams of powdered stem and leaves were separately packed in a Soxhlet apparatus and extracted with ethanol. The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract of stem (BNS) and leaves (BNL) were used for anticancer activity.

### Animals

Healthy male adult Swiss Albino mice (20-25gm) were used for the study. The animals were housed in microcolon boxes in a controlled environment (temperature  $25 \pm 20^\circ\text{C}$ ) and 12 hr dark/light cycle) with standard laboratory diet (Sai Durga feeds and foods, Bangalore) and water *ad libitum*. The mice were segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They were fed on healthy diet and maintained in hygienic environment in our animal house.

### Tumor cells

Ehrlich Ascites Carcinoma (EAC) cells were obtained from Division of Oncology Department of Biotechnology, Tamil Nadu, Veterinary and Animal Husbandry, Chennai, Tamil Nadu, India. The freshly drawn ascetic fluid was diluted in phosphate buffer solution pH (6.8) and aliquot of ( $1 \times 10^6$  cells 0.25 mL) of the diluted solution were injected intraperitoneal inoculation to mice belonging to age group of 5 to 6 weeks and weight (20 to 25 gms).

### Acute oral toxicity study

Acute oral toxicity was performed by following OECD guideline – 420<sup>7</sup> fixed dose procedure for ethanol extract of stem and leaves of *B. nervosa* and it was found that dose increasing up to 2000 mg / kg body weight, showed no toxicity or mortality in experimental mice.

### Antitumor activity

Healthy Swiss albino mice were divided into seven groups of six animals (n=6) each. The test samples were dissolved in isotonic saline (0.9% NaCl W/V) and used directly in the assay. EAC cells were collected from the donor mouse and were suspended in sterile isotonic saline. The viable EAC cells were counted (Trypan blue indicator) under the microscope and were adjusted at  $1 \times 10^6$  cells/ ml. 0.1 ml of EAC cells per 10g body weight of the animals were injected (i. p) to each mouse of each group except normal saline group (Group I). This was taken as Day 0. Group I served as a normal saline control (1mL/kg, p.o) and group II served as EAC bearing control. On day 1, the ethanol extract of *B. nervosa* leaf at a dose of 150 and 300mg/kg each of the Group III, IV and *B. nervosa* stem at a dose of 150 and 300mg/kg each of the Group V, VI were administered orally and continued for 14 consecutive days respectively. Group VII served as tumor induced

animal administered with vincristine (80mg/kg body weight) for 14 consecutive days. On day 15, half of the animals (n=3) in each case were sacrificed and the remaining animals were kept to observe the life span study of the tumor hosts. The effect of ethanol extract of *B. nervosa* on tumor growth and host's survival time were monitored by studying parameters like tumor volume, tumor cell count, viable tumor cell count, nonviable tumor cell count, mean survival time and increase in life span.<sup>8-9</sup>

### 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. Packed cell volume was determined by centrifuging the ascitic fluid at 1000 rpm for 5min.

### Determination of 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.

### Estimation of viable and non viable tumor cell count (Trypan blue dye assay)

The cells were then stained with trypan blue (0.4% normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were non viable. These viable and non viable cells were counted.

### Percentage increase of life span (% ILS)

The percentage increase in life span (% ILS) was calculated from the following equation.

$$\text{Increase in life span} = \frac{T - C}{C} \times 100$$

### Body weight

Body weight of the experimental mice was recorded both in the treated and control group at the beginning of the experiment (zero day) and sequentially on every 5th day during the treatment period.

### Haematological studies

At the end of the experimental period, all mice were sacrificed by cervical dislocation. Blood was collected from freely flowing tail vein and used for the estimation of Haemoglobin content (Hb), Red blood cell count (RBC) and White blood cell count (WBC). WBC differential count was carried out from Leishman stained blood smears.<sup>10</sup>

### Statistical analysis

The data were analyzed using student's t- test statistical methods. For the statistical tests, *p* values of less than 0.01 and 0.05 were taken as significant.

