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## Evaluation of Anticancer Activity of Ethanolic Extract of *Premna corymbosa* Rottl. Leaves

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#### ABSTRACT

To study the anticancer activity of ethanolic extract of *Premna corymbosa* Rottl. Leaves.

**Method:** The Ethanolic extract of *Premna corymbosa* Rottl. Leaves (EEPC) were evaluated for anti-cancer activity against Ehrlich ascites carcinoma (EAC) bearing swiss albino mice and for the present anticancer study we used two concentration of ethanolic extract of *Premna corymbosa* Rottl. (EEPC) in the dose of 100 mg/kg and 200 mg/kg in 1% carboxy methyl cellulose (CMC) and were given orally.

**Result:** The leaf of *Premna corymbosa* Rottl was selected for the study, on the basis of ethanobotanical information which reveals its uses against one of the most hazardous disease cancer which shown significant response.

**Conclusion:** The leaves of *Premna corymbosa* Rottl. Has been studied to compare and give detailed reports on pharmacological studied on it.

### INTRODUCTION

Herbal Medicine sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body [1]. Medicinal plants have been used as remedies for human diseases for centuries. The reason for using them as medicine lies in the fact that they contain chemical components of therapeutic value [2]. The medicinal value of plants lies in some chemical substances (usually secondary metabolites), that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic [3]. Cancer is the cause of more than six million deaths each year in the world. In 2001, about 1,268,000 new cancer cases and 553,400 deaths were reported in the United States [4]. For a long time, plants are being used in the treatment of cancer [5]. According to an estimate, 50% of breast cancer and 37% of prostate cancer patients use herbal products [6]. More than 60% of currently used anticancer agents are derived in one way or another from natural sources [7,8]. Biological active components from plants are significant and important source of new drugs that are likely to lead to new and better treatments for breast cancer. As chemotherapy destroys the normal cells along with cancer cells, sometimes cancer cells can develop resistance to treatment through mutations [9]. The World Health Organization (WHO) estimates that 4 billion people, 80% of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous people's traditional medicine and a common element in Ayurvedic, homeopathic, naturopathic, traditional oriental, and Native American Indian medicine. WHO notes that of 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures [10].

The leaf of *Premna corymbosa* Rottl belonging to family Verbenaceae. *Premna corymbosa* Rottl. is a large shrub or a small tree up to 9 m in height with yellowish lenticellate bark, spinous large branches and yellowish brown woody aromatic root. It is commonly found in India and the Andaman coasts. It also occurs in the plains of Assam and in Khasi hills, near the sea from

Bombay to Malacca, Ceylon, Andamans, Nicobars. *Premna corymbosa* Rottl was reported to contain sitosterol, the isoxazole alkaloid: Premnazole, flavonoids and luteolin and leaves of the plant are used for stomachic, carminative, galactagogue, useful in dyspepsia, flatulence, colic, cough and fever. Leaves are good as an external application to piles & tumors and root having taste like bitter, pungent, heating, laxative; Stomachic which are useful in anemia, vata, diabetes chyluria, inflammation, swellings and also useful in bronchitis, dyspepsia, piles, constipation, fever. The root is given in decoction as a cordial & tonic. They also useful in neuralgia, cardiac disorder, hepatopathy, cough, asthma, leprosy & skin disease. *Premna corymbosa* Rottl. has been used in different disease like Piles, Tumors, flatulence, Cough, Catarrh and Fever. It also useful in neuralgia, cardiac disorder, hepatopathy, coughs, asthma, leprosy & skin disease. Very less phytochemical and biological studies on leaves has been performed [11-14]. So, for that we are interested to report *Premna corymbosa* Rottl. leaves for anti-cancer activity.

## MATERIALS AND METHOD

### Collection of specimen

The plant *Premna corymbosa* Rottl. Belonging to family verbenaceae are widely found throughout India in the plains, along the Indian and the Andaman coasts, it also occurs in the plains of Assam and in Khasi hills. The species for the proposed study that is *Premna corymbosa* Rottl. was collected from Chennai in the month of July. Care was taken regarding the age and the health of the plant to obtain a best condition leaves part.

### Taxonomical identification

The species for the proposed study was identified and authenticated as *Premna corymbosa* Rottl by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Center (PARC), Chennai.

### Treatment

The leaves were washed with water & dried it in sunlight first hour & then it was dried in shade. The dried leaves were coarsely powdered by means of grinder and the powder was passed through the sieve no.60 for powder microscopy & coarse powder was used for further studies.

