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RESEARCH ARTICLE

Anticarcinogenic activity of *Leptadenia reticulata* against Dalton's Ascitic Lymphoma

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ABSTRACT

The aim of the present study is to evaluate the effect of ethanolic extract of leaves of *Leptadenia reticulata* leaves (LELR) against Dalton's Ascitic Lymphoma (DAL) in Swiss Albino mice. DAL cells were injected intraperitoneally (10^6 cells) to the mice. Two days after cells injection the animals were treated with 200 mg/kg of LELR for 8 days. Five-fluorouracil (20 mg/kg) was used as reference drug. On day 11, cancer cell number, packed cell volume, decrease in tumour weight of the mice, increase in life span and hematological parameters were evaluated and compared with the same parameters in control. A significant increase in the life span and a decrease in the cancer cell number and tumour weight were noted in the tumour-induced mice after treatment with LELR. The hematological parameters were also normalized by LELR in tumour-induced mice. These observations are suggestive of the protective effect of LELR against Dalton's Ascitic Lymphoma (DAL).

Keywords: *Leptadenia reticulata, Dalton's Ascitic Lymphoma, Anticancer agents*

Cancer is one of the ailments which cannot be completely subdued by chemotherapy. The chemotherapeutic agents though effective against various types of tumour are not totally free from side effects [1]. This fostered our attempts to evaluate some plant products against cancer, as they are less likely to cause serious side effects [1]. Many Indian plants like black pepper, asafoetida, pippali and garlic are quoted to be useful in different types of cancer [2,3]. One such plant is "*Jivanti*" (*Leptadenia reticulata*), belonging to family Asclepiadaceae, , well known for its tonic, restorative and stimulant property in the Indian system of medicine. This plant is distributed in the Southern parts of India. The main constituents reported are stigmasterol, β – sitosterol, flavonoids, pregnane glycosides and proteins [4]. Presence of triterpenes and steroids were also reported [4]. Aerial parts of *Leptadenia reticulata* is reported to contain tocopherol and possess several pharmacological activities such as galactogogue, antimicrobial and anti-inflammatory activity. Simiarenol (3 β – hydroxy-E: B-friedo-hop-5-ene), a rare triterpene alcohol was isolated from the leaves of *L.reticulata*. Seeds of *L.reticulata* are reported to contain hyperoside, a flavonoid glycoside. *L. reticulata* is claimed to have hypotensive effect in dogs [4].

Antioxidant principles derived from plants are reported to have antitumour activity [5]. Hence plants containing flavonoids are constantly being screened for antitumour activity [6]. Some of the active principles present in this plant are reported to be flavonoids [4]. It is also used by the tribals of Kolli Hills, Tamil Nadu, India for various types of tumors and by practitioners of traditional systems of medicine against acute tumours. Hence it was decided to illustrate the ethnobotanical use of the plant and the study was planned to evaluate the effect of leaf extract of *Leptadenia reticulata* against Dalton's Ascitic Lymphoma (DAL)

MATERIALS AND METHODS

The leaves of the plant were collected from Madurai district, Tamil Nadu, India, in the month of November – December and identified by Dr. Jeyavenkatesh, Professor, Department of Siddha Medicine, Madurai Kamaraj University and a voucher specimen was deposited in institutional herbarium (Voucher No. AS-5).

The study was carried out after obtaining permission from Institutional Animal Ethics Committee (No. 626/02/a/CPCSEA) and CPCSEA regulations were adhered to during the study. Male Swiss Albino mice (20-30 g) were selected for this study [1]. The animals were maintained under standard environmental conditions and fed with standard pellet feed and water *ad libitum*.

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Table 1. Effect of LELR on DAL induced mice

Groups	Cancer cell number (x 10^6)	Packed Cell Volume (%)	Increase in Tumour weight (g)	Number of Days survived	Increase in Life Span (%)
G1	1.82 ± 0.18	51.35 ±0.46	10.86 ± 0.48	20 ± 0.2	--
G2	0.94 ± 0.10*	37.80 ± 0.58**	5.64 ± 0.54**	25 ± 1.2*	26.6 %
G3	0.82 ± 0.36*	28.56 ± 0.18**	3.86 ± 0.40**	28 ± 3.2*	40 %

Values are represented as mean ± SEM of six animals.
One-way ANOVA followed by Newman-Keul's multiple comparison test
* $p < 0.05$, ** $p<0.001$ compared to G1.
G1 – Control (DAL induced, non-treated)
G2 – 200 mg/kg of LELR treated group
G3 – 20 mg/kg of 5-flourouracil treated group

Preparation of the drug

The shade-dried leaves of *Leptadenia reticulata* were powdered coarsely and about 500 g of this powder was extracted (Soxhlet) with 70% ethyl alcohol in 1:10 w/v ratio for 72 hrs. The yield was 20 g. The extract was dried in vacuum and dissolved in water before use. The phytochemical screening gave positive results for carbohydrates, alkaloids, flavonoids and glycosides [7, 8].

