

## CYTOTOXIC AND ANTI –TUMOUR PROPERTIES OF ETHANOLIC EXTRACT OF BACOPA MONNIERI (L) PENN

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**ABSTRACT:** *Bacopa Monnieri (L) penn.* belongs to the family *scrophulariaceae* is commonly used in Ayurvedic system of medicine to treat various disease. The present study examines the anti-tumour effect of ethanolic extract of whole plant *bacopa monnieri (L) (EEEM)* by *in vitro* short term chemosensitivity and *in vivo* tumour model test systems. *EEEM* dose dependently inhibited the proliferation of transformed cell lines significantly. Fifty percent dose on 3 hour exposure to *EEEM* was 150 µg/ml for Dalton's lymphoma ascites tumour cells (DLA). Oral administration of the *EEEM* retarded the development of solid tumour and restored the altered hematological parameters near to normal.

### INTRODUCTION

*Bacopa monnieri (L) penn.* syni *Herpestis monnieri (L) .B. & K.* named in various Indian languages as Brahmi, Jal Neem, Nir Brahmi, Sarasvati, sambarani chetty is small prostate herb belonging to the family *scrophulariaceae*. Grows wild in damp places and marsh lands in the major parts of plains of India (1) it is known to have anti tumour activity (2, 18).

The plant has been prescribed for nervous disorders such as insanity, epilepsy, neurasthenia, nervous breakdown etc. The fresh juice of the plant boiled in ghee may be given as such (brahmi oil). It is also used as brain tonic (1). Isolation of alkaloids brahmine, hespestine, mannitol and saponins by the earlier workers (3-8). Sedative effect on dogs and frogs, tranquillizing effect and learning tests on albino rats have been reported by earlier workers (9-11).

The present study evaluates the anti-cancer property of *Bacopa monnieri (L) Penn.*

### Materials and Methods

Whole plants of *Bacopa monnieri* were collected from Kinathukkadavu, 20km. away from Coimbatore, Tamil Nadu and identified by Dr. Mohan, Botanical survey of India, Coimbatore with the help of herbarium sheets.

Dalton's lymphoma ascites tumour cells (DLA) was obtained from Amala Cancer Research Centre, Amala Nagar Trichur, Kerala.

All the chemicals used in the present study of analytical reagent quality.

### Preparation of the drug

The fresh samples of plant (whole plant) were collected, air dried and powdered. The dried powder of the plant was cold extracted with 50% ethanol for seven days with occasional stirring. The process was repeated twice. (12). The pooled extracts were concentrated, evaporated to dryness

under reduced pressure. The extract was resuspended in 1% gum acacia and subjected to the various studies.

### **Determination of cytotoxicity**

Short term in vitro cytotoxicity assay was conducted using DLA tumour cells. 100, 200, 400 µg of EEBM and 0.1ml of 1% gum acacia (control) were incubated at 37°C with one million cells in 1ml PBS (Phosphate Buffer Saline) for three hours. (13, 17) after incubation, the percentage dead cell as determined using trypan blue exclusion method.

### **Tumour reduction experiment**

Swiss albino mice weighing 17 to 25 gm supplied from our animal house, J.S.S College of Pharmacy, Ooty, were housed in well ventilated cages and fed with normal mouse pellet feed (Lipton India) and water ad libitum were used for the study. DLA was propagated into the peritoneal cavity of the mice by injecting one million cells. The cells were aspirated from developed tumour and washed with PBS. The solid tumours developed in mice by injected one million cells subcutaneously on hind limbs. The animals were divided into three groups. Each group was further subdivided into two groups (Group a & b) were treated with 100, 200 mg/kg body wt of EEBM respectively.

Group 1 animals were treated 24 hours after tumour transplantation once daily for 10 days.

Group 2 animals were treated once daily for 10 days prior to tumour transplantation

Group 4 animals were served as control treated with gum acacia (1%).

