**Drosera indica** L: Potential effect on liver enzyme, lipid profile and hormone change in Dalton’s lymphoma ascites (DLA) bearing mice

Raju Asirvatham¹, Arockiasamy Josphin Maria Christina²

¹Shri Rawatpura Sarkar Institute of Pharmacy, Datia, MP, India.
²AIMST University, Malaysia

**Abstract**

Aim: In this study, the ethanol and aqueous extracts of *Drosera indica* L were prepared and cancer induced liver enzyme, lipid profile and hormone changes were studied in mice using the Dalton’s lymphoma ascites (DLA) cells.

Method: Animals were divided into seven groups as the normal control, DLA control, standard (5FU) and the ethanol and aqueous extracts (250 and 500mg/kg each) of *D. indica* L + DLA (four groups) were given the respective treatments 24 h after tumor cell inoculation, for 14 days.

Result: Both ethanol and aqueous extracts of *D. indica* L at doses of 250 and 500mg/kg extracts showed significant (p<0.001) effects on the elevated liver enzyme, lipid profile and hormonal changes to normal.

Conclusion: The results of the present study demonstrated that both extracts were able to normalize the cancer induced liver enzyme, lipid profile and hormone changes in DLA bearing mice.

**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]. The aim of naturopathic cancer treatment is to support normal metabolism, decrease side effects of treatment and boost the body's immune system. These treatments also provide strategies for long term health maintenance and improve energy, well-being and overall quality of life [2]. In certain cancer therapies there is an increased risk of development of characteristics metabolic syndrome. It will be important to evaluate cancer therapy itself to overcome a risk factor for the development of metabolic syndrome. Metabolic syndrome often associated with elevated triglycerides, reduced high density lipoprotein (HDL), low testosterone levels and overall connection with sex hormones [3]. These problems were overcome by plant drug treatment. Ayurvedic, a system of Indian medicine in which the treatment involves the use of whole plant extracts either alone or a combination of several plant extract for better efficacy and reduction in toxicity. Each herbal formulation contains multiple active principles that may operate synergistically, producing therapeutic benefits and lowering the risks on adverse effects [4].

*Drosera* is a cosmopolitan genus of insectivorous plants and consists of approximately 170 species. In India, *D. indica* L., *D. burmanii* and *D. peltata* J.E.Sm. ex Wild have been reported from many different locations. These species are used as vital components in an Ayurvedic preparation called ‘Swarnabhaisma’ (Golden...
Drosera indica L., has been commonly used for treatment of stomach, eczema, and hepatitis [7]. The major naphthoquinone found in D. indica is plumbagin (2-methyl-5-hydroxy-1,4-naphthoquinone) [8]. This present study was carried out to evaluate the effect of D. indica on development of metabolic syndrome by treatment with ethanol and aqueous extract of Drosera indica L., in Dalton lymphoma ascites (DLA) bearing mice mice.

MATERIAL AND METHODS

Plant material
The whole plant of D. indica L. was collected from the forests of Savanadurga, Karnataka, India during December 2010. The plant material was identified and authenticated by Dr. S.N.Yoganarasimhan, Taxonomist and Research Coordinator at M. S. Ramaiah College of Pharmacy, Bangalore, Karnataka, India. The material was washed with tap water, shade dried, powdered, passed through sieve no. 60 and stored in air tight containers for further experiments.

Preparation of the extracts
Alcoholic extract: A weighed quantity of the air-dried powdered drug was extracted with ethanol (90 %v/v) in a Soxhlet apparatus. The extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50°C. The ethanol extract was suspended in distilled water for experimental purposes.

Aqueous extract: The marc from the ethanol extract was macerated with chloroform-water for 24h to obtain the aqueous extract. Aqueous extract was concentrated under vacuum and dissolved in distilled water for experimental studies.

The ethanol (EEDI) and aqueous (AEDI) extracts of D. indica L. were stored in air tight containers.

Acute toxicity studies
Acute toxicity study was carried out on EEDI and AEDI following OECD guidelines (OECD 423) [9].

Induction of cancer using DLA cells
Dalton lymphoma ascites (DLA) cells were supplied by Amala Cancer Research Center, Trissur, Kerala, India. The cells maintained (in vivo) in Swiss albino mice by intraperitoneal transplantation (2X10^6 cells/ mouse) for 7 days. Treatment protocol [10, 11]

Mature male and female (virgin) Swiss Albino mice weighing 20-25g (n=10) were kept in identical laboratory condition and were fed with standard pellet diet and water ad libitum. Study protocol was approved by the Institution Animal Ethical Committee (Protocol.No.A.Raju 0903PH2254/ JNTUH 2009). They were divided into seven groups as Normal group (G1), DLA control group (G2), DLA + 20mg/kg of 5-Fluorouracil treated group (G3), 250,500mg/kg of EEDB (G4 and G5) and 250 and 500mg/kg AEDB (G6 and G7) of ten each and used for the study. The DLA cells were injected intraperitoneally (2X10^6 cells/ mouse) to all groups of animals except G1. On the second day the animals of G3 with 5-fluorouracil (20 mg/kg, i.p), G4 and G5 were treated with 250 and 500 mg/kg of EEDB and G6 and G7 with 250 and 500mg/kg of AEDB orally. The treatment was continued for 14 days. G1 was treated with vehicle.