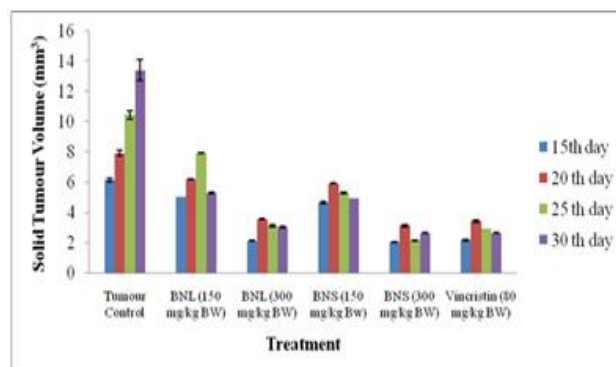


## RESULTS AND DISCUSSION

Cancer is a multi-mechanistic second largest disease in the world which requires a multidimensional approach for its treatment, control and prevention. There are various types of tumors such as sarcoma, lymphoma, carcinoma and leukemia. In our study, Ehrlich ascites carcinoma was used to induce cancer cells in mice. The ehrlich tumor was initially described as a spontaneous murine mammary adenocarcinoma. It is a rapidly growing carcinoma with very aggressive behavior and is able to grow in almost all strains of mice. In ascetic form it has been used as transplantable tumor model to investigate the antitumor effects of several substances.<sup>11</sup> Research is being carried out throughout the world to find a lead compound which can block the development of cancer in human. Nature has always been a great contributor towards this goal.

In the present study, vincristin was selected as anticancerous drug. The isolation of vinca alkaloids

(vincristin and vinblastin) from *Catharanthus roseus* introduced a new era in the use of plant material as anticancer agents. They were the first agents to advance into clinical use for the treatment of cancer.<sup>12</sup>



**Figure 1:** Effect of BNL and BNS extracts on solid tumor volume in EAC induced mice

**Table 1:** Effect of BNL and BNS extracts on Relative Organ Weight of tumor induced (EAC) and drug treated mice

Group	Dose (mg/kg body weight)	Relative Organ Weight (g /100 g body weight) after 10 days					
		Body weight	Spleen	Thymus	Liver	Kidney	Lungs
I	Normal Control	21.39±0.74	0.42±0.018	0.21±0.026	2.98±0.81	0.93±0.05	0.77±0.03
II	Tumour Control	43.12±0.81***	0.74±0.021**	0.34±0.05*	4.36±0.16*	1.83±0.07*	0.93±0.02ns
III	BNL (150)	36.05±0.42**	0.51±0.013ns	0.24±0.014ns	2.78±0.62a	1.132±0.06	0.86±0.02
IV	BNL (300)	20.89±0.18aa	0.52±0.026a	0.18±0.013aa	2.24±0.32a	0.81±0.07a	0.60±0.05
V	BNS(150)	26.51±0.38ns	0.69±0.014*ns	0.30±0.028	3.93±0.16ns	1.45±0.03	0.88±0.06
VI	BNS(300)	19.54±0.11aa	0.45±0.051a	0.23±0.024a	2.84±0.13a	0.81±0.03a	0.70±0.06
VII	Vincristin (80)	18.22±0.14aa	0.34±0.061aa	0.19±0.034aa	2.82±0.14a	0.83±0.07a	0.65±0.08

Each Value is SEM ± 6 individual observations : \* P < 0.05 ; \*\* P<0.01; \*\*\* P<0.01 Compared to normal control vs drug treated groups : a P < 0.05 ; aa P<0.01, Compared to EAC control vs drug treated groups: ns –non significant

**Table 2:** Effect of BNL and BNS extracts on the survival time, life span, packed cell volume, viable and non viable cell count

Group	Dose (mg/kg body weight)	Mean survival time (Days)	Increase of life span (%)	Packed cell volume (ml)	Viable cells (1 X10 <sup>6</sup> cells / ml)	Non Viable cells (1 X10 <sup>6</sup> cells / ml)
I	Normal Control	-	-	-	-	-
II	Tumour Control	16.54±0.12	-	4.93±0.014	18.93±0.65	0.93±0.024
III	BNL (150)	20.43±0.18ns	23.51	3.83±0.056	10.22±0.73ns	1.854±0.039*
IV	BNL (300)	26.53±0.84*	60.39	2.16±0.067**	6.84±0.49**	2.54±0.046**
V	BNS(150)	22.34±0.65**	35.06	2.52±0.031**	8.29±0.36*	1.48±0.033*
VI	BNS(300)	28.92±0.72***	74.84	1.85±0.015***	3.92±0.84***	1.42±0.075*
VII	Vincristin (80)	27.22±0.64***	64.57	1.65±0.071***	4.06±0.23***	1.38±0.032*

Each Value is SEM of 6 animals Significance between tumour induced control vs drug treated group \* P < 0.05; \*\* P < 0.01;\*\*\* P < 0.001

