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# *In vivo* anticancer activity of *Clerodendrum serratum* (L) moon.

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**ABSTRACT**

The roots of *Clerodendrum serratum* commonly known as Bharangi is used in treatment of fevers, rheumatism and dyspepsia. Aqueous extract (AE) and methanolic extract(ME) of roots of *Clerodendrum serratum* were screened for *in vivo* anticancer activity using DLA cell model at the dose 100 mg and 200mg/kg body weight. The parameters were analyzed mean survival time, percentage increase in life span, body weight analysis, hematological parameters and biochemical parameters. The study revealed that methanolic extract exhibit significant anticancer activity as compared to aqueous extract.

**Key words:** Anticancer, Dalton's Lymphoma Ascites, *Clerodendrum serratum* (CS) MST (Mean Survival Time), %ILS (Increase in Life Span).

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## INTRODUCTION

Cancer is a disease in which there is an uncontrolled multiplication and spread, within the body, of abnormal forms of the body's own cells. Uncontrolled replication of cells causes a man of tissues to forms, commonly known as tumor. Cancer is a genetic term for a wide variety of malignant neoplasm that may result in deleterious effects on the host due to their invasive and metastasizing [1]. Drug discovery from medicinal plants has played an important role in the treatment of cancer and most clinical applications of plant secondary metabolites and their derivatives over the half century have been applied towards combating cancer. Of all available anticancer drugs between 1940 and 2002, 40% were natural products or natural product derived, with another 8% considered natural product mimics [2]. An analysis of the number of chemotherapeutic agents and their sources indicates that over 60% of approved drugs are derived from natural compounds [3]. Plants have a long history of use in the treatment of cancer, though many of the claims for the efficacy of such treatment should be viewed with some skepticism because cancer, as a specific disease entity, is likely to be poorly defined in terms of folkore and traditional medicine [4]. One of the best approaches in search for anticancer agents from plant resources is the selection of the plants based on ethno medical leads and testing the selected plants efficacy and safety in light of modern sciences [5]. *Clerodendrum serratum* Linn. commonly known as Bharangi belonging to family Verbenaceae is a large shrub with quadrangular stems found in South India, Bangalore, Bellary and Chickmanglore [6, 7]. Pharmacognostic features of the roots showed that they were roughly circular in cross sectional outline with shallow fissures and thin membranous peeling of phellem tissue [8]. Beta-sitosterol, 24(s)-ethyl cholesta-5, 22, 25-trien-3 beta-ol, 5 hydroxy-7, 4'-dimethoxy flavone, luteolin, apigenin, scutellarine and urosolic acid were reported in the stem of *Clerodendrum serratum* [9]. They were reported to contain crude saponins and have a pungent, bitter and acrid taste. Traditionally, they were used in treatment of fevers, rheumatism, dyspepsia, to relieve nausea, malaria and tumors [6, 10, 11, 12, and 13]. Owing to such importance of the plant in the traditional medicine, the present study was aimed to screen its antitumor activity using DLA (Dalton's Lymphoma Ascites) model.

## MATERIALS AND METHODS

### Plant Material

The plant was collected from Nagercoil, Dist. Kanyakumari, Tamilnadu and was identified and authenticated at Chunkankadi, Tamilnadu by Dr.Girija Kumari. The specimen was retained at J.S.S. College of Pharmacy, Ooty. The roots were cleaned, shade dried and pulverized. The 250g powdered roots of the plant were subjected to the cold maceration using methanol and distilled water and was kept in solvent for 7 days with intermittent shaking. The individual extracts were concentrated under the reduced pressure to obtained dry viscous mass. The semisolid material was kept in vacuum dessicator for drying. The percentage yield of crude extract was 2.81 and 10.63%w/w using methanol and distilled water (aqueous) respectively.

**Animals**

Studies were carried out using Swiss Albino mice. They were obtained from animal house of J.S.S. College of Pharmacy, Ooty. The mice were grouped and house in polyacrylic cages and maintained under Std. Laboratory conditions. They were allowed free access to Std. dry pellet diet and water *ad libitum*. All procedures were carried out in strict accordance with the guidelines prescribed by the committee for the purpose of control and supervision on experimentation on animals were approved by the IAEC (JSSCP/IAEC/M.Pharm/PH.COG/06/2006-07)

**Tumor cells**

The DLA cells were procured from National Cell Sciences, Pune and maintained in the Biotechnology department at J.S.S. College of Pharmacy, Ooty.