On day 15, the mice were sacrificed before that blood was withdrawn by retro - orbital plexus method and the following parameters were measured.

Assay of Hormones [12, 13]
Hormones such as LH, FSH, E2and progesterone levels in virgin female mice blood were measured by RIA method using commercially available standard kits according to the manufacturer’s instruction (Sigma, US)

Lipid profile [14]
Cholesterol, triglyceride, HDL cholesterol was estimated using kits from Agappe Diagnostics, Kerala, India. The estimation was carried out on fully automated analyzer Hitachi 717(Italy)

Liver marker enzymes [15]
Serum enzymes such as Aspartate amino Transferase (AST), Alanine amino Transferase (ALT), Alkaline Phosphatase (ALP) and Lactate dehydrogenase (LDH) were analyzed using Agappe Diagnostics, Kerala, India.

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

RESULT

Acute toxicity studies
Ethanol and aqueous extract of D. indica L. were administered separately up to 3000 mg/kg body weight and since these extracts did not produce any toxic manifestation like increased motor activity, salivation, acute convulsion, coma and death. Hence they were considered safe for further pharmacological screening.
### Table 1. Effect of EEDI and AEDI on Liver enzyme of DLA bearing mice

| Parameters                | AST (U/I)      | ALT (U/I)      | ALP (U/I)     | LDH (U/I)     |
|---------------------------|----------------|----------------|---------------|---------------|
| Normal                    | 70.55±0.93     | 25.1±0.17      | 0.98±0.05     | 293.4±12.63   |
| DLA control               | 134.9±2.76     | 16.33±1.48     | 5.7±0.1       | 563.3±19.19   |
| DLA+5FU (20mg/kg)         | 67.18±1.9      | 24.1±0.27      | 1±0.09        | 297.97±6.32   |
| DLA+EEDI (250mg/kg)       | 107.38±4.6a    | 23.5±1.01a     | 3.13±0.05a    | 373.2±28.4a   |
| DLA+EEDI (500mg/kg)       | 68.7±1.1a      | 23.7±0.45a     | 0.93±0.06a    | 310.9±3.45a   |
| DLA+AEDI (250mg/kg)       | 117.2±1.76a    | 21.15±0.26a    | 4.05±0.06a    | 462.5±6.87a   |
| DLA+AEDI (500mg/kg)       | 87.62±1.94a    | 22.4±0.36a     | 2.3±0.17a     | 386.4±6.32a   |

The data were expressed as mean ±S.E.M. n = 10. The data analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keul’s multiple comparison test:

a- ***p<0.001, compared to the DLA control group

### Table 2. Effect of EEDI and AEDI on Hormone level of DLA bearing mice

| Parameters                | LH (ng/ml)X10^2 | FSH (ng/ml) | E2 (β-estradiol) pg/ml | Progesterone (ng/ml) |
|---------------------------|-----------------|-------------|------------------------|----------------------|
| Normal                    | 20±1.74         | 6.7±0.23    | 24.1±1.32              | 15.5±0.14            |
| DLA control               | 35.6±2.2        | 1.63±0.2    | 5.33±0.15              | 5.05±0.34            |
| DLA+5FU (20mg/kg)         | 16.42±0.51      | 6.27±0.32   | 20.53±1.11             | 14.82±0.23           |
| DLA+EEDI (250mg/kg)       | 24.07±0.9a      | 4.1±0.09a   | 12.83±1.37             | 10.57±0.19           |
| DLA+EEDI (500mg/kg)       | 23.95±0.96a     | 5.7±0.17a   | 21.72±0.58             | 15.27±0.49           |
| DLA+AEDI (250mg/kg)       | 23.4±1.85a      | 2.95±0.23a  | 10.75±1.01b            | 8.67±0.21            |
| DLA+AEDI (500mg/kg)       | 22.57±1.1a      | 3.95±0.06a  | 12.9±1.38              | 12.82±0.39           |