Antitumor activity of ethanol extract of *B. nervosa* stem (BNS) and leaves (BNL) against EAC (Ehrlich Ascites Carcinoma) tumor bearing mice was assessed by the parameters such as relative organ weight, tumor volume, viable and non viable cell count, mean survival time and percentage increase in life span. Usually the parameters for evaluating the antitumor activity of a drug are

prolongation of life span, reduction in tumor volume and the improvement in hematological parameters of the host. Generally, the result obtained in the present study at the dose level of 300 mg/kg body weight was highly significant and comparable to that of standard drug. This observation is supported by Muthuraman *et al.*<sup>13</sup> The extract of *B.nervosa* did not provoke any gross behavioral

changes or manifestations of toxic symptoms in the animals. The extracts were non-lethal even at the maximum single oral dose of 2000 mg/kg

There was a significant decrease in the body weight of BNS and BNL treated groups compared to the tumor control. A highly significant ( $p < 0.01$ ) decrease was observed in groups IV (BNL) and VI (BNS) treated with 300 mg/kg of extract (Table 1). The administration of the extract indicated a dose dependent decrease in the weight of different organs. The relative organ weight of all the vital organs were restored to normal on treatment with 300 mg/kg body weight of the extract. Similar reduction was noticed on treatment with the standard drug Vincristin (80 mg/kg body weight).

An increase in the relative organ weight of immunologically important organs like spleen and thymus in the tumor control can be attributed to their increased activity and production of immunocompetent cells. It may also be due to the accumulation of fluids. The administration of the extract indicated a dose dependent

decrease in the weight of lymphoid organs which may be due to the bioactive compounds present in the extract supporting the activity of the immune system. The reduction in weight of liver and kidney on treatment may be the result of removal of toxic fluids by the action of the extracts.<sup>14</sup>

Tumor volume was observed on 15<sup>th</sup>, 20<sup>th</sup>, 25<sup>th</sup>, and 30<sup>th</sup> day in the tumor control and drug treated mice. Treatment with BNL and BNS extract at the dose of 300mg/kg body weight for a period of 14 consecutive days in EAC induced solid tumor bearing mice showed a significant ( $p < 0.001$ ) reduction in tumor volume ( $3.02 \pm 0.054$  and  $2.63 \pm 0.026$ ) respectively when compared to the tumor alone group ( $13.4 \pm 0.68$ ) on 30<sup>th</sup> day. The administration of the extract showed a dose dependent decrease in tumor volume. The animals treated with the standard drug (Vincristin 80 mg/kg body weight) were found to be also efficient ( $2.64 \pm 0.013$ ) in preventing the development of solid tumor on the same day as shown in Figure 1.

**Table 3:** Effect of BNL and BNS extracts on Hematological parameters in EAC tumor bearing mice

Group	Dose (mg/kg body weight)	Hb (gm %)	RBC (million /mm <sup>3</sup> )	WBC (10 <sup>3</sup> cells / mm <sup>3</sup> )	Differential Count (%)		
					Lymphocytes	Neutrophils	Eosinophils
I	Normal Control	10.29±0.65	3.84±0.13	10.21±0.22	60.30±0.98	34.56±0.62	5.14±0.31
II	Tumour Control	8.53±0.43*	3.61±0.24	13.56±0.78	51.42±0.28*	40.22±0.19*	8.36±0.78
III	BNL (150)	9.98±0.15	3.95±0.13	10.27±0.62	52.55±0.84	42.08±0.76	5.37±0.45
IV	BNL (300)	12.63±0.74a	4.88±0.28a	11.89±0.13a	56.83±0.91*	38.66±0.66	4.51±0.32
V	BNS(150)	11.94±0.65	4.17±0.56	11.63±0.66a	55.59±0.83*	40.74±0.21	3.67±0.11
VI	BNS(300)	12.68±0.48a	4.96±0.69a	12.43±0.36a	42.66±0.54a	52.93±0.62a	4.41±0.96a
VII	Vincristin (80)	12.93±0.38a	4.73±0.18	12.82±0.59a	44.38±0.29a	50.67±0.38a	4.95±0.87a

Each Value is SEM of 6 animals Significance between tumour induced control Vs. drug treated group \*  $P < 0.05$ ; \*\*  $P < 0.01$ .