**Maintenance of DLA cell lines**

The DLA cells were propagated in the peritoneal cavity of the mice by injecting $10^6$ cells. The cells were aspirated aseptically from developed tumor mice, during the log phase on the $15^{th}$ day of tumor transplantation using 18 gauge needle by withdrawing the fluid from peritoneal cavity. The ascitic fluid was washed three times in PBS (phosphate buffer saline) and the cell pellet was resuspended in PBS. The tumor cell count was done using tryphan blue dye exclusion method in a heamocytometer. The cell suspension was diluted to get $2x10^6$/0.1 ml.

***In vivo* anticancer activity**

The inhibition or reduction of tumor development of aqueous and methanolic extract was determined by Dalton's Lymphoma Ascites (DLA cell model).

Male Swiss Albino mice were divided into 7 groups (n = 6). All the groups were injected with DLA cells (0.2 ml 2 X $10^6$ cells/mice) intraperitoneally except normal group. This was taken at day zero. The group first was normal group receiving normal saline. The next group was cancer control (DLA control). Std. group receiving cyclophosphamide (25 mg/kg/day i.p.) [14]. The aqueous and methanolic extract of *Clerodendrum serratum* at different doses (100 and 200mg/kg/day) [25] were administered in groups 4, 5, 6, and 7 respectively for 14 days orally. After the last dose, for the study of extract, the blood was collected for hematological and biochemical parameters.

**Determination of MST and % ILS** [15].

At the end of the treatment, the survival time of DLA tumor bearing mice were counted and the average survival time and the % increase in life span (%ILS) were calculated

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$$\% \text{ ILS} = \frac{\text{MST of treated group} - \text{MST of control group}}{\text{MST of the control group}} \times 100$$

**Body weight analysis** [16]

All mice were weighed on the day of tumor inoculation and the weekly intervals until survival of animals. Body weights of mice were recorded daily. Average body weight and % decrease in body weight was calculated with formula

$$\% \text{ Decrease in body weight} = \frac{\text{Gain in body weight of control} - \text{Gain in body weight of treated group}}{\text{Gain in body weight of control}} \times 100$$

**Heamatological parameters** [17]

Effect of extracts and standard drug on hematological parameters on DLA tumor model on 14$^{th}$ day of tumor inoculation was determined.The hematological parameters were analyzed by using cell analyzer. The parameters were analyzed RBC, WBC and Hb.

**Biochemical parameters** [18]

Blood was collected on 14$^{th}$ day of tumor inoculation by sino orbital puncture and the serum was separated at 2000 rpm for 30 minutes. The effects of extract drug, standard drug on the various biochemical parameters like ALAT, ASAT and Total Protein were determined.

**Statistical analysis**

The experimental results were expressed as ± S.E.M. Data was assessed by one way ANOVA followed by t-test, p-value $p< 0.05$ was considered as statistically significant.

## RESULT

**Mean survival and % ILS**

The MST was significant increased to 29 ± 0.63 (% ILS = 30.63) and 30.2 ± 0.48 (% ILS = 36.03) on administration of 100 mg ME and 200 mg ME respectively when compared with cancer control group. 200 mg ME 30.2 ± 0.48 ($P <0.001$) shown significant increase in MST when compared with 200 mg AE treated group 26 ± 0.63 (% ILS = 17.11). 200 mg ME 30.2 ± 0.48 ($P <0.001$) shown significant increase in MST when compared with std. control group. The results are compiled in Table no.1

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**Table no. 1. Effect of AE and ME of CS on Mean Survival Time and % ILS of DLA bearing mice.**

| Groups | Mean Survival Time | % ILS |
|---|---|---|
| Cancer control | 22.2 ± 0.58 | - |
| Std. Control | 32.4 ± 0.40 *** | 45.94 |
| 100 mg AE | 24 ± 0.63 | 8.10 |
| 200 mg AE | 26 ± 0.63** | 17.11 |
| 100 mg ME | 29 ± 0.63*** | 30.63 |
| 200 mg ME | 30.2 ± 0.48*** | 36.03 |