Acute toxicity studies

Acute toxicity study was carried out on leaf extract of *Leptadenia reticulata (*LELR) following OECD guidelines [9]. The extract was found to be safe up to 2000 mg/kg of body weight.

Determination of antitumour activity

The animals were acclimatized to our laboratory conditions. They were divided into three groups viz. Control (G1), 200 mg/kg of LELR treated (G2), and 20 mg/kg of 5-Fluorouracil treated group (G3) of six each and used for the study [10]. The DAL cells were procured from Amala Cancer Institute, Thrissur, Kerala and injected intraperitoneally (10^6 cells/mice) to all groups of animals. On the second day the animals of G2 were treated with 200 mg/kg of LELR while G3 with 5-fluorouracil (20 mg/kg) and the treatment was continued for next 10 days. G1 was not allocated any treatment after inoculation with DAL cells. The mice were observed for next 10 days for the development of ascitic tumour. On day 11, the following parameters were estimated.

1-Cancer cell number
2-Packed cell volume (PCV)
3-Decrease in tumour weight of the mice
4-Increase in life span (ILS)

Determination of hematological parameters [1]

Apart from the above mentioned parameters, the effects of LELR on hematological parameters were also studied in the mice of all the groups. Blood was collected from all groups of animals by retro-orbital puncture and counted for RBC and WBC. For comparison a normal control group (G4) was used which was neither inoculated with cancer cells nor treated *Statistical analysis* [1].

The results are expressed as mean ± S.E.M. The evaluation of the data was done using one way ANOVA followed by Newman-Keul's multiple comparison test; $p< 0.05$ implied significance.

RESULTS

The extract reduced the cancer cell number to 0.94 ± 0.10 x 10^6 cells in the treated mice (Table 1). Following inoculation with DAL cells, there was profound proliferation of tumour cells in the peritoneal cavity of the mice. As a result the PCV in the tumour control mice was found to be high (51.35%). Intraperitoneal administration of the extract had reduced the PCV to 37.8%. Also a decrease in tumour weight was noted in the LELR treated mice (Table 1). The percentage increase in lifespan (ILS) of the LELR-treated mice increased by 26.6% (Table 1).

Regarding the effect of LELR on the hematological parameters, it was found that the tumour bearing mice showed reduced number of RBC but an increase in WBC compared to normal control mice. Following treatment with LELR, RBC count was elevated to 4.62 ± 0.22 x $10^5 \mu l^{-1}$ whereas WBC count was reduced to 5.26 ± 0.62 x 10^3 μl^{-1} (Table 2).

Table 2. Effect of LELR on hematological parameters

Groups	Total WBC (x 10^3)μl^{-1}	Total RBC (x 10^5) μl^{-1}
G1	7.65 ± 0.10	2.84 ± 0.40
G2	5.26 ± 0.62**	4.62 ± 0.22*
G3	5.12 ± 0.58**	4.74 ± 0.64*
G4	4.64 ± 0.64**	5.12 ± 0.42**

Values are represented as mean ± SEM of six animals.
One-way ANOVA followed by Newman-Keul's multiple comparison test.
*$p<0.05$, ** $p<0.01$ compared to G1.
G1 – Control (DAL induced, non-treated)
G2 – 200 mg/kg of LELR treated group
G3 – 20 mg/kg of 5-flourouracil treated group
G4 – Normal control

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DISCUSSION

Cancer is a group of more than 100 different diseases characterized by uncontrolled cellular growth, local tissue invasion and distant metastases [11] and the free radicals have been implicated in carcinogenesis [12]. Supportive to this, many plant extracts containing antioxidant principles have been reported to possess antitumour activity [5]. Based on this, it was contemplated to carry out this study.

In the present study, intraperitoneal inoculation of DAL cells in the mice produced an enormous increase in the cancer cell count, which indicated that there is progression of cancer in the animals. The decrease in the cancer cell number observed in the LELR treated group G2 indicates that the test drug is having significant inhibitory effect on the tumour cell proliferation. The increase in tumour weight of G2 may be due to accumulation of peritoneal fluid as an abnormal enlargement of peritoneal cavity was observed in tumour-induced mice. Treatment with LELR reduced the tumour weight and hence increased the lifespan. From the hematological studies it is understood that the significant rise in WBC in G1, might be a defensive mechanism against cancer cells. As the progression of cancer was brought under control by LELR the WBC count got reduced in G2. These observations on the effect of LELR on various parameters studied to evaluate the antitumour activity enabled us to conclude that it has significant antitumour activity. However, further investigations are essential for the isolation of the active principles of LELR and its mechanism of action.

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