### Preparation of extracts

#### Ethanol extract

The shade dried coarse powder of the root (300 gm) was packed well in soxhlet apparatus and was subjected for continuous hot extraction with 90% ethanol up to 18-20 hr. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely, dried and kept in a desiccator. Obtained extract (dark green) was weighed and percentage yield was calculated in terms of air-dried powdered crude material [15].

### Acute oral toxicity (LD<sub>50</sub>) study

In the assessment and evaluation of the toxic characteristics of a substance, determination of acute oral toxicity is usually an initial step. Acute oral toxicity was performed by using OECD guidelines-423 (Organization of Economic Co- Operation and Development) - Fixed dose procedure (FDP). The Fixed Dose Procedure (FDP) is a method for assessing acute oral toxicity that involves the identification of a dose level that causes evidence of non-lethal toxicity (Evident toxicity) which describing clear signs of toxicity following administration of test substance, such that an increase to the next highest fixed dose would result in the development of severe toxic signs and probably mortality LD<sub>50</sub> (median lethal dose oral, is a statistically derived single dose of a substance that can be expected to cause death in 50 percent of animals when administered by the oral route. The LD<sub>50</sub> value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg) [16].

### Observation

No toxicity or death was observed for these given dose levels, in the selected and treated animals. So the LD<sub>50</sub> of the ethanolic extract of *Premna corymbosa* Rottl. (EEPC) as per OECD guidelines-420 is greater than 2000 mg/kg (LD<sub>50</sub> > 2000 mg/kg). Hence the biological dose was fixed 200 mg/kg for ethanolic extract.

### Preparation of extract drug and mode of administration

For the present anticancer study we used two concentration of ethanolic extract of *Premna corymbosa* Rottl. (EEPC) in the dose of 100 mg/kg and 200 mg/kg in 1% carboxy methyl cellulose (CMC) and were given orally.

### Animals

Studies were carried out by using adult Swiss albino mice weighing between 18-25 gms. They were obtained from Periyar College of Pharmacy, Trichy, Tamilnadu. The mice were grouped and housed in polypropylene cage containing paddy husk as bedding throughout the experiment and maintained under standard laboratory condition (Temp-25°C ± 2°C) and light (14 hr and 10 hr of light and dark, respectively). The mice were fed with a standard pellet diet and given water ad libitum. The study was approved by the Institutional Animal Ethical Periyar College of Pharmacy, Trichy, Tamilnadu

## ANTICANCER ACTIVITY

The Ethanolic extract of *Premna corymbosa* Rottl. Leaves (EEPC) were evaluated for anti-cancer activity against Ehrlich ascites carcinoma (EAC) bearing swiss albino mice.

### Cancer cell line

Ehrlich ascites carcinoma (EAC) cells were procured from the courtesy of Amla Cancer Research Centre, Trissure, India. The Ehrlich ascites carcinoma (EAC) cells were transplanted to animal as per the procedure mentioned below<sup>[17]</sup>.

### Tumour transplantation procedure

All experiment has been performed on swiss mice of both sex 5-6 weeks old, weighing 18-25 gms.

Ehrlich ascites carcinoma (EAC) in ascites form was selected for the present study where the tumour cell was known to grow as uniform cell suspension in the abdominal cavity of the host. Ehrlich ascites carcinoma(EAC) being maintained in the ascites form in swiss mice by serial transplantation of tumour cell (I.P.) in 5-6 week old mice well developed ascitic tumour containing large number of neoplastic monoclear cell produced around the 10-14 day of transplantation. The ascites fluid of Ehrlich ascites carcinoma (EAC) was drawn out from donor mice was diluted in saline pH (0.9%) and aliquot ( $1 \times 10^6$  cells / 0.25 ml) of the diluted solution were injected into the mice.

### Treatment schedule<sup>[18-20]</sup>

Swiss albino mice weighing 18-25 gms were divided into five groups each consisting of 12 animals (n = 12).

The groups under treatment were designated as follows.

Group 1 -Served as normal (Saline)

Group 2 -Served as tumour control ( $1 \times 10^6$  EAC cells/ml)

Group 3 -Received ethanolic extract 100 mg/kg orally

Group 4 -Received ethanolic extract 200 mg/kg orally

Group 5-Served as reference standard (5 Fluorouracil-20 mg/kg IP)

➤ All the treatment was continued for 14 days

➤ After the last dose of 18 hour fasting six mice from each group were sacrificed for the study of anti-tumour activity and Hematological parameters.

➤ The rest of animal group were kept to check the mean survival time (MST) and increase in the lifespan (% ILS) of tumour scaring mice.