**Values are mean S.E.M., n= 6, * p< 0.05, ** p< 0.01, *** p< 0.001.**

## Body weight analysis

The average body weight was found to have a significant increase in the cancer control group (40.4 g) as compared with the normal group. Treatment with 100 mg ME (35.4 g) ($p<0.05$) and 200 mg ME (32.8 g) ($p <0.001$) in DLA bearing mice it was found that significant reduction in the body weight when compared with cancer control group. On administration of std. drug significant reduction in body weight in the std. control group ($P <0.001$) was observed. 200 mg ME treated group significantly ($p <0.001$) reduced the body weight when compared with all other extract treated groups. The results are compiled in Table no.2

**Table no. 2. Anticancer effect of AE and ME of Clerodendrum serratum on Body Weight of DLAbering mice.**

| Groups | Average body weight in gms |
|---|---|
| Normal | 30.0 ± 0.90 |
| Cancer control | 40.4 ± 0.74*** |
| Std. Control | 30.2 ± 1.24*** |
| 100 mg AE | 38.2 ± 0.67 |
| 200 mg AE | 37.8 ± 0.96 |
| 100 mg ME | 35.4 ± 1.07* |
| 200 mg ME | 32.8 ± 1.02*** |

**Values are mean S.E.M., n= 6, * p< 0.05, ** p< 0.01, *** p< 0.001.**

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**Hematological parameters**

The RBC count in the cancer control group was decrease as compared to the normal group. Treatment with 100mg AE and 200mg AE increased the RBC count to more or less normal levels. Also treatment with 100mg ME and 200mg ME treated group increased the RBC count to normal levels. Treatment with standard drug shown increase in RBC count in the Std. Control group. 200 mg ME treated group had shown increase in RBC count when compared with all other extract treated groups. The total WBC count was found to have increased significantly in the cancer control group when compared with normal group ($p < 0.001$).

Administration of 100mg AE and 200mg AE in DLA bearing mice reduced the WBC count as compared with cancer control group. Also administration of 100 mg ME ($P < 0.01$) and 200mg ME ($p < 0.001$) in DLA bearing mice significantly reduced the WBC count as compared with cancer control group. 200mg ME treated group had shown significantly reduced in the WBC count when compared all other extract treated groups.

The hemoglobin content in cancer control group was significantly ($p<0.001$) decreased as compared to the normal group. Treament with 100mg ME ($p<0.05$) and 200mg ME ($p<0.001$) treated group significantly increased the hemoglobin content to more or less equal to normal levels. On treatment with standard drug significant increase in hemoglobin content in Std. control group ($P<0.001$) was observed. 200mg ME treated group ($p<0.001$) had shown significant increase in hemoglobin content when compared with Std. control groups. The results are compiled in Table no.3.

**Table no 3. Effect of AE and ME extract of *Clerodendrum serratum* on hematological parameters of DLA bearing mice.**

| Groups | RBC ($X10^9$/ml) | WBC ($X10^6$/ml) | Hb (g %) |
|---|---|---|---|
| Normal | 12.03 ± 0.30 | 7.64 ± 0.56 | 14.46 ± 0.43 |
| Cancer control | 7.33 ± 0.45 | 16.48 ± 0.53*** | 8.7 ± 0.74*** |
| Std Control | 10.59 ± 0.28 | 8.32 ± 0.39*** | 13.9 ± 0.44*** |
| 100 mg AE | 8.49 ± 0.50 | 15.34 ± 0.37 | 9.02 ± 0.77 |
| 200 mg AE | 9.32 ± 0.87 | 12.78 ± 2.16 | 9.94 ± 0.35 |
| 100 mg ME | 9.69 ± 0.66 | 10.96 ± 0.27** | 10.16 ± 0.20* |
| 200 mg ME | 10.25 ± 0.48 | 9.44 ± 0.29*** | 12.94 ± 0.41*** |