To investigate the inhibitory effect on ascetic tumor was local or systematic; the effect of administration of *B. nervosa* was tested against solid tumor induced by EAC cell lines. The abnormal mass of tissue that does not contain cyst or liquid is referred as solid tumor and is mostly epithelial in nature.<sup>15</sup> There was a significant inhibition of solid tumor volume and reduction in body weight in solid tumor bearing animals treated with extract when compare to tumor control. This undoubtedly suggests that the inhibitory effect of *B. nervosa* is systematic, not only related to its local cytotoxic effect. This inhibitory effect on tumor volume and protection of hematopoietic system was comparable with the result produced by the standard drug Vincristin. The extract administration reduced tumor volume indicating inhibition in the growth and multiplication of tumor cells which may be due to the decrease in the ascites fluid acting as a direct nutritional source or the presence of compounds inhibiting mitosis, DNA synthesis or replication via enzyme pathway.<sup>16</sup>

The effect of BNL and BNS extract at the doses of 150 and 300 mg/kg body weight on the mean survival time and increase in life span of EAC bearing mice is shown in Table

2. In the EAC control group, the mean survival time was  $16.54 \pm 0.12$  (days) while it increased to  $20.43 \pm 0.18$  (150 mg/kg),  $26.53 \pm 0.84$  (300 mg/kg) in BNL treated groups and  $22.34 \pm 0.65$  (150 mg/kg),  $28.92 \pm 0.72$  (300 mg/kg) in BNS treated groups. The group treated with the standard drug Vincristin (80 mg/kg) showed  $27.22 \pm 0.64$  days for the same. The increase in the life span of tumor bearing mice treated with BNL and BNS extract at different doses (150 and 300 mg/kg BW) and the standard Vincristin was found to be 23.51%, 60.39%, 35.06%, 74.84% and 64.57% respectively. In the present study, animals administered with different doses of *B. nervosa* extract showed a dose dependent increase in mean survival time and the life span with respect to control. Administration of BNL and BNS extract at the dose of 300 mg/kg body weight significantly ( $p < 0.01$  and  $p < 0.001$ ) decreased the packed cell volume and viable cell count. Furthermore, non viable tumor cell count at the dose of 300mg/kg body weight of BNL extract were significantly ( $p < 0.01$ ) increased in a dose dependent manner.

The cytokines produced in the body by the lymphocytes are known as interleukins. The reduction in viable cell count and increased non viable cell count towards normal



in tumor host suggest that extract stimulate the growth and activity of immune cells by the production of interleukins, which target tumor cells and cause lysis of the tumor cells by indirect cytotoxic mechanism. Furthermore, the reduced volume of tumor and increased survival time of the mice suggest that the extract might have exerted a delay in vascular permeability to the cells.<sup>17</sup>

The effect of BNL and BNS extract treated animals was shown in Table 3. Hematological parameters of tumor bearing mice were found to be altered compared to normal group. The hemoglobin content and RBC count in the EAC control group was decreased as compared to the normal group. Treatment with BNL and BNS extract at the dose of 300 mg/kg significantly ( $p < 0.05$ ) increased the hemoglobin content and RBC count to more or less normal levels. In differential count of WBC, the percentage of neutrophils and eosinophils increased while the lymphocyte count decreased in the EAC control group. Groups treated with BNL and BNS extract showed restoration of these hematological parameters in dose dependent manner.

Myelosuppression and anemia (reduced hemoglobin) have been frequently observed in ascites carcinoma.<sup>18-19</sup> Anemia encountered in ascites carcinoma mainly due to iron deficiency either by haemolytic or myelopathic conditions which finally lead to reduced RBC number.<sup>20</sup>

Plant derived natural products such as flavonoids, terpenoids and steroids have received considerable attention due to their diverse pharmacological properties which include cytotoxic and chemo preventive effects.<sup>21</sup> *B. nervosa* is one of the best medicinal plants which belong to the family Periplocaceae. It is endemic to Nilgiri Biosphere Reserve (NBR). Thirty compounds were isolated from ethanol extract of stem and leaves of *B. nervosa* by GC-MS analysis. Among the thirty compounds, piperine, stigmastene 3 one, phytol, widdrol, tumerone, phenol, 2, 5 bis (1,1 dimethyl ethyl), benzaldehyde 2,5 – dimethyl, lupeol and caryophyllene showed anticancer activities.<sup>22</sup>

## CONCLUSION

The outcome of present investigation undoubtedly indicates that, the treatment of *B. nervosa* was effective on inhibiting the tumor progression in *in vivo* models. Further studies are needed to characterize the anticancer activity of the selected extract to find out the exact mechanism involved so that it can be formulated and may be tried clinically.

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