### Tumour growth response

Anticancer effect of Ethanolic Extract of *Premna corymbosa* Rottl, (EEPC) was assessed by observation of change with respect to body weight, ascites tumour volume, viable and non-viable tumour cell count, Mean survival time (MST) and percentage increase in life span (% ILS).

### Tumour volume and count

Six mice from each group were sacrificed at the end of 14 days and tumour volume was measured. The tumour smears was prepared in slide and were stained with Leishmans stain, the tumour cell were examined under light microscopy and the hemogram was determined.

### Hematological studies

For the hematological studies the blood was obtained from the freely flowing tail vein blood, 18 hrs after the last dose. For the total count of Red blood cells and white blood cells, the blood was drawn in to RBC and WBC pipette respectively, then diluted and counted in a Neuber counting chamber. The Hemoglobin concentration was determined by using sahli's hemoglobin meter. Deferential count of Leukocytes was done on freshly drawn blood film using Leishman's stain A. The RBC, WBC, Hb%, and differential count were estimated from the normal, EAC (control), standard and ethanolic extract of *Premna corymbosa* Rottl. (EEPC) treated groups<sup>[21,22]</sup>.

## RESULTS

**Pharmacological studies** were performed stepwise starting from acute toxicity studies and then screening of anticancer activities simultaneously in detail.

**Acute oral toxicity studies** were performed for ethanolic extract following OECD guidelines 420. Fixed dose procedure

where fixed dose levels of extracts starting from 50,20,200,500,1000 increasing upto 2000 mg/kg body weight was given and sign and symptoms of toxicity was observed for next 48 hrs no toxicity or death was observed in experimental rats and LD50 of extracts was found greater than 2000 mg/kg. Hence the biological dose was fixed 200 mg/kg of ethanolic extract.

### Screening of anticancer activity

The present investigation indicates that the ethanolic extract of *Premna corymbosa* Rottl. (EEPC) showed significant anticancer activity in EAC bearing mice. The effects of EEPC at the dose of 100 mg/kg and 200 mg/kg on various parameters are shown as follows.

### Effect of mean survival time and tumour growth

Treatment with ethanolic extract of *Premna corymbosa* Rottl. (EEPC) at the doses of 100 and 200 mg/kg significantly reduced the tumour volume, and viable cell count in a dose dependent manner as compared to that of EAC control groups. Furthermore, nonviable tumour cell counts at different doses of EEPC were increased when compared with the EAC control group as shown in **Table 1**.

**Table 1.** Effect of the ethanolic extract of leaf of *Premna corymbosa* Rottl (Eepc) on tumour growth of EAC bearing mice.

Groups	EAC Control	Ethanolic Extract (100 mg/kg)	Change over control (%)	Ethanolic Extract (200 mg/kg)	Change over control (%)	5 Fluoro uracil (20 mg/kg)	Change over control (%)
Tumour volume (ml)	4.8 ± 0.94	3.4* ± 0.93	29.16	2.3**± 0.1	52.3	1.9**	60.41
Viable tumour count ×10 <sup>7</sup> cells/ml	10.4 ± 0.31	5.2*± 0.05	50	3.5** ± 0.08	66.34	2.8** ± 0.17	73.07
Non-viable tumour cells count × 10 <sup>7</sup> cells/ml	0.4 ± 0.03	0.5 ± 0.03	25	0.7 0.04	75	0.8 ± 0.01	100

Value are mean ± SEM. Number of mice in each groups (n = 6)

\*P < 0.01 vs. Control

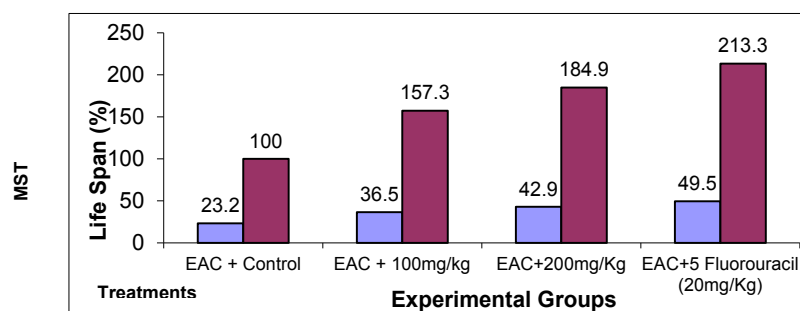
\*\* P < 0.001 vs. Control by students 't' test

**Table 2.** Effect of ethanolic extract of leaves of *Premna corymbosa* Rottl. (eepc) on mean survival time on EAC bearing mice.