Solid tumours were measured from day 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, 28<sup>th</sup> of transplantation. The volume was calculated using the formula  $V = \frac{4}{3}\pi r_1^2 r_2^2$  where  $r_1^2$   $r_2^2$  are radii of tumours. (14)

### **Haematological studies.**

In order to detect the influence of the EEBM on the hematological status of DLA bearing mice a comparison was made amongst the above mentioned four groups of mice on the 14<sup>th</sup> day after transplantation with normal mice. Blood was drawn from each mouse in the conventional way and white blood cells count (WBC), red blood cells count (RBC), differential count and hemoglobin (Hb) percentage were estimated. (15)

### **Results**

#### **Short term in vitro cytotoxicity assay**

The EEBM prepared was checked for their cytotoxicity by in vitro short term incubation with tumour cells (table 1) there was a dose dependent cytotoxicity to DLA tumour cells in vitro. The cytotoxicity was found to be 150µg/ml in vitro to these cells. It was found that treatment with 400 µg/ml produced a 70% death of DLA cells.

#### **Tumour Reduction experiment**

The effect of EEBM on tumour reduction is shown in (Table 2). Administration of EEBM reduced solid tumour volume in mice considerably. The maximum anti-tumour activity was shown in post treatment with 200mg/kg body wt for 10 days once daily 24 hrs after tumour transplantation group.

For example, tumour volume of untreated mice was 0.19ml on 7<sup>th</sup> day, 1.9ml on the 28<sup>th</sup> while for the treated mice the tumour volume was nil on the 7<sup>th</sup> as well as on the 14<sup>th</sup> day, 0.54 ml on the 21<sup>st</sup> day and 1.8ml

on the 28<sup>th</sup> day. These data indicate a considerable reduction in tumour volume during the post treatment with 200mg/kg for 10 days 24hrs after tumour transplantation.

### Haematological Parameters

Haematological Parameters (table 3), of tumour bearing mice on day 14 were found to be significantly altered from the normal group. The total WBC count was found to be increase. In a differential count of WBC, the percent of neutrophils increased while the lymphocyte count decreased. At the same time interval, EEBM (200mg/kg/da/oral) treatment for 10 days after tumour transplantation could restore those altered parameters near to normal.

### Discussion and Conclusion

The use of chemotherapeutic drugs in cancer therapy involves the risk of life threatening host toxicity. Therefore the research goes on to develop the drugs which selectively act on tumour cells. The plant *Bacopa Monnieri* belonging to the family *scophulariaceae* have high medicinal properties. The present study also revealed its potential cytotoxic and anti-tumour

properties. The EEBM has shown significant cytotoxicity assay was found to have significant cytotoxicity towards transformed cell lines. In solid tumour reduction studies the treatment of EEBM exhibited significant tumour reducing property analysis of the haematological parameters at 14 days after transplantation of EEBM treated groups were able to reverse the changes in the haematological parameters consequent to tumour transplantation. The possible mechanism of anti-tumour action of EEBM against DLA cells may be due to radiomimetic, nucleotoxic and cytotoxic effect and acts in a manner similar to that of a spindle poison and inhibits cell mitosis. (16, 17).

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**Table No. 1**  
**Short term in Vitro Cytotoxicity of EEBM**

| Treatment µg/ml                 | Percentage Cell Death |
|---------------------------------|-----------------------|
| 100                             | 25%                   |
| 200                             | 60%                   |
| 400                             | 70%                   |
| Control (0.1 ml 1 % Gum acacia) | 7%                    |

**Table No. 2**  
**Effect of EEBM on Solid Tumour Model**

| Group Treatment Mg/kg | Tumour Volume In Cubin Centimetre |                      |                      |                      |
|-----------------------|-----------------------------------|----------------------|----------------------|----------------------|
|                       | 7 <sup>th</sup> Day               | 14 <sup>th</sup> Day | 21 <sup>st</sup> Day | 28 <sup>th</sup> Day |
|                       |                                   |                      |                      |                      |