**Values are mean S.E.M., n= 6, * p< 0.05, ** p< 0.01, *** p< 0.001.**

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**Biochemical parameters**

In the biochemical parameters ALAT, ASAT and Total protein activity was analysed. The ALAT activity was found to be significantly increased in the cancer control group when compared with the normal group ($p<0.001$). Administration if 100 mg AE and 200 mg AE ($p<0.001$) significantly reduced the ALAT activity in DLA bearing mice as compared with cancer control group. Also, Administration of 100 mg ME ($P<0.001$) and 200 mg ME ($P<0.001$) in DLA bearing mice significantly reduced the ALAT activity as compared with cancer control group. 200 mg ME had shown significant reduction in ALAT activity when compared with all other extracts treated groups.

The ASAT activity was found to be significantly increased in the cancer control group when compared with the normal group ($p<0.001$). Treatment with 100 mg AE ($p<0.001$) and 200 mg AE ($p<0.001$) significantly reduced the ASAT activity when compared with cancer control group. Also treatment with 100 mg ME and 200 mg ME ($p<0.001$) significantly reduced the ASAT activity as compared with cancer control group. 200 mg ME ($p<0.001$) had shown significant reduction in ASAT activity when compared with all other extract treated groups.

The total protein was found to be increased significantly in the cancer control group when compared with the normal group ($p<0.001$). Administration of 100 mg AE and 200 mg AE treated groups had shown reduction in the total protein as compared with cancer control. Also Administration of 100 mg ME ($p<0.001$) and 200 mg ME ($p<0.001$) treated groups in DLA bearing mice significantly reduced the total protein as compared with cancer control group. 200 mg ME treated group had shown significant reduction in total protein when compared with all other extract treated groups. The results are compiled in Table no.4

**Table no. 4. Anticancer effect of AE and ME of CS on Biochemical parameters of DLA bearing mice.**

| Groups | ALAT (U/L) | ASAT (U/L) | Total Protein(g/dl) |
|---|---|---|---|
| Normal | 32.2 ± 0.58 | 85.6 ± 0.81 | 3.4 ± 0.24 |
| Cancer control | 50.8 ± 0.86*** | 103.6 ± 1.20*** | 7.4 ± 0.24*** |
| Std. control | 33.6 ± 0.58*** | 88.8 ± 0.73*** | 4.4 ± 0.24*** |
| 100mg AE | 48.8 ± 0.86 | 97.6 ± 0.92*** | 7.2 ± 0.20 |
| 200mg AE | 44.6 ± 0.97*** | 90.8 ± 0.58*** | 6.6 ± 0.24 |
| 100mg ME | 40 ± 1.04*** | 87 ± 0.70*** | 5.4 ± 0.24*** |
| 200mg ME | 35.6 ± 1.20*** | 82.6 ± 1.24*** | 4.6 ± 0.24*** |

**Values are mean S.E.M., n= 6, * p< 0.05, ** p< 0.01, *** p< 0.001.**

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## DISCUSSION

In the present *in vivo* anticancer study of roots of *Clerodendrum serratum* was evaluated against a Dalton's lymphoma ascites. The reliable criteria for judging the value of any anticancer drug is the prolongation of life span of animal and disappearance of leukemic cells from blood [18]. In DLA ascite model following the inoculation of tumor cell lines (DLA) a marked decrease in life span and increase in body weight of mice were observed. Following the administration of AE and ME, in ME treated groups had shown the MST and % ILS were considerable increased in MST and duration of life span and the body weight was significantly decreased. (Due to reduction in Ascitic fluid volume). Ascites fluid is the direct nutritional source to tumor growth could possibly be a makes to meet more nutritional requirement of tumor cells. A regular rapid increase in ascites tumor volume was noted in tumor bearing mice [19]. Linear progression in the body weight of tumor bearing mice with advancement of duration was observed. Such increase in body weight was significantly retarded following the ME treatment. Most anticancer drugs are antiproliferative and will also affect radically dividing normal cells. Bone marrow depression occurs within 10-14 days after a single dose of a cytotoxic drug indicated by a fall in circulating leukocytes and platelets. This may lead to bleeding disorders, increased susceptibility to infection and bone marrow aplasia [20, 21]. The elevated WBC count in tumor bearing mice was significantly reduced by ME treatment. The ME treatment inhibits tumor cell growth, enhances the survival of treated mice and restore the hematological parameters.