Experimental Groups	Mean Survival Time (days) (MST)	Life span (%)	Increase Life span
EAC + Control	23.2 ± 1.51	100	-
EAC + 100 mg/kg	36.5 ± 2.9	157.3	57.3
EAC + 200 mg/kg	42.9 ± 3.9	184.91	84.9
EAC + 5 Fluorouracil (20 mg/kg)	49.5 ±4.1	213.36	113.3

Value are mean ± SEM. Number of mice in each groups (n = 6) P < 0.01, Experimental Groups compared with control

The effect of EEPC at the dose of 100 mg/kg and 200 mg/kg on the mean survival time and % increase in life span of EAC bearing mice was shown in **Table 2 and Graph 1**. In the EAC control group the mean survival time was 23.2 ± 1.51 days, while it increased to 36.5 ± 2.9 (100 mg/kg) and 42.9 ± 3.9 (200 mg/kg) days, respectively in the EEPC treated groups, where as the standard drug 5 Fluorouracil (20 mg/kg), treated group had a mean survival time of 49.5 ± 4.1 days.



**Graph 1.** Graphical representation of ethanolic extract of leaves of *Premna corymbosa* Rottl. (eepc) on mean survival time of mice.

### Effect on hematological parameter

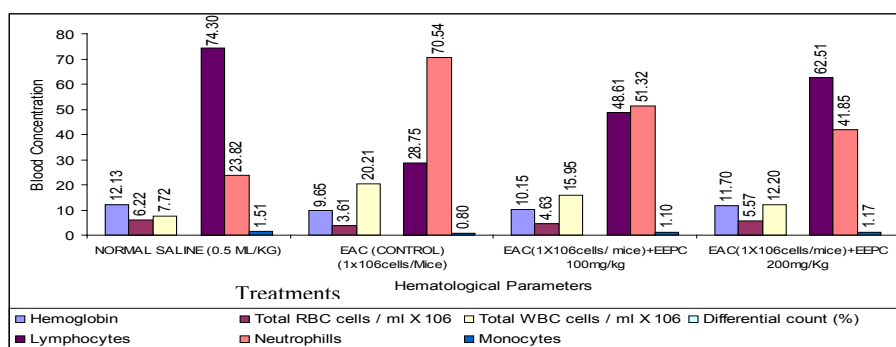
As shown in **Table 3 and Graph 2** the hemoglobin content in EAC control mice (9.65 g%) was significantly decreased when compared with normal mice. EEPC at the dose of 100 and 200 mg/kg the hemoglobin content in EAC treated mice were increased (10.15 g and 11.70 g% respectively). Moderate change in the RBC count was observed in the extract treated mice. The total WBC count was significantly increased in EAC bearing mice as compared to normal mice; whereas, EEPC treated mice significantly

decreased the WBC count as compared to control. In differential leukocyte count, the percentage of lymphocyte decreased and percentage of Neutrophils increase in EAC control; while EEPC treated mice, lymphocytes was increase and Neutrophils was decreased significantly as compared with EAC control.

**Table 3.** Effect of ethanolic extract of leaves of *Premna corymbosa* Rottl.(eepc) on hematological parameters of EAC treated mice.

Parameters	Normal saline (0.5 ml/kg)	EAC (Control) (1 × 10 <sup>6</sup> cells/mice)	EAC (1 × 10 <sup>6</sup> cells/mice) + EEPC 100 mg/kg	EAC (1 × 10 <sup>6</sup> cells/mice) + EEPC 200 mg/kg
Hemoglobin (g%)	12.13 ± 0.12	9.65 ± 0.32	10.15 ± 0.12	11.70 ± 0.15
Total RBC cells/ml × 10 <sup>6</sup>	6.22 ± 0.09	3.61 ± 0.12	4.63 ± 0.13	5.57 ± 0.21*
Total WBC cells/ml × 10 <sup>6</sup>	7.72 ± 0.06	20.21 ± 1.68*	15.95 ± 1.16	12.20 ± 0.07
Lymphocytes	74.30 ± 1.54	28.75 ± 1.32*	48.61 ± 2.14*	62.51 ± 1.94*
Neutrophills	23.82 ± 1.6	70.54 ± 0.92*	51.32 ± 1.93	41.85 ± 3.40*
Monocytes	1.51 ± 0.02	0.80 ± 0.02	1.10 ± 0.08*	1.17 ± 0.04