|                           |                 |                       |                       |                       |
|---------------------------|-----------------|-----------------------|-----------------------|-----------------------|
| Gr. Ia 100                | ----            | -----                 | $0.66 \pm 0.09^{**}$  | $2.4 \pm 0.20^{***}$  |
| Gr. Ib 200                | ----            | -----                 | $0.54 \pm 0.04^{***}$ | $1.82 \pm 0.10^{***}$ |
| Gr. IIa 100               | Not determined  | $1.22 \pm 0.09^{***}$ | $1.94 \pm 0.07^{***}$ | $3.48 \pm 0.07^{***}$ |
| Gr. IIb 200               | Not determined  | $1.08 \pm 0.07^{***}$ | $1.56 \pm 0.07^{***}$ | $3.00 \pm 0.08^{***}$ |
| Gr. IIIa 100              | -----           | $1.50 \pm 0.2^*$      | $3.29 \pm 0.2^*$      | $6.6 \pm 0.25$        |
| Gr. IIIb 200              | -----           | $1.6 \pm 0.12^*$      | $2.96 \pm 0.2^*$      | $6.4 \pm 0.35^*$      |
| Gr. IV<br>1%Gum<br>Acacia | $0.19 \pm 0.02$ | $1.90 \pm 0.29$       | $3.30 \pm 0.35$       | $6.70 \pm 0.26$       |

Group I: Received treatment 24 hrs after Tumour Transplantation.

Group II: Received treatment 10 days after Tumour Transplantation.

Group III: Received treatment 10 days prior to Tumour Transplantation.

Values are the mean  $\pm$  S.E.M. of six animal in group.

Group I, II, III were compared with IV Group.

Values marked with \*, \*\*, \*\*\*, indicate the significance ( $P < 0.5$ ,  $p < 0.01$ ,  $P < 0.001$ , respectively).

**Table No.3**  
**Effect of EEBM on Haematological Parameters**

| Group & Treatment    | Total EBS cells/ml x 10 <sup>6</sup> | Total RBC cells/ml x 10 <sup>10</sup> | Hb (g%)      | Differential Count (%) |               |             |
|----------------------|--------------------------------------|---------------------------------------|--------------|------------------------|---------------|-------------|
|                      |                                      |                                       |              | Lymphocyte             | Neutrophil    | Monocyte    |
| Normal               | 12.7 ± 0.76                          | 8.81 ± 0.52                           | 13.6 ± 1.3   | 65.8 ± 1.67            | 32 ± 1.51     | 2 ± 0.17    |
| Gr. I 100 mg/kg      | 23.3 ± 0.97 *                        | 8.46 ± 0.53 *                         | 12.9 ± 0.43* | 47 ± 1.02***           | 50 ± 1.76 *** | 3.1 ± 0.42* |
| Gr. I 200 mg/kg      | 21.1 ± 0.53 **                       | 8.7 ± 0.49 *                          | 13.4 ± 0.62* | 50 ± 20.1***           | 46 ± 1.28 *** | 3.9 ± 0.31* |
| Gr. II 100 mg/kg     | 21.3 ± 0.04 **                       | 8.96 ± 0.46 *                         | 12.6 ± 0.51* | 42 ± 1.79**            | 53 ± 1.59***  | 4.1 ± 0.51* |
| Gr. II 200 mg/kg     | 18.7 ± 0.48 ***                      | 9.15 ± 0.25 *                         | 12.4 ± 0.5 * | 47 ± 1.55***           | 51 ± 1.17 *** | 2.2 ± 0.31* |
| Gr. III 100 mg/kg    | 26.7 ± 0.92*                         | 9.1 ± 0.56 *                          | 13.9 ± 0.36* | 38.7 ± 2.7*            | 60 ± 1.8*     | 2.1 ± 0.1*  |
| Gr. III 200 mg/kg    | 25.3 ± 1.22*                         | 8.85 ± 0.66*                          | 13.4 ± 0.33* | 31.9 ± 1.9*            | 65.5 ± 2.9*   | 3.2 ± 0.4*  |
| Gr. IV 1% Gum Acacia | 27.0 ± 1.51 ***                      | 8.43 ± 0.5*                           | 13.1 ± 0.47* | 36 ± 1.29 ***          | 62 ± 1.68 *** | 2.1 ± 0.2*  |

Group I: Received treatment 24 hrs after Tumour Transplantation.

Group II: Received treatment 10 days after Tumour Transplantation.

Group III: Received treatment 10 days prior to Tumour Transplantation.

Normal, Group 1, 2 and 3 compared with Group 4

Values marked with \*, \*\*, \*\*\*, indicate the significance (P<0.5, p<0.01, P<0.001, respectively).

Values are the mean ± S.E.M. of six animal in group.

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