Enzymes in serum have been studied for many years as possible early indicators of neoplasia and as aids in following the progression and regression of disease [22]. It was reported that the presence of tumor in the humans or experimental animals is known to affect many functions of the vital organs especially in the liver, even when the site of the tumor does not interfere directly with organ functions [23]. There are a number of possible mechanisms for the appearance of abnormal activities of enzymes in serum [24].

These includes:-

a. Over production of enzyme by the tumor.
b. Tumor blockage of the duct system through which enzyme passes into the blood.
c. Induction of enzyme by the presence of tumor
d. Change in permeability of the cell allowing leakage of soluble enzymes into the circulation.

In the study, the ME treated groups shown significant decrease in ALAT and ASAT activities. The tumor bearing mice group had shown the increase in the total protein content [17].

In conclusion the *in vivo* anticancer study confirmed that the ME of *Clerodendrum serratum* exhibits anticancer activity at the dose 100mg and 200mg/kg body weight. Further studies are currently in way to isolate and characterize the active principle responsible for antitumor activity.

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**Fig. no. 1. Effect of AE and ME of *Clerodendrum serratum* on Mean survival time of DLA bearing mice.**

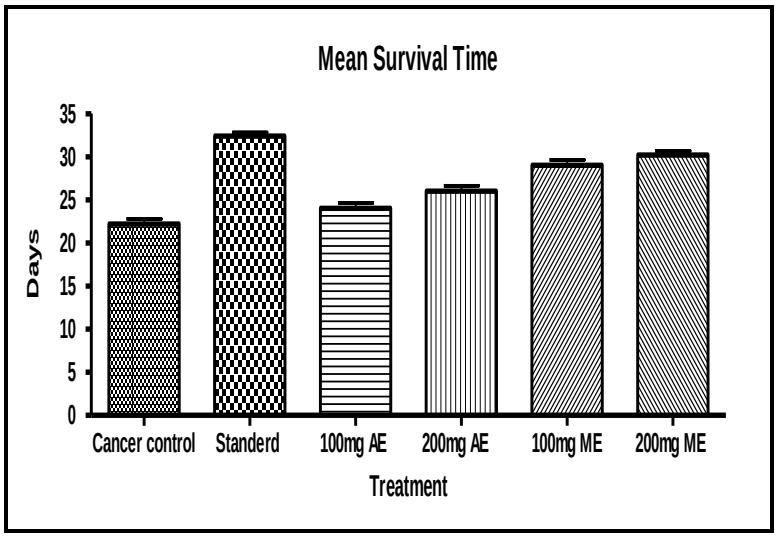
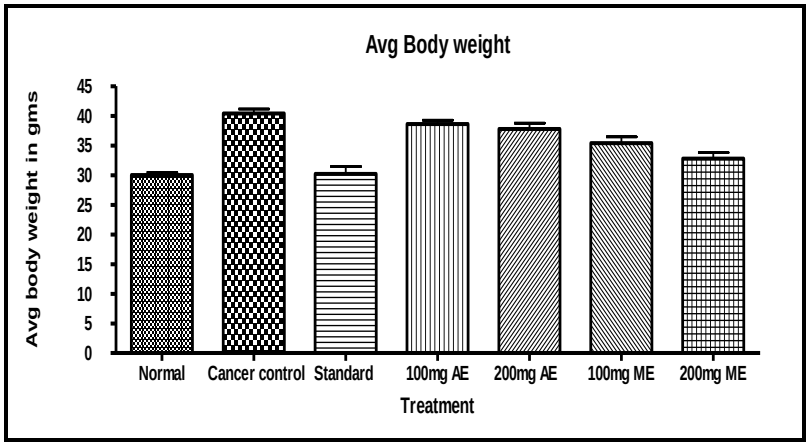



**Fig.no.2. Effect of AE and ME of *Clerodendrum serratum* on body weight of DLA bearing mice**.

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