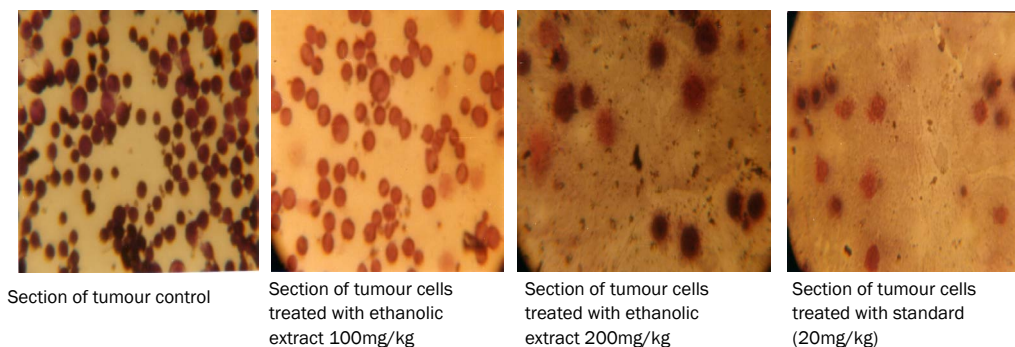
Value are mean ± SEM. Number of mice in each groups (n= 6) EAC control group compared with normal group experimental group compared with EAC control, P < 0.01, \* P < 0.05



**Graph 2.** Graphical representation of ethanolic extract of leaves of *Premna corymbosa* Rottl. (eepc) on hematological parameters.

**Viable and non-viable cell count**

For viable and non-viable cell counting, the ascites cell were stained by the trypan blue (0.4 % in normal saline), dye exclusion test and count was determined in a Neuber counting chamber. The cells that did not take up the dye were viable and those that took the stain were non-viable (**Figure 1**).



**Figure 1.** Effect of ethanolic extract of leaves of *Premna corymbosa* Rottl. on tumour cells (EAC).

**DISCUSSION**

Herbal Medicine sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body. Herbalists use the leaves, flowers, stems, berries, and roots of plants to prevent, relieve, and treat illness. From a "scientific" perspective, many herbal treatments are considered experimental. The reality is, however, that herbal medicine has a long and respected history. Many familiar medications of the twentieth century were developed from ancient healing traditions that treated health problems with specific plants. Today, science has isolated the medicinal properties of a large number of botanicals, and their healing components have been extracted and analyzed. Many plant components are now synthesized in large laboratories for use in pharmaceutical preparations. For example, vincristine (an antitumour drug), digitalis (a heart regulator), and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants. The leaf of *Premna corymbosa* Rottl was selected for our project, on the basis of ethanobotanical information which reveals its use against one of the most hazardous disease cancer. Literature survey showed that very less work has been performed on this plant and ethnobotanical information about the uses of the leaf part of the plant showing activities against various diseases. So we can validate scientifically for folk claim for its therapeutic activity.

The present study was carried out to evaluate the anticancer effect of EEPC in EAC bearing mice. The EEPC treated mice at the dose of 100 and 200 mg/kg significantly inhibit the tumour volume, tumour cell count and brought back the hematological parameter to more or less normal level. In EAC bearing mice, a regular rapid increase in ascites tumour volume was noted. Ascites fluid is the direct nutritional source for tumour cells and a rapid increase in ascitic fluid with tumour growth would be a means to meet the nutritional requirement of tumour cells. Treatment with EEPC inhibited the tumour volume, tumour cell count, and increased the percentage of trypan blue positive stained dead cells in tumour bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the life span of animals. The EEPC decreased the ascitic fluid volume, viable cell count and increased the percentage of life span. The anemia encountered in tumour bearing mice is mainly due to reduction in RBC or hemoglobin percentage, and this may occur either due to iron deficiency or due to hemolytic or myelopathic condition. Treatment with EEPC brought back the hemoglobin content, RBC and WBC count more or less to normal level.

## CONCLUSION

The acute oral toxicity studies following OECD guidelines-423, fixed dose procedure, showed that ethanolic extracts up to 2000 mg/kg are non-toxic and safe. The pharmacological studies of ethanolic extracts showed that it possessed anticancer activity to varying extent. Ethanolic extract showed these activities which might be due to flavonoids, triterpenoid and steroids present in the extract. The ethanolic extract (200 mg/kg) showed significant effect on blood parameters like RBC and hemoglobin percentage and also the prolongation of the life span of animals thus it could serve as good adjuvant to other anticancer agents and seems to be promising for the development of phytomedicines for cancer. Thus the result of present studies justifies the use of plant for treating cancer as suggested in folklore remedies. Further investigation on different biological activities of this plant with different modes will not only validate the types of activities claimed by ayurvedic, siddha and folk and traditional practitioners, but also will bring out innovations in the field of Pharmacy.

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