The data were expressed as mean ±S.E.M. n = 10. The data analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keul’s multiple comparison test:

a- ***p<0.001, compared to the DLA control group

b- **p<0.01, compared to the DLA control group

### Table 3. Effect of EEDI and AEDI on Lipid profile of DLA bearing mice

| Parameters                | Cholesterol (mg/dl) | Triglyceride (mg/dl) | HDL(mg/dl) | LDL(mg/dl) |
|---------------------------|---------------------|----------------------|------------|------------|
| Normal                    | 152.7±5.3           | 95.58±1.3            | 34.6±0.4   | 117.7±1.2  |
| DLA control               | 118.3±3.08          | 127.8±0.7            | 21.5±0.8   | 79.5±2.6   |
| DLA+5FU (20mg/kg)         | 142.9±0.9           | 89.05±1.5            | 32.2±1.1   | 115.1±0.9  |
| DLA+EEDI (250mg/kg)       | 135.3±1.4b          | 98.35±0.6a           | 30.5±0.9   | 116.7±0.7b |
| DLA+EEDI (500mg/kg)       | 148.05±2.0a         | 94.75±0.8a           | 34.9±0.8   | 119.5±0.4a |
| DLA+AEDI (250mg/kg)       | 128.7±3.8b          | 114.1±0.7a           | 28.6±0.5   | 104.7±1.1b |
| DLA+AEDI (500mg/kg)       | 144.4±0.9a          | 103.5±1.3a           | 30.9±0.9   | 112.05±1.5a|

The data were expressed as mean ±S.E.M. n = 10. The data analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keul’s multiple comparison test:

a- ***p<0.001, compared to the DLA control group
Administration of daily doses of 250, 500 mg/kg for 14 days altered the serum liver enzyme, as shown in Table 1. There is an increased AST, ALP and LDH and there is a decreased ALT in DLA bearing mice. Treatment with both the doses of EEDI and AEDI significantly (p<0.001) reduced the elevated levels of the altered parameters to normal level.

The serum hormone levels in virgin female mice were greatly altered in DLA bearing mice (Table 2). Continuous 14 days oral administration of 250 and 500mg/kg of EEDB and AEDB restored the hormone level near to normal in female virgin mice.

The serum Cholesterol, HDL and LDL cholesterol significantly (p<0.001) decreased in DLA control mice and were restored by the doses of EEDI and AEDI treatment. Serum triglycerides values were significantly elevated in DLA control mice and were brought back to normal value by the extract treatment (Table 3).

DISCUSSION

The present study was carried out to evaluate the extracts of D. indica L on liver enzyme, lipid profile and hormone changes in DLA bearing mice, doses of 250 and 500 mg/kg of EEDB and AEDB were given orally for 14days to DLA bearing mice.

Endogenous level of sex steroids and gonadotrophic hormones and increased receptor population are associated with cancer development [12]. The steroids which are likely to influence tumorogenesis, are namely estrogen, progesterone. The steroids are synthesized and secreted mainly from ovaries and adrenal glands on the stimulation of follicle stimulating hormone (FSH) and lutenizing hormone(LH), are involved in regulation of steroidogenesis. In this study DLA-bearing virgin female mouse showed altered hormone level which was brought back to normal.

Since liver is considered to be the main organ of drug detoxifying organ, some liver marker enzyme levels were measured from serum. AST, ALP and LDH levels were increased in DAL controlled mice, whereas ALT level was decreased. Yet there is no explanation has been given such changes but Abu Sienna et al., 2003 suggested that, the consumption of free amino acid for building the proteins of rapidly dividing tumor cells might result in the disturbance of the enzyme activity in the liver. On treatment with 250, 500mg/kg of EEDI and AEDI, altered liver enzyme level was restored as that of the normal group. It was compared with DAL control group, this result indicating that the plant extracts play a protective role on the liver.

Several studies have reported clear relationship between low cholesterol levels and cancer [14,16,17].Alterations of cholesterol metabolism, including increased cholesterol synthesis and accumulation of cholesterol esters in tumor tissues associated with a decrease of high density lipoprotein cholesterol in serum, were previously observed in different models of neoplastic cell proliferation including haematological malignancies [18].In our study showed that the serum cholesterol, HDL cholesterol and LDL cholesterol showed significantly decreased in DLA control mice which was restored by the doses of 250,500 mg/kg of EEDI and AEDB treatment, whereas the serum triglycerides values were significantly elevated in DLA control mice and are brought back to normal value by the extract treatment.

CONCLUSION

Results concluded that the ethanol and aqueous extract of D. indica L was effective in inhibiting the tumor growth (reported) in DLA-bearing mice. The results of the present study demonstrated that both extracts normalized the cancer induced metabolic change and lipid profile. The higher dose of ethanol extract showed a significant good activity when compared with the lower dose, similarly higher dose of aqueous extract showed better activity than lower dose but ethanol extract was comparatively better than aqueous extract.

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