Antitumour Activity of *Elephantopus scaber* Linn Against Dalton's Ascitic Lymphoma

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The antitumour activity of the leaves of *Elephantopus scaber* has been evaluated against Dalton's ascitic lymphoma (DAL) in Swiss albino mice. A significant enhancement of mean survival time of *Elephantopus scaber* treated tumour bearing mice was found with respect to control group. *Elephantopus scaber* treatment was found to enhance peritoneal cell counts. When these *Elephantopus scaber* treated animals underwent i.p. inoculation with DAL cells, tumour cell growth was found to be inhibited. Fourteen days after transplantation, *Elephantopus scaber* treated group were able to reverse the changed in the haematological parameters, protein and PCV consequent to tumour inoculation.

The plant *Elephantopus scaber* Linn (Compositae) is a small herb, grows in throughout India, tropical Asia, Australia and America. This plant considered to be indigenous to India, has been used in the Indian medicinal system as an analgesic, diuretic, astringent and antiemetic [1,2]. Leaves of this plant are mainly prescribed for abdominal cancer in Siddha medicinal system[3]. The phytochemical review indicated the presence of elephantopin, triterpenes, stigmosterol epifriedelionol, lupeol and a mixture of triacontan-1-ol and dotriacontan-1-ol [4,5]. The present study is focussed on evaluation of the anticancer activity of the leaves of *Elephantopus scaber* against Dalton's Ascitic Lymphoma in mice.

The leaves of *Elephantopus scaber* were collected from Kolli Hills, Tamil Nadu, and were authenticated by the Botany department, Tamil Nadu Agriculture University, Coimbatore. A voucher specimen has been preserved in our laboratory. The powdered leaves were macertated by using water and the extract (ESE) was used for the present study.

Swiss albino mice (20-25 g) were used throughout the study. They were housed in standard microlon boxes and were given standard laboratory diet and water. Dalton's Ascitic Lymphoma (DAL) cell were obtained through the courtesy of Cancer Research Institute, Adayar, Chennai. DAL cells were maintained by weekly intraperitoneal inoculation of 10^6 cells/ mouse.

Animals were inoculated (i.p.) with 2×10^5 cells/ mouse in phosphate buffered saline on day 0 and treatment with ESE was started 24 h after inoculation, at a dose of 200 mg/kg/day i.p.[6] (group – A). The control group (group – B) was treated with same volume of 0.9% sodium chloride. All treatments were continued for 9 d. Median survivals times (MST) for each group, containing 9-11 mice, were noted. The animals surviving more than 60 d were considered to be cured. The antitumour efficacy of ESE(200 mg/kg/day i.p. for 9 d) was compared with that of 5-fluorouracil (5-FU 20 mg/kg/day i.p. for 9 d). MST were noted with reference control. Survival times of treated groups (T) were compared with those of control group(C).

Three group of normal mice (n=5) were used for determining the effect of ESE on normal peritoneal cells. One group was treated once with 200 mg/kg i.p. of ESE while the second group received the same treatment for 2 consecutive days. The untreated third group was used as control. Peritoneal exudate cells were counted 24 h after treatment for each of the treated group and compared with those of the untreated group.

In order to detect the influence of ESE on the

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TABLE 1: EFFECT OF INOCULATION WITH *ELEPHANTOPUS SCABER* AND 5-FU-TREATED DAL.

Treatment	Median survial Time (d)	Increase in Life Span (T/C %)
Elephantopus scaber (200 mg/kg,i.p.)	32	139.1 *
5FU (20 mg/kg, i.p.)	40	173.9
Sodium Chloride (0.9%)	23	100

Number of animal in each group were 10 and days of drug treatment were 9. *P<0.01 when compared to the control.

haemotological status of DAL bearing mice, comparisions were made amongst three groups (n=5) of mice on day 14 after transplantation. The three groups comprised of tumour bearing mice tumour bearing mice treated with ESE 200 mg/kg/day i.p. (9 d) and normal mice. Blood was drawn from each mouse in the conventional way and the white blood cell cound (WBC), red blood cell count (RBC), haemoglobin (HB), protein and packed cell volume (PVC) were determined[7,8]. The average of five determinations were computed.

