ORIGINAL ARTICLE

# Antitumour activity of ethanolic extract of leaves of *Holoptelea integrifolia* on Dalton's ascitic lymphoma in Swiss albino mice

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

**Key words:** *Holoptelea integrifolia*, Dalton's ascitic lymphoma, lifespan, tumour volume

## INTRODUCTION

A number of natural products have been studied for anticancer activity on various experimental models. This has resulted in the availability of nearly 30 effective anticancer drugs.[1] *Holoptelea integrifolia*, Planch (Family: Ulmaceae) is a large deciduous tree distributed throughout the greater part of India. The plant is popularly known as Aya in Tamil.[2] Some recent explorations to appraise its use in traditional medicine have been reported in which antiviral,[3] antioxidant, antimicrobial and wound healing activity[4] were comprehensively emphasized. Ethno medically, the leaves and stem bark of this plant were used by tribals for skin diseases, obesity[5] and in the management of cancer.[6] This study aims to evaluate antitumour activity of ethanolic extract of leaves of *Holoptelea integrifolia* (EHI) on Dalton's ascitic lymphoma (DAL) in Swiss albino mice.

## MATERIALS AND METHODS

### Plant Material and Extraction

The leaves of *Holoptelea integrifolia* Planch. were collected from the foot hills of Yercaud, Tamil Nadu, India, in the month of September, 2008, and identified and authenticated at the Plant Anatomy Research Centre (PARC), Pharmacognosy Institute, Chennai, India. A voucher specimen (HIP/Ph/2008/46) has been kept in our laboratory for future reference. The air-dried and coarsely powdered leaves (400 g) were extracted successively with 1.5 L each of petroleum ether (60-80°C) and ethanol in a Soxhlet extractor for 72 hours. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40-50°C). The petroleum ether extract yielded a yellowish green sticky semisolid, weighing 3 g (3%). The ethanol extracts yielded brown and semi-solid residues weighing 7.0 g (7.0%).

### Animals

The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the SRM University, Kattankulathur, India. Mature male Swiss albino mice weighing 20-25 g were housed in standard isolation cages (45×35×25 cm) under environmentally controlled conditions with 12-hour light/12-hour dark cycle. They were allowed free access to water, standard laboratory chow (Hindustan Liver Pvt. Ltd, Mumbai) given food and water *ad libitum*. After a sufficient period of acclimatization, they were used to evaluate antitumour activity.

### Tumour Cell Line

Dalton's ascitic lymphoma (DAL) cells were obtained through the courtesy of the Cancer Research Centre, Adyar, Chennai, India. DAL cells were maintained by weekly intraperitoneal (i.p.) inoculation of $1\times10^6$ cells/mouse.[7]

### Antitumour Activity

After acclimatization, mature male Swiss albino mice were divided into four groups (n=10). All the groups [Table 1], except group I, were injected with DAL cells ($1\times10^6$ cells/mouse.i.p.). This was taken as day 0. Group I served as

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**Received:** 22-04-2009; **Accepted:** 06-10-2009; DOI: 10.4103/0973-8258.62164

**Table 1: Effect of ethanolic extract of *Holoptelea integrifolia* on survival time, life**

| Span, tumour volume, viable and non-viable cell count in dal bearing mice | Servival time (Days) | Increase of life span (%) | Tumour volume (ml) | Viable cell count × $10^6$ cells/ml | Non-viable cell count × $10^6$ cells/ml |
|---|---|---|---|---|---|
| Normal saline (5 ml/kg p.o) | - | - | - | - | - |
| DAL control ($1\times10^6$ cell) | 21.40±1.41 | - | 3.68±0.11 | 9.42±0.14 | 3.41±0.21 |
| DAL ($1\times10^6$ cell)+ EHI (250 mg/kg p.o) | 30.81±1.02* | 43.50 | 2.40±0.11* | 3.89±0.04* | 1.91±0.09* |
| DAL ($1\times10^6$ cell)+ EHI (500 mg/kg p.o) | 35.64±1.05* | 66.35 | 1.01±0.04* | 2.62±0.09* | 2.14±0.19* |

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

normal saline control (5 ml/kg, p.o.) and group II served as DAL bearing control. On day 1, the EHI at a dose of 250 and 500 mg/kg body weight to the Group III and IV were administered orally and continued for 14 consecutive days respectively. The dose of EHI was selected based on our previous study on anti-inflammatory activity.[8] On day 15, five mice of each group were sacrificed 24 hours 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 tumour hosts. The effect of ethanol extract on tumour growth and host's survival time were monitored by studying parameters like tumour volume, tumour cell count, viable tumour cell count, nonviable tumour cell count, mean survival time and increase in life span.[7,9]

**Determination of Tumour 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 five minutes.

**Determination of Tumour Cell Count**

The ascitic fluid was taken in a hematocrit (micro) tube and diluted 1000 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the cells in 64 small squares were counted.

**Estimation of Viable Tumour Cell Count**

The cells were then stained with 0.4% Trypan blue in physiological saline. The dye was counted as viable and nonviable cell count.