The effect of ESE on the survival of tumour bearing mice showed the MST for the control group was 23 d, while it was 2 d and 40 d for the groups treated with ESE (200 mg/kg/day,i.p.) and 5-FU (20 mg/kg/day i.p.), respectively, as shown in Table 1. The average number of peritoneal exudate cells per normal mouse was found to be $5.8 \pm 0.8 \times 10^6$ (200 mg/kg i.p.), ESE treatment increased the number of peritoneal cells as shown in Table 2. Single treatment enhanced peritoneal cells to $8.7 \pm 1.8 \times 10^6$. While two consecutive treatments enhanced the number to $12.9 \pm 2.1 \times 10^6$.

TABLE 2: EFFECT OF *ELEPHANTOPUS SCABER* TREATMENT ON PERITONEAL CELL COUNT IN NORMAL MICE.

Treatment	Number of Peritoneal Cells/ Mouse x 10^6
Control	$5.8 \pm 0.8 \times 10^6$
Treated once	$8.7 \pm 1.8 \times 10^6$
Treated twice on two consecutive days	$12.9 \pm 2.2^* \times 10^6$

Number of animals in each group were 5 and the dose was 200 mg/kg/day i.p., *P<0.01 when compared to control. Values are expressed as mean ± SEM

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, protein and PCV were found to be increased with a reduction of the haemoglobin content of RBC. The total RBC count showed a modest change. In a differential count of WBC, the present of neutrophils increased while the lymphocyte count decreased. At the same time interval, ESE (200 mg/kg/day, i.p) treatment could restore those altered parameters to normal.

The reliable criterion for judging the value of any anticancer drug is the prolongation of life span of the animal and disappearance of leukemic cells from blood[9,10]. The

TABLE 3: EFFECT ON *ELEPHANTOPUS SCABER* ON HAEMATOLOGICAL PARAMETERS.

Treatment	Hb (g%)	Total RBC Cells/ml x 10^{10}	Total WBC Cells/ml x 10^6	Protein g%	PCV (mm)	Differential Count (%)		
						Lymphocytes	Neutrophils	Monocytes
Normal mice	16.5± 0.5	1.42±0.5	6.5±0.6	8.6± 0.3	17± 0.5	68± 2.2	30± 2.2	2±1.0
Tumour bearing mice (14d)	12.0±0.8	1.22±0.3	15.8± 0.3	12.40±0.5	26±0.5	26±31	68±5.1	1±0.7
Treated tumour bearing mice	14.4±0.8	1.32±0.5	8.5±0.5*	8.9±0.2	20±0.2	58±2.1*	41±2.4*	2±0.6

Number of animals in each group were 5 and the dose administered was 200 mg/kg/day.i.p., *P<0.01 when compared to control. Values are expressed as mean ± SEM

above results demonstrated the antitumour effect of ESE against DAL is Swiss albino mice. A significant enhancement of MST and peritoneal cells counts were observed (Tables 1 and 2).

To evaluate whether ESE treatment indirectly inhibited tumour cell growth, the effect of ESE treatment was examined on the peritoneal exudate cells of normal mice. Normally each mouse contains about $5x10^6$ intraperitoneal cells, 50% of which are macrophages. ESE treatment was found to enhance peritoneal cell counts. These results demonstrated the indirect effect of ESE in DAL cells, probably medicated through enhancement and activation of macrophages or through some cytokine product inside the peritoneal cavity produced by ESE treatment.

Analysis of the haematological parameters showed a minimum toxic effect in mice which were cured by ESE treatment. Fourteen days after transplantaion, ESE treated groups were able to reverse the changes in the haematological parameters consequent to tumour inoculation.

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New Spectrophotometric Methods for the Determination of Roxithromycin

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Two simple spectrophotometric methods (A and B) have been developed for the determination of roxithromycin in pure and its pharmaceutical formulations. Method A is based on the formation of a blood red coloured complex with ferric chloride and 1,10-phenanthroline with absorption maximum at 520 nm. In method B, roxithromycin forms blue coloured complex with Folin-Ciocalteu (FC) reagent in the presence of sodium carbonate exhibiting maximum absorption at 760 nm. The chromogens obey Beer's law in the concentration ranges of 2.5 to 40 µg/ml and 2.5 to 12.5 µg/ml for method A and B, respectively.

Roxithromycin is a broad spectrum semisynthetic macrolide antibiotic and chemically it is erythromycin-9-[O-(2-methoxyethoxy) methyl]oxime[1,2]. Very few analytical methods have been reported for the determination of roxithromycin which include HPLC[3-6] and visible spectrophotometric[7] methods. The authors have developed two simple sensitive and reproducible spectrophotometric methods (A and B) for the determination of roxithromycin.

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