**Percent of Increase in Life Span**

Recording of mortality monitored the effect of the EHI on tumour growth and per cent of increase in the life span (ILS %) were calculated.[10]

ILS (%) = [(Mean survival of treated group/ Mean survival of control group) -1] ×100. Mean survival time = [1$^{st}$ Death + Last Death]/2

**Hematological Studies**

Red blood cell count (RBC), hemoglobin content and white blood cell (WBC) counts were measured from freely flowing tail vein blood. WBC differential count was carried out from Leishman stained blood smears. Protein conc. was estimated by Lowry's method.[11] One millilitre of peritoneal fluid was withdrawn and centrifuged at 3000 rpm for 30 min according to the method described by Docie *et al*.[12]

**Statistical Analysis**

The experimental results were expressed as the mean±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, indicate the presence of alkaloids, glycosides, sterols, flavonoids, tannins and saponins. The effect of EHI on the survival of tumour bearing mice showed MST (% ILS) [Table 1] for the tumour control group (DAL treated) to be 21.40±1.41 days, while it was 30.81±1.02 days (43.50%) and 35.64±1.05 days (66.35%) for the group treated with EHI at the dose of 250 and 500 mg/kg respectively. The average number of tumour volume [Table 1] in DAL treated animals was found to be 3.68±0.11. EHI treatment at both dose levels significantly ($P$<0.05) reduced tumour volume, which was found to be 2.40±0.11 and 1.01±0.04 respectively. Viable cell count of the tumour bearing mice was significantly decreased while non-viable cell count were increased in EHI 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 hemoglobin and RBC. In a differential count of WBC, the per cent of neutrophils increased while the lymphocyte count decreased. At the same time interval, EHI treatment could change those altered parameters to

**Table 2: Effect of ethanolic extract of *Holoptelea integrifolia* on hematological parameters in DAL bearing mice**

| Treatment group | Hb (g %) | RBC (million/mm$^3$) | WBC (10$^3$cells/mm$^3$) | Proteins (g %) | PCV (mm) | Differential count % | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Lymphocytes | Neutrophils | Monocytes |
| Normal saline (5 ml/kg) | 14.5±0.2 | 6.5±0.2 | 7.2±0.2 | 8.5±0.2 | 17.8±0.7 | 70.2±1.31 | 29.8±1.1 | 2.2±0.4 |
| DAL control (1×10$^6$ cell) | 7.8±0.6$^{a**}$ | 3.8±0.1$^{a**}$ | 15.2±1.3$^{a**}$ | 14.6±1.4$^{a**}$ | 27.5±0.4$^{a**}$ | 30.3±0.4$^{a**}$ | 68.6±1.6$^{a**}$ | 3.8±0.5$^{a*}$ |
| DAL (1×10$^6$ cell)+ EHI (250 mg/kg p.o) | 10.2±0.6$^{b**}$ | 5.1±0.5$^{b**}$ | 11.2±0.7$^{b*}$ | 11.8±0.1$^{b*}$ | 21.4±0.4$^{b**}$ | 55.8±1.1$^{b**}$ | 42.1±1.3$^{b**}$ | 2.9±0.4$^{ns}$ |
| DAL (1×10$^6$ cell)+ EHI (500 mg/kg p.o) | 12.4±0.4$^{b**}$ | 5.8±0.3$^{b**}$ | 8.6±0.7$^{b**}$ | 9.2±0.1$^{b**}$ | 18.4±0.1$^{b**}$ | 67.3±2.1$^{b**}$ | 30.1±2.2$^{b**}$ | 2.8±0.3$^{ns}$ |

Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's test. *$P$<0.05, **$P$<0.01, $^{ns}P$>0.05; a *vs*. Normal group, b *vs*. DAL control. n=5.

near normal.

## DISCUSSION

The reliable criterion for judging the value of any anticancer drug is the prolongation of lifespan of the animal and decreased WBC count from blood.[13,14] The above results demonstrated the antitumour effect of EHI against DAL is Swiss albino mice. A significant ($P$<0.05) percent increase in the life span and non-viable cell count in peritoneal exudates ($P$<0.05) was observed due to EHI treatment. To evaluate whether EHI treatment indirectly inhibited tumour cell growth, the effect of EHI treatment was examined on the viable and non-viable cell counts against tumour bearing mice. Normally, each mouse contains about 5 × 10$^6$ intraperitoneal cells, 50% of which are macrophage. EHI treatment was found to enhance non-viable cell counts in peritoneal exudates and decrease the viable cell count. It might be due to the absorption of EHI by viable cells which leads to lysis of cell through to the activation of macrophages or some cytokine production in peritoneal cavity.

Usually, in cancer chemotherapy, the major problems encountered are myelosuppression and anaemia.[15,16] The results have, however, clearly shown that EHI 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 EHI. After 14 days of transplantation, EHI-treated groups were able to reverse the changes in the hematological parameters consequent to tumour inoculation.

All these data point to the possibility of developing an ethanolic extract of leaves of *Holoptelea integrifolia* as a novel, potential agent in the area of cancer chemotherapy. The phytochemical study indicated the presence of flavonoids, alkaloids and terpenoids in EHI. Flavonoids have been shown to possess antimutagenic and antimalignant effects.[17,18] Further, flavonoids have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation[19] and angiogenesis.[20] According to the previous reports, *Holoptelea integrifolia* possess antioxidant ability.[4] Thus, antitumour effects produced by the EHI may be due to flavonoids as well as its antioxidant potential. The ethanolic extract of *Holoptelea integrifolia* restores the mean survival time and decreases tumour volume count in treated mice. Our study suggests that EHI possess potent anticancer activity and increase life span. Further studies to characterize the active principle and elucidate the mechanism of action of EHI are in progress using different cell lines.

## ACKNOWLEDGMENT

The financial assistance of SRM College of Pharmacy, SRM University, Kattankulathur, Tamil Nadu, India, is gratefully acknowledged.

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**Source of Support:** SRM College of Pharmacy, SRM University, Kattankulathur, Tamil Nadu, **Conflict of Interest:** None declared.